OPEN ACCESS MACEDONIAN JOURNAL OF MEDICAL SCIENCES

eISSN 1857-9655

oa mjms

http://www.mjms.mk http://www.id-press.eu/mjms

OA Maced J Med Sci. 2016 Sep 15; 4(3):319-533

Open Access Maced J Med Sci (OAMJMS) is an international peer-reviewed, Open Access journal published four times per year. ***Indexed in:*** Bielefeld Academic Search Engine (BASE), CAB Direct, CiteFactor, CNKI Scholar, CrossRef DOI, DeepDyve, Directory of Open Access Journals (DOAJ), Directory of Open Access scholarly, Resources (ROAD), Europe PMC, Global Health database, Global Impact Factor (GIF), Google Scholar, Harvard Library [HOLLIS +], HINARI Access to Research Initiative, JournalTOCs, Mendeley, NLM Catalog, OpenAIRE, Open Academic Journals Index (OAJI), Open Access Library (OALib), pubget, PubMed, PubMed Central, PubMed Central CANADA, SciLit, SciRew, Scopus, SouthEast European Science Advanced through Evaluation (SEESAmE), The Knowledge Network, Virtual Library of Macedonia (COBISS.MK), Western Theological Seminary, WorldCat. ***Publisher:*** ID Design 2012/DOOEL Skopje, Rajko Zhinzifov No. 48, 1000 Skopje, Republic of Macedonia. OAMJMS Online (eISSN 1857-9655) offers free access to all articles at http://www.id-press.eu/mjms/

Editorial Team

Editorial Office:

ID Design 2012/DOOEL Skopje, Rajko Zhinzifov No. 48, 1000 Skopje, Republic of Macedonia | Telephone: +389 70 255155. | e-mail: mspiroski@id-press.eu | URL: http://www.id-press.eu/mjms/

Publisher:

ID Design 2012/DOOEL Skopje, Rajko Zhinzifov No. 48, 1000 Skopje, Republic of Macedonia.

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):319-323.
http://dx.doi.org/10.3889/oamjms.2016.072
eISSN: 1857-9655
Basic Science

Neck Circumference in Overweight/Obese Subjects who Visited the Binjai Supermall in Indonesia

Dharma Lindarto*, Shierly, Santi Syafril

Department of Internal Medicine, Faculty of Medicine, North Sumatra University - H. Adam Malik Hospital, Jalan Bunga Lau No 17, Medan 20136, Indonesia

Citation: Lindarto D, Shierly, Syafril S. Neck Circumference in Overweight/Obese Subjects who Visited the Binjai Supermall in Indonesia. Open Access Maced J Med Sci. 2016 Sep 15; 4(3):319-323. http://dx.doi.org/10.3889/oamjms.2016.072

Keywords: obesity; neck circumference; cutoff point; overweight; BMI.

***Correspondence:** Dharma Lindarto. Department of Internal Medicine, Faculty of Medicine, North Sumatra University - H. Adam Malik Hospital, Jalan Bunga Lau No 17, Medan 20136, Indonesia. Phone +62811631514. E-mail: dharmalindarto22@gmail.com

Received: 13-May-2016; **Revised:** 25-Jun-2016; **Accepted:** 26-Jun-2016; **Online first:** 16-Jul-2016

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: Neck circumference (NC) is a simple screening measure for identifying overweight and obesity, it reflects upper-body fat distribution and central obesity.

AIM: To determine whether a single measure of NC might be used to identify overweight/obesity.

MATERIAL AND METHODS: An observational, analytical, cross-sectional study was done. The subjects consisted of all consecutive subjects who visited Binjai Supermall (North Sumatera Province, Indonesia) between 23rd and 29th September 2015 and agreed to participate in the study. NC, weight, height, body mass index (BMI), and waist circumference (WC) were measured. Overweight and obesity were defined as BMIs of 23.0–24.9 and ≥ 25 kg/m^2, respectively.

RESULTS: In total, 1554 subjects participated. Of these, 1238 (79.7%) were overweight/obese. NC correlated significantly with weight, height, BMI, and WC. Receiver operating characteristic (ROC) analysis showed that for all men and women, the area under the curve of overweight/obesity for NC was 0.83 and 0.79, respectively. The best NC cutoff points for males and females that indicated overweight/obesity were ≥ 37 cm (sensitivity, 78.3% and specificity, 75.5%) and ≥ 33.5 cm (sensitivity, 76.6% and specificity, 66.7%), respectively.

CONCLUSION: The NC cutoffs that were identified may be useful for screening for overweight/obesity and related co-morbidities.

Introduction

Obesity is defined as an excessively high amount of body fat or adipose tissue in relation to lean body mass [1]. Current estimates; in the US, are that 69% of adults are either overweight or obese, with approximately 35% being obese [2]. Obesity raises the risk of hypertension, dyslipidemia, type 2 diabetes mellitus (T2DM), coronary heart disease, stroke, gallbladder disease, osteoarthritis, sleep apnea, respiratory problems, and some cancers [3]. In developing countries, people with a high socioeconomic status are most likely to be obese. This may be due to their occupation, education level, physical activity, and tendency to smoke [4]. In 2013, the prevalence of obesity in North Sumatra Province, Indonesia was about 27%. This is markedly greater than the national prevalence of obesity (20%) [5].

When assessing obesity, various techniques are used. These include measuring BMI, the waist circumference (WC), waist/hip ratio, mid-upper arm circumference, subscapular/triceps ratio, and neck circumference (NC) [6]. NC is a particularly simple and rapidly obtained anthropometric measurement that can be used to screen for overweight/obese people because it reflects upper-body fat distribution and central obesity [7, 8]. The Framingham Heart Study showed that NC is an index of central obesity as it associates independently with visceral adiposity and body mass index (BMI). Moreover, NC associates independently with cardiometabolic risk factors (*e.g.*, systolic and diastolic blood pressure) even after adjusting for visceral adiposity or BMI and WC [9]. Similarly, NC with metabolic syndrome correlated better among females than males. The cross - sectional study was conducted in a tertiary care hospital in South India, showed that men with NC >37

cm and women with NC >34 cm are more prone to cardiometabolic syndrome and require additional evaluation [10]. A study of 3182 diabetic Chinese patients showed that NC correlated positively with BMI, WC, and metabolic syndrome [11]. However, the usefulness of the NC measurement in diverse healthy and clinical populations in Indonesia has not yet been reported.

The aim of this study was to determine whether a single measure of NC might be used to identify overweight/obese and to define NC cutoff levels for overweight/obesity for men and women according to existing BMI as standards.

Material and Methods

An observational, analytical, cross-sectional study was done. The study was approved by local of the Research and Ethics Committee and was conducted according to the principles of Helsinki and its revisions. All participants provided written informed consent to participate in the study before enrollment in the study.

Subjects

The subjects were all consecutive participants who visited Binjai Supermall (North Sumatera Province, Indonesia) between 23 ^{to} and 29th September 2015 and agreed to participate in the study after its objectives and methods were explained. The subjects were recruited by a team composed of three trained interviewers who stood near the main entrance of the Supermall next to a 3×4 m billboard bearing the words “Survey Obesity” (“Survey of Obesity”). The interviewers explained the purpose of the study to passers-by. If people agreed to participate, they were led to a secluded room staffed by three medical professionals who took the subject’s medical history and determined their eligibility to participate. Subjects were excluded if they were pregnant or breastfeeding, had a history of neck disease (*e.g.*, thyroid disorders, neck surgery, or neck malignancy), had a history of lung diseases such as chronic obstructive pulmonary disease, asthma, or pulmonary fibrosis, or had any anatomical disorder. Written informed consent was obtained. Thereafter, the anthropometric measurements (height, weight, BMI, WC, and NC) were obtained.

Anthropometric Evaluation

The anthropometric measurements were obtained using standard techniques. WC was measured at the midpoint between the lowest costal margin and the iliac crest at the end of normal expiration [3]. For women, NC was measured in the middle of the neck, namely, between the mid cervical spine and the mid anterior neck. For men, NC was measured just below the laryngeal prominence (Adam’s apple) [12]. All measurements were taken in the standing position. Weight was measured with a digital scale while the patient was wearing light clothing and lacked shoes. The digital scale measured weight to the nearest 100 g. Height was measured with a stadiometer while the subject was without shoes.

Definitions of Anthropometric Measurement Cutoffs

Healthy WC limits are 90 cm for men and 80 cm for women.BMI was calculated by dividing weight (kg) by the square of height (m^2). Normal weight and underweight were considered to be BMIs of 18.5–22.9 and < 18.5 kg/m^2, respectively. Overweight and obesity were considered to BMIs of 23.0 – 24.9 and ≥ 25 kg/m^2, respectively. These weight cutoffs were specified for the Asia-Pacific population by the Western Pacific Regional Office of the World Health Organisation [13].

Statistical Analysis

Data are shown as mean ±SD unless otherwise specified. Independent t-test and Pearson’s correlation were the tests of significance done to analyse the quantitative data. The NC cutoffs for overweight/obesity against BMI in males and females were identified by receiver operator characteristic (ROC) analysis. A p-value of less than 0.05 was considered statistically significant. All statistical analysis was performed using SPSS 22.0 software.

Results

Characteristics of the Participants

In total, the study sample of 1554 subjects who visited Binjai Supermall. Of these, 1238 (79.7%) were overweight/obese (554 males and 684 females, aged between 25 and 70 years old).

Table 1: Anthropometric measurements of the overweight/obese participants in the study population

Characteristics	All Overweight/Obese *Mean* ± SD	Male *Mean* ± SD	Females *Mean* ± SD	P-value
Sex (M/F)	1238	554	684	
Age (yr)	41.6 ± 10.34	41.6 ± 10.72	41.6 ± 10.03	0.965
Weight (kg)	72.4 ± 11.89	78.2 ± 11.89	67.8 ± 9.67	0.000**
Height (cm)	161 ± 9.00	167.6 ± 6.85	155.5 ± 6.65	0.000**
NC (cm)	37.4 ± 3.46	39.1 ± 2.86	36.2 ± 3.32	0.000**
WC (cm)	92.7 ± 10.00	95.9 ± 9.77	90.1 ± 9.42	0.000**
BMI (kg/m^2)	27.9 ± 3.58	27.7 ± 3.52	28.1 ± 3,63	0.180

Data are expressed in mean ± SD. Abbreviations: NC: neck circumference; BMI: body mass index; WC: waist circumference; * P<0.05, ** P<0.01.

The overweight/obese males and females did not differ significantly in terms of age, but the males were significantly heavier and taller and had larger NCs and WCs. However, the males and females did not differ in terms of BMI (Table1).

Correlation analyses in the whole cohort (n = 1238), all males, and all females, NC correlated positively and significantly with weight (males: r = 0.621, P = 0.000; females: r = 0.452, P = 0.000), height (males: r = 0.218, P = 0.000; females: r = 0.195, P = 0.000), WC (males: r = 0.650, P = 0.000; females: r = 0.458, P = 0.000), and BMI (males: r = 0.578, P = 0.000; females: r = 0.373, P = 0.000) (Table 2).

Table 2: Correlations between neck circumference and other anthropometric variables in the whole cohort and in males and females only

Correlation	Total (n =1238)		Males (n = 554)		Females (n = 684)	
	r	P	r	P	r	P
Weight	0.595	0.000**	0.621	0.000**	0.452	0.000**
Height	0.452	0.000**	0.218	0.000**	0.195	0.000**
WC	0.570	0.000**	0.650	0.000**	0.458	0.000**
BMI	0.357	0.000**	0.578	0.000**	0.373	0.000**

Abbreviations: NC: neck circumference; BMI: body mass index; WC: waist circumference; * P<0.05, ** P<0.01.

ROC analysis showed that the area under the curve (AUC) of overweight/obesity for NC and BMI was 0.83; 95% CI 0.78-0.87; P < 0.001 (Figure 1) and 0.79; 95% CI 0.75–0.82; P < 0.001 (Figure 2) for men and women, respectively.

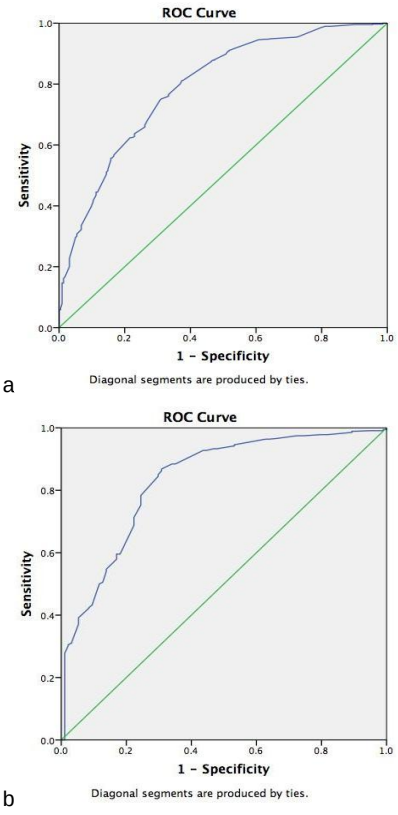

Figure 1: Receiver operating characteristic curves related to neck circumference and BMI in males (a) and females (b)

When BMI served as the standard of overweight/obese, the best NC cutoff points for males and females were ≥ 37 cm (sensitivity, 78.3% and specificity, 75.5%), and ≥ 33.5 cm (sensitivity, 76.6% and specificity, 66.7%), respectively (Table 3).

Table 3: Neck circumference cutoff levels that indicate overweight/obese males and females, as determined by Receiver Operating Characteristic analysis

	Males			Females	
Cutoff	Sensitivity (%)	Specificity (%)	*Cutoff*	Sensitivity (%)	Specificity (%)
36.45	88.4	66.0	33.05	81.3	62.2
36.55	86.8	69.1	33.15	81.1	62.6
36.65	86.3	69.1	33.25	80.1	63.1
36.80	85.0	70.2	33.35	79.6	63.5
36.95	84.5	70.2	33.45	79.2	64.0
37.05	**78.3**	**75.5**	**33.55**	**76.6**	**66.7**
37.15	78.0	75.5	33.65	76.3	66.7
37.30	76.2	75.5	33.75	76.0	66.7
37.45	75.3	75.5	33.85	75.1	68.9
37.55	71.3	77.7	33.95	74.5	69.4
37.65	70.8	77.7	34.05	67.5	73.4

Comparison of this study with previously conducted studies in Table 4.

Table 4: Comparison of the results of this study with previously conducted studies

Parameter	Sex	This Study N = 1238	Aswathappa et al [19] N = 1351	Hingorjo et al [20] N = 155	Ang NS & Raboca JC [21] N = 224
Study Population	Male	554	840	41	116
	Female	684	511	109	108
BMI (kg/m^2)	Male	27.9 ± 3.58	24.8 ± 4.40	21.69 ± 4.93	27.8 ± 3.68
	Female	27.7 ± 3.52	25.38 ± 4.89	21.04 ± 3.82	25.38 ± 4.89
WC (cm)	Male	95.9 ± 9.77	90.47 ± 12.09	80.67 ± 12.94	95.0 ± 8.73
	Female	90.1 ± 9.42	86.56 ± 12.09	78.17 ± 9.12	86.56 ± 12.09
NC (cm)	Male	39.1 ± 2.86	36.4 ± 5.70	35.56 ± 2.77	37.6 ± 3.81
	Female	36.2 ± 3.32	34.1 ± 5.70	31.52 ± 1.96	34.12 ± 5.70
Cutoff NC (cm)	Male	≥ 37	≥ 36	≥ 35.5	≥ 40
	Female	≥ 33.5	≥ 32	≥ 32	≥ 33.8
Determining the overweight/ obese		BMI ≥ 23 kg/m^2	BMI ≥ 23 kg/m^2	BMI ≥ 23kg/m^2	WC ≥ 90cm (males)

Abbreviations: NC: neck circumference; BMI: body mass index; WC: waist circumference.

Discussion

Obesity is now reaching pandemic proportions across much of the world and will impose an unprecedented health, financial, and social burden on the general public unless effective actions are taken to reverse the trend [14]. In 1956, Vague was the first researcher to find that the thickness of a neck skinfold, as measured by callipers, is a marker of upper-body fat distribution [15]. Thereafter, it was shown that NC can serve as an anthropometric marker to screen for central obesity and upper-body subcutaneous fat and their related co-morbidities [14]. Indeed, NC seems superior to other central obesity indicators in terms of predicting related co-morbidities: after adjustment for BMI and WC, NC was found to be the only risk factor for T2DM [14]. Similarly, Vallianou *et al.* showed that NC associated independently with high-density lipoprotein cholesterol, glucose, triglyceride, and uric acid levels, even after adjusting for BMI and WC. This indicates that NC may also be

useful for screening atherogenic dyslipidemia [16].The usefulness of this variable is heightened by the fact that it is easy to measure and thus can be used for self-monitoring by lay people.

The present study showed that NC correlated strongly and positively with weight, height, BMI, and WC in both male and female subjects. Several studies have also examined the association between conventional anthropometric measures of obesity and NC [6, 11, 17, 18]. In particular, Papandreou *et al* reported that NC was found to be independently associated with obesity levels in Emirati college students [19], Onat *et al.* reported that NC correlated strongly with BMI, WC, homeostatic model-assessed insulin resistance, and blood pressure [17]. Similarly, Yang *et al*.showed that, in Chinese subjects with T2DM, NC correlated positively with BMI, WC, and metabolic syndrome [11].

The present study showed that the NC cutoff points that best-indicated overweight/obese males and females were ≥ 37 cm (sensitivity, 78.3% and specificity, 75.5%) and ≥ 33.5 cm (sensitivity, 76.6% and specificity, 66.7%), respectively. These findings are consistent with similar studies performed in India [20] and Pakistan [21]. However, our cutoff points were lower than those generated by studies in countries such as the Philippines [22] and Iran [23]. This may be due to the fact that the present study used Asian BMI cutoff values to identify the NC cutoff points: in Asians, the BMI cutoff that indicates overweight/obesity is > 23.0 kg/m^2, whereas, in Caucasians, it is > 25 kg/m^2.

The study has several limitations that should be addressed in future research. In particular, the subjects who were included in this study were not evaluated in terms of their metabolic profile or thyroid function. Thus, whether the NC cutoffs we identified could be used to predict the risk of metabolic syndrome is not yet clear.

In conclusion, this study indicated that 79.7% of the visitors to Binjai Supermall during a 7 days period in 2015 were overweight/obese, as indicated by their BMI. NC correlated strongly with weight, height, BMI, and WC. When BMI cutoffs for the Asia-Pacific population were used in ROC analysis, the best NC cutoffs for indicating overweight/obesity in males and females were ≥ 37 cm (sensitivity, 78.3% and specificity, 75.5%) and ≥ 33.5 cm (sensitivity, 76.6% and specificity, 66.7%), respectively. These values may be useful because NC predicts overweight and obesity and related co-morbidities and can be used as an initial screening tool for the purpose. This usefulness is enhanced by the fact that it is a straightforward and inexpensive test that can be performed in any office with a tape measure.

Acknowledgements

Authors are greatly thankful for all subjects included in this study, the staffs and employees of Binjai Supermall for the opportunity and implementation that enabled us to use the data to establish this study.

References

1. Stunkard AJ, Wadden TA. Obesity: Theory and Therapy. Second ed. New York, NY: Raven Press, 1993:163-79.

2. Flegal KM, Carroll MD, Kit BK, Ogden CL. Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. JAMA. 2012;307:491-7. http://dx.doi.org/10.1001/jama.2012.39 PMid:22253363

3. Clinical guidelines on the Identification, evaluation, and treatment of overweight and obesity in Adults the evidence report. National Institutes of health Obes Res. 1998;6(Suppl 2):51s–209s. PMid:9813653

4. Alaba O, Chola L. Socioeconomic inequalities in adult obesity prevalence in south Africa: a decomposition analysis. Int J Environ Res Public Health. 2014;11:3387-406. http://dx.doi.org/10.3390/ijerph110303387 PMid:24662998 PMCid:PMC3987040

5. RISKESDAS. Badan Penelitian dan Pengembangan Kesehatan, Kementerian Kesehatan RI. Riset kesehatan dasar 2013. Jakarta: Badan Penelitian dan Pengembangan Kesehatan, KementerianKesehatan RI. 2013;224.

6. Hatipoğlu N, Mazıcıoğlu M, Kurtoğlu, Kendirci M. Neck circumference: an additional tool of screening overweight and obesity in chilhood. Eur J Pediatr. 2010;169:733-9. http://dx.doi.org/10.1007/s00431-009-1104-z PMid:19936785

7. Ben-Noun L, Sohar E, Laor A. Neck circumference as a simple screening measure for identifying overweight and obese patients. Obesıty Research. 2001;9 (8):470-7. http://dx.doi.org/10.1038/oby.2001.61 PMid:11500527

8. Ben-Noun L, Laor A. Relationship between changes in NC and change blood pressure. Am J of Hypertention. 2004;17(5):409-14. http://dx.doi.org/10.1016/j.amjhyper.2004.02.005 PMid:15110899

9. Preis SR, Massaro JM, Hoffmann U, D'Agostino RB Sr, Levy D, Robins SJ, et al: Neck circumference as a novel measure of cardiometabolic risk: the Framingham Heart study. J Clin Endocrinol Metab. 2010;95(8):3701–10. http://dx.doi.org/10.1210/jc.2009-1779 PMid:20484490 PMCid:PMC2913042

10. Kumar NV, Ismail MH, Mahesha P, Girish M, Tripathy M. Neck Circumference and Cardio-Metabolic Syndrome. Journal of Clinical and Diagnostic Research. 2014;8(7):MC23-MC5. PMid:25177592 PMCid:PMC4149098

11. Yang GR, Yuan SY, Fu HJ, Wan G, Zhu LX, Bu XL, et al. Beijing Community Diabetes Study Group: Neck circumference positively related with central obesity, overweight, and metabolic syndrome in Chinese subjects with type 2 diabetes: Beijing Community Diabetes Study 4. Diabetes Care. 2010; 33(11):2465–7. http://dx.doi.org/10.2337/dc10-0798 PMid:20724650 PMCid:PMC2963514

12. Din ASE, Hassan N, El-Masry S, Al-Tohamy M. Neck circumference as a simple screening measure for indentifyingegyptian overweight and obese adults. Maced J Med Sci 2013;6(3):232-7. http://dx.doi.org/10.3889/MJMS.1857-5773.2013.0309

13. WHO/IASO/IOTF. The Asia-Pacific Perspective: Redefining

Obesity and itsTreatment. Health Communications Australia Pty Ltd, 2000:18.

14. Preis SR, Pencina MJ, D'Agostino RB, Meigs JB, Vasan RS, and Fox CS. Neck circumference and the development of cardiovascular disease risk factors in the Framingham Heart Study. Diabetes Care. 2013;1(36):e2. http://dx.doi.org/10.2337/dc12-0738

15. Vague J. The degree of masculine differentiation of obesities: a factor determining predisposition to diabetes, atherosclerosis, gout, and uric calculous disease. Am J Clin Nutr. 1956;4:20–4. PMid:13282851

16. Vallianou NG, Evangelopoulos AA, Bountziouka V, Vogiatzakis ED, Bonou MS, Barbetseas J, et al. Neck circumference is correlated with triglycerides and inversely related with HDL cholesterol beyond BMI and waist circumference. Diabetes Metab Res Rev. 2013;29(1):90-7. http://dx.doi.org/10.1002/dmrr.2369 PMid:23135790

17. Onat A, Hergenc G, Yuksel H, Can G, Ayhan E, Kaya Z. Neck circumference as a measure of central obesity: Associations with metabolic syndrome and obstructive sleep apnea syndrome beyond waist circumference. Clin Nutr. 2009;28:46-51. http://dx.doi.org/10.1016/j.clnu.2008.10.006 PMid:19010573

18. Nafiu OO, Burke C, Lee J, Voepel-Lewis T, Malviya S, Tremper KK. Neck circumference as a screening measure for identifying children with high body mass index. Pediatrics. 2010;126: e306-e10. http://dx.doi.org/10.1542/peds.2010-0242 PMid:20603254

19. Papandreou D, Noor ZT, Rashed M, Jaberi HA. Association of Neck Circumference with Obesity in Female College Students. Open Access Maced J Med Sci. 2015;3(4):578-81. http://dx.doi.org/10.3889/oamjms.2015.118 PMid:27275290 PMCid:PMC4877890

20. Aswathappa J, Garg S, Kutty K, Shankar K. Utility of Neck Circumference, A Simple And Novel Measure As Anthropometric Marker Of Obesity In Adults. World Journal Of Pharmacy And Pharmaceutical Sciences. 2014;3(3):1618-1629.

21. Hingorjo MR, Qureshi MA, Mehdhi A. Neck circumference as a useful marker of obesity: a comparison with body mass index and waist circumference. J Park Med Assoc. 2012;62(1):36-44. PMid:22352099

22. Ang NS, Raboca JC. Neck circumference as a screening measure for abdominal obesity and its association with metabolic syndrome among high risk filipino patients in makati medical center-a pilot study. Journal of the Asean Federation of Endocrine Societies. 2011;26(2):150-8. http://dx.doi.org/10.15605/jafes.026.02.13

23. Hatamizadeh M, Ranjbar H, Arab M, Abbaszadeh A, Ranjbar A, Soltanahmadi J. Association between neck circumference and cardiovascular risk factors in patients with acute coronary syndrome. Annals of Military and Health Sciences Research. 2014;12(3):100-5.

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):324-328.
http://dx.doi.org/10.3889/oamjms.2016.074
eISSN: 1857-9655
Basic Science

Serum Interleukin-18 and Its Gene Haplotypes Profile as Predictors in Patients with Diabetic Nephropathy

Ahmed I. Abd Elneam[1*], Nahla M. Mansour[2], Nayel A. Zaki[3], Mohamed A. Taher[4]

[1]Molecular Genetics and Enzymology Dept., Human Genetics Division, National Research Centre, 33 El Bohouth St. (former El Tahrir St.), Dokki 12622, Cairo, Egypt (Affiliation ID 60014618); [2]Gut Microbiology and Immunology Group, Chemistry of Natural and Microbial Products Department, Pharmaceutical Industries Research Division, National Research Centre, 33 El Bohouth St. (former El Tahrir St.), Dokki 12622, Cairo, Egypt; [3]Internal Medicine Department, Faculty of Medicine, Sohag University, Sohag, Egypt; [4]Medical Biochemistry Department, Sohag Faculty of Medicine, Sohag University, Egypt

Citation: Abd Elneam AI, Mansour NM, Zaki NA, Taher MA. Serum Interleukin-18 and Its Gene Haplotypes Profile as Predictors in Patients with Diabetic Nephropathy. Open Access Maced J Med Sci. 2016 Sep 15; 4(3):324-328. http://dx.doi.org/10.3889/oamjms.2016.074

Keywords: Diabetic Nephropathy; IL-18- polymorphisms; PCR; RFLP; haplotype.

***Correspondence:** Ahmed I. Abd Elneam. Molecular Genetics and Enzymology Department, Human Genetics Division, National Research Centre, Cairo, Egypt (Affiliation ID 60014618). E-mail: drahmedabdelneam@gmail.com

Received: 13-May-2016; **Revised:** 25-Jun-2016; **Accepted:** 26-Jun-2016; **Online first:** 21-Jul-2016

Competing Interests: The authors have declared that no competing interests exist.

Abbreviations: DM: diabetes mellitus, DN: diabetic nephropathy, GFR: glomerular filtration rate, IL-18: Interleukin- 18, NKC: natural killer cell, IFN-γ : interferon-γ, ICAM-1 : Intercellular Adhesion Molecule 1

Abstract

BACKGROUND: Diabetic nephropathy (DN) is known as an acute microvascular complexity as a subsequence progression in diabetes mellitus type 1 and 2. Many evidence pointed that the proinflammatory cytokine Interleukin (IL)-18 might be involved in the pathogenesis of DN.

AIM: The current study aimed to evaluate the association of serum IL-18 and its promoter gene polymorphisms with diabetic nephropathy.

METHODS: This study included 62 diabetic nephropathy patients (DN group) compared to 52 diabetes mellitus patients (DM group). The two groups were subjected to anthropometry assessment, molecular studies including SNP genotyping by RFLP and finally statistical analysis.

RESULTS: The assessment of the serum IL-18 level and the frequencies of its allele and haplotype: *-137G/C, -607C/A* and *-656G/T* among the DN and DM subjects revealed that *-137G* allele has significant variation between DN and DM subjects (about 80.8%, P = 0.05) but, no significant variation in *-607* or *-656* alleles *IL-18 gene* promoter.

CONCLUSION: These data confirm the impact of high serum IL-18 and the haplotype of the polymorphism located in the promoter region of the *IL-18 gene* with the DN.

Introduction

Diabetic nephropathy (DN) is a chronic complication of both type 1 and 2 diabetes mellitus (DM) [1, 2]. The complications of this disease include kidney failure and high risk to macrovascular problems which may lead to death [2]. Not only diabetic kidney diseases such as Kimmelstiel Wilson disease but also, intercapillary glomerulonephritis include the redundant drain of protein into the urine, hypertension, and steadily defective kidney activity [**3**]. In severe Kimmelstiel-Wilson syndrome, end-stage renal disease, kidney failure, renal dialysis and the kidney transplant became their occurrence of order.

However, the actual molecular process causing the DN is not extensively clear even though many classic processes and pathways have been suggested to have an impact on DN development. Recently new molecular and epigenetic mechanisms showed evidence that inflammation including secreted proinflammatory cytokines and chemokines is related to DN [4]. Several proinflammatory cytokines such as interleukins (IL-6, IL-8 and IL-18) and tumour necrosis factor-α (TNF-α) are raised in patients with DN [**5**]. The mode of action for increasing these

proinflammatory cytokines is still unpredictable. The oxidative stress has a significant role in raising the nuclear factor-kappaB (NF-κB) [6].

Interleukin-18 (IL-18) is a multiple phenotypic cytokines produced by activated monocytes, dendritic cells and glial cells and it shows impact in numerous inflammatory processes [7]. It belongs to the IL-1 superfamily and it works with IL-12 to induce cell-mediated immunity following infection with microbial products like lipopolysaccharide (LPS).The combination of these two cytokines has been shown to inhibit the IL-4 dependent IgE and IgG1 production and enhance IgG2a production in B cells [8].

Serum IL-18 levels have been showed increasing in patients with DN [9]. IL-18 is known to lead the production of other proinflammatory cytokines [10], endothelial apoptosis [11], upregulation of ICAM-1 [12], and hyperhomocysteinemia [13]. Thus, IL-18 might be an important factor not only in the atherosclerosis processing but also, in the development and progression of diabetic nephropathy.

Two polymorphisms in the *IL-18* gene promoter region at positions *-607* and *-137,* seem to correlate effectively on the genotype and serum concentrations of IL-18 [14]. Hernesniemi et al. (2008) also reported that *IL-18* promoter *G-137C* polymorphism is an important predictor of sudden cardiac death in patients with and without underlying coronary heart disease. In spite of the link between IL-18 promoter polymorphism, diabetes and cardiovascular disease, the relation between an IL-18 promoter polymorphism and cardiovascular disease has not been studied in diabetic nephropathy patients [15].

Position of *IL-18* gene is at chromosome 4q13–21 and several polymorphisms in its promoter region have been identified as – *607(C/A)* (dbSNP: rs1946518), *-137(G/C)* (dbSNP: rs187238) and *-656(G/T)* [16]. These common polymorphisms have been shown to regulate the IL-18 production of monocytes and are associated with transcriptional activity of the *IL-18* gene.

This study aimed to determine the association of serum IL-18 level and its gene promoter polymorphism *-607(C/A), -137(G/C)* and *-656(G/T)* with diabetic nephropathy in the Saudi Arabia population as a step towards finding a reliable biomarker for diagnosis the DN disease.

Material and Methods

Subjects

Fifty-two patients (40 male and 12 females) diagnosed with Diabetic nephropathy were selected from outpatient Clinic of General AL-Dawadmie Hospital KSA. The diagnosis of DN was done by microalbuminuria (30-300 mg/day) or macroalbuminuria (>300 mg/day) with or without a decrease in glomerular filtration rate (GFR) or arterial hypertension as described by Eknoyan et al., (2003) [17]. Sixty-two diabetic patients without evidence of renal affection were selected as a control group. Both groups were undergone a complete physical and clinical examinations and fasting blood samples were collected. This study was approved by the Local Medical Ethical Committee and according to their instructions. All patients included in the study gave written informed consent. Glycosylated haemoglobin (HbA_{1c}), total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglyceride (TG) were determined by standard biochemical methods.

Serum IL-18 assay

The IL-18 was determined in serum samples for each subject by enzyme-linked immune assay (ELISA) (MyBioSource, Cat. No. MBS396299 San Diego, California, USA) according to the manufacture's instruction.

DNA extraction

Genomic DNA was purified from whole blood samples with the QIAamp® DNA Blood Mini Kit, Holliston, MA, USA). DNA was eluted in 150 µl elution buffer and examined on 1% agarose gel and stored at –20°C for analysis.

SNP genotyping and haplotypes reconstruction

PCR-RFLP based method was used to detect polymorphism in an IL-18 gene, for each polymorphism, a specific PCR-RFLP was done. SNPs for, rs1946519 (*−656G/T*) and rs187238 (*−137G/C*) were done by using the method described previously by Folwaczny et al. (2005) [18] while the *IL-18 -607* polymorphism (*C/A*) was identified as described by Kumar et al. (2014) [19]. The primers used for detection are listed in (Table 1) and were ordered commercially from Sigma-Aldrich. PCR reactions were carried on a thermocycler (Bio-Rad, USA) using 2X master mix (Qiagen, Cat No. 206143 Valencia, USA) according to the manufacturer's instructions. The PCR amplified products were run on 1.5% agarose gel and the bands corresponding to the predicted size were cut and purified using the gel extraction kit (QIA quick columns, Qiagen, Cat No. 28104, Valencia,USA) following the manufacturer's instructions.

Anthropometry assessment

Anthropometric evaluation was performed for all patients in both groups. Body weight, height and waist circumference were measured following the recommendations of the International Biological Program [20].

Table 1: The primers used in polymerase chain reactions (PCR) for amplification the target region from IL-18 gene

SNPs	PCR primers	Annealing temperature (°C)	Restriction enzymes	Fragment sizes (bp)
−656 (G/T)	F:5'AGGTCAGTCTTTGCTATCATTCCAGG'3 R:5'CTGCAACAGAAAGTAAGCTTGCGGAGA GG'3	60	Mwo I	G: 96 + 24 T: 120
−137 (G/C)	F:5'CACAGAGCCCCAACTTTTTACGGCAGA GAA'3 R:5'GACTGCTGTCGGCACTCCTTGG'3	60	Mbo II	G: 116 + 39 C: 155
-607 (C/A)	F:5'TTCTGTTGCAGAAAGTGTAAAAATTTT'3 R: 5'AAAGGATAGTTGATACAGGCCATT'3	55	Dra I	C: 154 A: 125 +28

Body weight was determined to the nearest 0.01 kg using a Seca Scale Balance, with minimal wear and without shoes. Body height was measured to the nearest 0.1 cm using a Holtain portable Anthropometer. Waist circumference was measured at the level of the umbilicus with the standing position, the face directed forward, shoulders relaxed, and normal breathing by using non-stretchable plastic tape to the nearest 0.1 cm. Body mass index (BMI) was calculated as body weight divided by height squared (kg/m^2).

Statistical analysis

Allele's frequency, genotypes, Linkage disequilibrium and haplotypes were computed using the Arlequin software (version 3.1) and SNPstats (http://bioinfo.iconcologia.net/SN Pstats). Data was presented by means ± SD and percentages .The compiled data were computerised and analysed by SPSS V 12. The following tests of significance were used: t-test between means we used analyses mean difference, t-test between percentage to analyse percent difference and chi –square. A level of significance with p > 0.005 was considered insignificant.

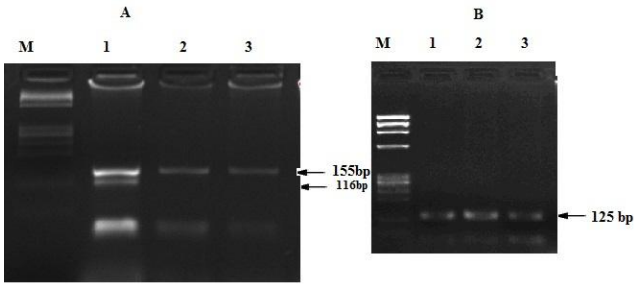

Figure 1: PCR digestion for -137 polymorphism and -607 polymorphism interleukin 18 genes. (A) line 1, -137 GC and lines 2 and 3 -137 CC. M line: ϕx 174 Marker. (B) lines 1,2 and 3 – 607 TT. M line: ϕx 174 Marker

Results

In the present work, we analysed serum *IL-18* and three functional polymorphisms, *-137G/C*, *-607C/A* and *-656G/T* at the promoter region of the IL18 gene in 62 DM and 52 DN patients Figure 1. General and clinical characteristics of all the subjects enrolled in this study are shown in Table 2. High serum IL-18 in DN patients in comparison to DM patients is observed as shown in (Table 2). There was significant positive co-relation between serum IL-18 and HBA_{1c} (R^2 = 0.04, Figure 2).

Table 2: General and laboratory characteristics of diabetes mellitus (DM) and diabetic nephropathy (DN) patients

Variable	DM (mean ± SD)	DN (mean ± SD)	P –value
Age (years)	52.8 ± 7.42	52.9 ± 9.15	0.5
Sex (M/F)	47/15	40/12	--
BMI (kg/m^2)	24.1 ± 5.1	28.3 ± 5.2	0.05*
SBP (mmHg)	133.6 ± 20.1	147.6 ± 27.9	0.005*
DBP (mmHg)	80.1 ± 15.5	99.0 ± 15.2	0.005*
Smoking habit %	40.3%	63.5%	0.005*
S. cholesterol (mmol/l)	3.6 ± 1.1	3.8 ± 1.3	0.2
S. LDL (mmol/l)	2.3 ± 0.9	2.5 ± 1.1	0.2
S. HDL (mmol/l)	1 ± 0.4	1.1 ± 0.4	0.1
S. triglyceride (mmol/l)	1.5 ± 0.5	1.7 ± 0.6	0.1
S. urea (mg/dl)	33.6 ± 12.3	47.3 ± 20.7	0.05*
S. creatinine (µmol/l)	77.3 ± 21.1	130.5 ± 27.6	0.04*
Cr Cl (mL/min)	122.9 ± 15.3	68.8 ± 9.9	0.005*
UACR (mg/mmol/L)	25.4 ± 10.1	53.6 ± 13.3	0.04*
IL-18 (pg/ml)	3.1 ± 0.4	5.2 ± 1.5	0.01*
HbA_{1c}	8.4 ± 2.5	8.9 ± 2.1	0.4
Oral drug therapy	57 (91.9%)	39 (75%)	--
Insulin drug therapy	5 (8.1%)	13 (25%)	--
Albuminuria (mg/24h)	24.5 ± 5.2	373.7 ± 44.2	0.001*

Cr Cl (Creatinine clearance) = 1.23 x (140-age in years) x weight (kg) / s. creatinine (µmol/l); * Significance between DM and DN patients (P < 0.05).

Comparing the allele frequencies of *-137G/C*, *607C/A* and *656G/T* at the *IL-18* promoters polymorphisms among the DM and DN patients was indicated in Table 3 and Table 4 revealed significant ratio of *-137G* allele (about 80.8%) more than *C* allele in DN (P < 0.05) and in DN than DM patients (P < 0.05) while no significant variation between both groups in *-607C/A* and *–656G/T* polymorphism was observed.

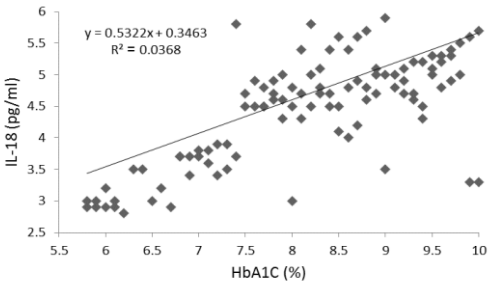

Figure 2: The relation between serum IL-18 and HbA_{1c}

Discussion

The patients with diabetic nephropathy, in general, suffer from interruption of angiogenesis, permeability, apoptosis, and multiplication which could

be as a result of inflammatory processes which affected the immune cells and cause the fibrotic phenomenon. This disease is characterised by the evolution from normoalbuminuria to microalbuminuria followed by continuous diminish in the glomerular filtration rate (GFR), and high arterial blood pressure.

Table 3: Comparing alleles of IL-18 promoter polymorphisms in DM and DN

Alleles	DM (%)	DN (%)
-656		
G	(51.6%)	(48.1%)
T	(48.4%)	(51.9%)
-607		
C	(46.8%)	(50%)
A	(53.2%)	(50%)
-137		
C	(53.2%)	(19.2%)**
G	(46.8%)*	(80.8%)

*: Significance between DM and DN in *-137 G* allele (P < 0.05); **: Significance between *C* and *G* alleles of -137 in DN patients (P < 0.05).

IL-18 is a dominant inflammatory cytokine that induces IFN-γ [21] which in turn induces functional chemokine receptor expression in human mesangial cells [22].

Table 4: *IL-18* Haplotypes distribution in diabetes mellitus (DM) and diabetic nephropathy (DN)

Haplotypes	DM N (%)	DN N (%)
-137C/-607A/-656G	9 (14.5 %)	3 (5.8 %)*
-137C/-607A/-656T	10 (16.1 %)	3 (5.8 %)*
-137C/-607C/-656G	7 (11.3 %)	2 (3.8 %)*
-137C/-607C/-656T	7 (11.3 %)	2 (3.8 %)*
-137G/-607A/-656G	7 (11.3 %)	10 (19.2%)
-137G/-607A/-656T	7 (11.3 %)	10 (19.2 %)
-137G/-607C/-656G	7 (11.3 %)	12 (23.1)
-137G/-607C/-656T	8 (12.9 %)	10 (9.2%)

*: Significance between DM and DN in the same haplotype (P < 0.05).

In addition, IL-18 leads to the production of IL-1, and TNF–γ and upregulation of ICAM-1, as well as apoptosis of endothelial cells [23]. IL-18 is constitutively expressed in renal tubular epithelia [17], infiltrating monocytes, macrophages, and T cells, endothelial cells of interstitial vessels along with proximal renal tubular cells, are potential sources of this cytokine [24]. In this study, *IL-18* promoter polymorphism *-137* is associated with the development of nephropathy in diabetic patients.

The distribution of genotypes in the current study is similar to previous reports on Chinese subjects. For example, we found that the *-137G* allele (*CG* or *GG* genotypes) was more common in DN than *C* allele (*CG* or *CC* genotypes) which is similar to Dong et al., 2007 and Szeto et al., 2009. Dong et al., 2007 found that *GG, GC* and *CC* genotypes at the *-137* site were 71.8%, 25.0% and 3.2% respectively but 78.7%, 20.0% and 1.3% respectively as reported by Szeto et al., 2009 [25, 26].

Further, among white males, the *C* allele carriers at the *-137* position had a high mortality risk but that with normal renal function and associated with high risk of cardiovascular disease [15]. A change at *IL-18* gene promoter at *-137* from *G* to *C* can change the histone 4 transcription factor-1 (H4TF-1) nuclear factor binding site to a binding site for an unknown factor found in the granulocyte-monocyte colony stimulating factor (GM-CSF) promoter [19]. Similarly, we found that the *-607(C/A)* and -656(G/T) had no significant difference in DM and DN which is similar to data reported by Dong et al., 2007 and Szeto et al., 2009 [25, 26]. Although other factors may also affect *IL-18* gene expression, available data suggest that promoter polymorphism is the major determinant of IL-18 production. Hyperglycemia stimulates the synthesis of IL-18 [27].

In the current study, it was found a statistically significant relationship between serum of IL-8 and the levels of glycosylated haemoglobin, in patients with diabetic nephropathy. Not only hyperglycemia but also albuminuria stimulates *IL-18* expression in proximal tubular cells [28] which is correlated with our results.

Acknowledgments

The authors are very grateful to patients and their family for their participation and cooperation during this study. We did not have funding body for this study, all was funded by authors. However, the authors are thankful to Shaqra University, Ministry of Higher Education, Kingdom of Saudi Arabia for laboratory facility to do this research. We are also very much thankful to the Department of Clinical Biochemistry Laboratory, Faculty of Medicine AlDawadmi, Shaqra University Kingdom of Saudi Arabia for their help with lab facility. We may not proceed to this present work if they do not allow us. We again thank their ethical committee for approving the work.

References

1. Moresco RN, Sangoi MB, De Carvalho JA, Tatsch E, Bochi GV. Diabetic nephropathy: traditional to proteomic markers. Clin Chim Acta. 2013;421:17-30. http://dx.doi.org/10.1016/j.cca.2013.02.019 PMid:23485645

2. Dabhi B, Mistry KN. Oxidative stress and its association with TNF-α-308 G/C and IL-1α-889 C/T gene polymorphisms in patients with diabetes and diabetic nephropathy. Gene. 2015;562:197–202 . http://dx.doi.org/10.1016/j.gene.2015.02.069 PMid:25732517

3. Ramanjaneyulu M, Kumar KA, M. Kumar MS, Reddy S, Kara S, Divyadhatri, Pravallika M, Rohith R, Chandaka M. Target identification and validation for diabetic nephropathy using molecular docking studies. Der Pharma Chemica. 2013;5(6):353.

4. Wang, Y, Harris DC. Macrophages in renal disease. J Am Soc Nephrol. 2011;22:21–27. http://dx.doi.org/10.1681/ASN.2010030269 PMid:21209251

5. Donate-Correa J, Martin-Nunez E, Muros-de-Fuentes M, et al: Inflammatory cytokines in diabetic nephropathy. J Diabetes Res. 2015; 948417. http://dx.doi.org/10.1155/2015/948417

6. Pan HZ, Zhang L, Guo MY, Sui H, Li H, Wu WH, Qu NQ, Liang MH, Chang D. The oxidative stress status in diabetes mellitus and diabetic nephropathy. Acta Diabetol. 2010;47(Suppl 1):71-6.

http://dx.doi.org/10.1007/s00592-009-0128-1 PMid:19475334

7. McInnes IB, Gracie JA, Leung BP, Wei XQ, Liew FY. Interleukin 18: a pleiotropic participant in chronic inflammation. Immunol Today. 2000;21(7):312-5. http://dx.doi.org/10.1016/S0167-5699(00)01648-0

8. Huang HY, Yu HT, Chan SH, Lee CL, Wang HS, Soong YK. Eutopic endometrial interleukin-18 system mRNA and protein expression at the level of endometrial-myometrial interface in adenomyosis patients. Fertil Steril. 2010; 94(1):339. http://dx.doi.org/10.1016/j.fertnstert.2009.01.132 PMid:19394601

9. Moriwaki Y, Yamamoto T, Shibutani Y, Aoki E, Tsutsumi Z, Takahashi S, Okamura H, Koga M, Fukuchi M, Hada T. Elevated levels of interleukin-18 and tumor necrosis factor-alpha in serum of patients with type 2 diabetes mellitus: relationship with diabetic nephropathy. Metabolism. 2003; 52: 605 –608. http://dx.doi.org/10.1053/meta.2003.50096 PMid:12759891

10. Dai SM, Matsuno H, Nakamura H, Nishioka K, Yudoh K. Interleukin-18 enhances monocyte tumor necrosis factor alpha and interleukin-1beta production induced by direct contact with T lymphocytes: implications in rheumatoid arthritis. Arthritis Rheum. 2004;50: 432–443. http://dx.doi.org/10.1002/art.20064 PMid:14872485

11. Marino E, Cardier JE. Differential effect of IL-18 on endothelial cell apoptosis mediated by TNF-alpha and Fas (CD95). Cytokine. 2003;22:142 –148. http://dx.doi.org/10.1016/S1043-4666(03)00150-9

12. Stuyt RJ, Netea MG, Geijtenbeek TB, Kullberg BJ, Dinarello CA, van der Meer JW. Selective regulation of intercellular adhesion molecule-1 expression by interleukin-18 and interleukin-12 on human monocytes. Immunology. 2003; 110:329–334. http://dx.doi.org/10.1046/j.1365-2567.2003.01747.x PMid:14632660 PMCid:PMC1783062

13. Aso Y, Okumura K, Takebayashi K, Wakabayashi S, Inukai T. Relationships of plasma interleukin-18 concentrations to hyperhomocysteinemia and carotid intimal-media wall thickness in patients with type 2 diabetes. Diabetes Care. 2003; 26:2622 –2627. http://dx.doi.org/10.2337/diacare.26.9.2622 PMid:12941729

14. Kretowski A, Mironczuk K, Karpinska A, Bojaryn U, Kinalski M, Puchalski Z, Kinalska I. Interleukin–18 promoter polymorphisms in type 1 diabetes. Diabetes. 2002;51:3347-3349. http://dx.doi.org/10.2337/diabetes.51.11.3347 PMid:12401730

15. Hernesniemi JA, Karhunen PJ, Rontu R, Ilveskoski E, Kajander O, Goebeler S, Viiri LE, Pessi T, Hurme M, Lehtimäki T. Interleukin-18 promoter polymorphism associates with the occurrence of sudden cardiac death among Caucasian males: The Helsinki Sudden Death Study. Atherosclerosis. 2008;196: 643–9. http://dx.doi.org/10.1016/j.atherosclerosis.2007.07.018 PMid:17765248

16. Boraska V, Terzić J, Škrabić V, Čaćev T, Bučević-Popović V, Peruzović M, Zemunik T. Neuro D1 Gene and Interleukin-18 Gene Polymorphisms in Type 1 Diabetes in the Dalmatian Population of Southern Croatia. Croatian Medical Journal. 2006; 47(4):571–578. PMid:16909454 PMCid:PMC2080453

17. Eknoyan G, Hostetter T, Bakris GL, Hebert L, Levey AS, Parving HH, Steffes MW, Toto R. Proteinuria and other markers of chronic kidney disease: a position statement of the national kidney foundation (NKF) and the national institute of diabetes and digestive and kidney diseases (NIDDK). Am J Kidney Dis. 2003;42(4):617-22. http://dx.doi.org/10.1016/S0272-6386(03)00826-6

18. Folwaczny M, Glas J, Török H-P, et al. Polymorphisms of the interleukin-18 gene in periodontitis patients. Journal of Clinical Periodontology. 2005;32(5): 530–534. http://dx.doi.org/10.1111/j.1600-051X.2005.00711.x PMid:15842270

19. Kumar D, Tiwary P, Chakravarty J, Sundar S. Association of Interleukin 18 Gene Polymorphism with Susceptibility to Viscera Leishmaniasis in Endemic Area of Bihar, an Indian Population Scientific World Journal. 2014; 2014: 852104.

20. Hiernaux J.M. , Tanner J.S., Weiner S.A. Lourie (Eds.), Human Biology: A Guide to Field MethodsIBP/Blackwell Scientific Publications, London/Oxford, UK (1969)

21. Arend WP, Palmer G, Gabay C. IL-1, IL-18, and IL-33 families of cytokines, Immunol Rev. 2008;22320–38. http://dx.doi.org/10.1111/j.1600-065x.2008.00624.x

22. Schwarz M, Wahl M, Resch K, Radeke HH. IFNγ induces functional chemokine receptor expression in human mesangial cells. Clinical and Experimental Immunology, 2002;128(2):285–294. http://dx.doi.org/10.1046/j.1365-2249.2002.01829.x PMid:11985519 PMCid:PMC1906381

23. Sprague AH, Khalil RA. Inflammatory Cytokines in Vascular Dysfunction and Vascular Disease. Biochemical Pharmacology. 2009;78(6):539–552. http://dx.doi.org/10.1016/j.bcp.2009.04.029 PMid:19413999 PMCid:PMC2730638

24. Akcay A, Nguyen Q, Edelstein CL. Mediators of inflammation in acute kidney injury. Mediators Inflamm. 2009;2009:137072. http://dx.doi.org/10.1155/2009/137072 PMid:20182538 PMCid:PMC2825552

25. Dong GP, Yu ZS, Liang L, Zou CC, Fu JF, Wang CL. IL-18 gene promoter -37C/G and -607C/A polymorphisms in Chinese Han children with type 1 diabetes mellitus. Int J Immunogenet. 2007;34:75–9. http://dx.doi.org/10.1111/j.1744-313X.2007.00665.x PMid:17373930

26. Szeto CC, Chow KM, Poon PY, Kwan BC, Li PK. Association of interleukin-18 promoter polymorphism and atherosclerotic diseases in Chinese patients with diabetic nephropathy. Nephrology (Carlton). 2009;14(6):606-12. http://dx.doi.org/10.1111/j.1440-1797.2008.01075.x PMid:19712260

27. Harms RZ, Yarde DN, Guinn Z, Lorenzo-Arteaga KM, Corley KP, Cabrera MS, Sarvetnick NE. Increased expression of IL-18 in the serum and islets of type 1 diabetics. Mol Immunol. 2015;64(2):306-12. http://dx.doi.org/10.1016/j.molimm.2014.12.012 PMid:25576800 PMCid:PMC4315765

28. Liu D, Xu M, Ding L-H, Lv L-L, Liu H, Ma K-L, Liu BC. Activation of the Nlrp3 inflammasome by mitochondrial reactive oxygen species: A novel mechanism of albumin-induced tubulointerstitial inflammation. The International Journal of Biochemistry & Cell Biology. 2014;57:7–19. http://dx.doi.org/10.1016/j.biocel.2014.09.018 PMid:25281528 PMCid:PMC4414121

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):329-336.
http://dx.doi.org/10.3889/oamjms.2016.082
eISSN: 1857-9655
Basic Science

Protective Effects of Vasodilatory Beta-Blockers Carvedilol and Nebivolol against Glycerol Model of Rhabdomyolysis-Induced Acute Renal Failure in Rats

Ahmed Atwa[1], Rehab Hegazy[2*], Nermeen Shaffie[3], Neamat Yassin[2], Sanaa Kenawy[4]

[1]Egyptian Russian University, Badr City, Egypt; [2]Pharmacology Department, Medical Division, National Research Center, Giza, Egypt; [3]Pathology Department, Medical Division, National Research Center, Giza, Egypt; [4]Pharmacology and Toxicology Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt

Citation: Atwa A, Hegazy R, Shaffie N, Yassin N, Kenawy S. Protective Effects of Vasodilatory Beta-Blockers Carvedilol and Nebivolol against Glycerol Model of Rhabdomyolysis-Induced Acute Renal Failure in Rats. Open Access Maced J Med Sci. 2016 Sep 15; 4(3):329-336. http://dx.doi.org/10.3889/oamjms.2016.082

Keywords: Rhabdomyolysis; Acute renal failure; Carvedilol; Nebivolol; Glycerol; NO; Rat.

***Correspondence:** Rehab Hegazy, PhD Pharmacology Department, Medical Division, National Research Centre, Bohoth st., 12622, Dokki, Giza, Egypt. Tel: +201001507676. E-mail: rehab_hegazy@hotmail.com

Received: 21-Jun-2016; **Revised:** 09-Jul-2016; **Accepted:** 10-Jul-2016; **Online first:** 01-Aug-2016

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: Rhabdomyolysis (RM)-induced acute renal failure (ARF) accounts for about 10–40% of all cases of ARF.

AIM: The present study investigated the possible protective effect of two nitric oxides (NO)-releasing third generation β-blockers, carvedilol (Carv) and nebivolol (Nebi), against RM-mimicking glycerol (Gly)-induced ARF in rats.

MATERIAL AND METHODS: After 24 h dehydration, rats received a single dose of 50% Gly (8 ml/kg, im). They were treated with vehicle, Carv (2.5 mg/kg/day, po) or Nebi (10 mg/kg, po) for 3 successive days starting from an hour prior to Gly injection. Evaluation of blood pressure and locomotor activity was performed during the experiment. 72 h following Gly administration, total protein in the urine, serum levels of creatinine, blood urea nitrogen, sodium and potassium as well as the renal contents of malondialdehyde, reduced glutathione and NO were assessed, together with a histopathological examination of renal tissues.

RESULTS: Carv and Nebi attenuated Gly-induced renal dysfunction and histopathological alterations. They decreased the Gly-induced oxidative stress and increased renal NO concentration. Restoration of normal blood pressure and improvement of locomotor activity were also observed.

CONCLUSION: The results clearly demonstrate protective effects of Carv and Nebi against renal damage involved in RM-induced ARF and suggest a role of their antioxidant and NO-releasing properties.

Introduction

Rhabdomyolysis (RM) is an important cause of acute renal failure (ARF). It results in about 10-40% of all cases [1]. The term rhabdomyolysis refers to the disintegration of skeletal muscles leading to the release of intracellular myoglobin (Mb), enzymes and electrolytes from myocytes into blood circulation [2]. It may be caused by trauma, ischemia, some drugs, toxins, metabolic disorders, or infections [3].

Many factors are known to contribute to RM-induced ARF; one of them is hypovolemia that results from the accumulation of a large amount of intravascular fluid in the space created from the damage of muscular tissue [4]. This hypovolemia resulted in a considerable reduction in renal blood flow (RBF) and glomerular filtration rate (GFR) that lead to ARF [5]. Hypovolemia is also associated with the sympathetic nervous system and reticular angiotensin aldosterone system (RAAS) activation with increased production of vasoconstricting molecules and inhibition of production of vasodilatory prostaglandins [6]. These, together with the vasoconstricting endotoxins and cytokines released into the systemic circulation after muscle damage, lead to renal hypoperfusion and tissue injury [7]. Another important factor contributes to RM-induced ARF is Mb that released by the dead myocytes. Mb scavenges nitric oxide (NO) which is the most potent endogenous vasodilatory factor, and this contributes

to the renal hypoperfusion and tissue injury in the setting of RM [8]. In addition, the intracellular degradation of Mb at the urinary pH to globin and ferriheme leads to free iron overloading of tubular cells [8]. Free iron is an oxidative metal that either facilitates the production of oxygen free radicals or acts as a free radical by itself [9]. This oxidative stress generated in the cytoplasm of tubular cells increases oxidation of lipids, proteins and DNA that resulting in ARF [10]. Lipid peroxidation occurs in the kidney markedly increases isoprostanes which are also potent vasoconstrictors [11].

Carvedilol (Carv) is a third-generation, non-selective β-blocker that also possesses α_1-adrenergic blocking activity [12]. It is indicated for the treatment of essential hypertension, heart failure, and post-myocardial infarction left ventricular dysfunction [13]. Data indicate that the vasodilation effect of Carv is mediated through both α_1-adrenergic receptor blockade and enhanced endothelial NO release [14]. Carv also has a number of ancillary activities including antioxidant, anti-inflammatory, anti-apoptotic, anti-ischemic, anti-proliferative, and Ca^{2+} antagonist properties [15]. These properties may provide protection for several major organ systems including the heart, blood vessels, kidneys and brain [16]. Carv has been found to decrease renal vascular resistance and improve renal hemodynamics by improving RBF and GFR [17]. Other renoprotective effects of Carv were found to be independent of its vasodilatory effect rather than its antioxidant and antiproliferative properties as well as its capability to reduce expression of profibrotic factors [18].

Nebivolol (Nebi) is a third generation selective β_1-adrenergic receptor blocker with vasodilator properties mediated by a direct stimulatory effect on the endothelial nitric oxide synthase (eNOS) (L-arginine-NO pathway) [19]. Nebi has also been shown to reduce the expression and protein levels of molecules involved in adhesion, inflammation, hypertension, and vascular remodelling that are induced by oxidative stress [20]. Treatment with Nebi has been shown to decrease renal fibrosis and glomerular injury as well as improving endothelial dysfunction. These effects have been attributed to vasodilatation, reduction in oxidative stress in addition to the enhancement of NO bioavailability [21].

Taken together, these pharmacological properties of both drugs, Carv and Nebi, with their renoprotective effects could be of potential interest in patients with renovascular diseases such as RM-induced ARF. For that, the present study was performed to investigate the possible protective effects of them against an RM-mimicking Gly-induced ARF in rats.

Material and Methods

Animals

Adult male Wistar rats weighing 150-200 g were utilised in the present study. Standard food pellets and tap water were supplied *ad libitum* unless otherwise stated. Animals and food pellets were obtained from the animal house colony of the National Research Center (NRC) (Cairo, Egypt). All the animal experiments were carried out in accordance with guidelines evaluated and approved by the ethics committee of NRC (Cairo, Egypt).

Drugs

Carv and Nebi were obtained from Sigma-Aldrich (USA). They were available as a powder, and used in the current study at doses of 2.5 mg/kg, po [22] and 10 mg/kg, po [23]**,** respectively**.** Drugs were freshly prepared at the beginning of each experiment by being suspended in distilled water and volumes were adjusted so each rat received 1 ml suspension/100 g body weight. All other chemicals used were of the highest purity available.

Experimental Design

RM-induced ARF in rats was induced using a single dose of hypertonic glycerol (Gly) solution (50% v/v in sterile saline) following 24 h of dehydration [24]. Animals were randomly allocated into four groups; each group consisted of 10 rats. The rats received an injection of Gly solution (8 ml/kg, i.m.) or equal volume of saline for animals of the 1st group, which served as the normal control. The injected volume was divided equally between the two hind limbs. Administration of drugs was carried out daily for 3 successive days, starting 60 min prior to the Gly injection. The first 2 groups, normal and RM-ARF groups, received saline orally, and the other 2 groups received Carv (2.5 mg/kg/d, po) and Nebi (10 mg/kg/d, po), respectively. Animals were allowed free access to food and tap water during the course of the experiment, while rats of the last 3 groups were deprived of drinking water for 24 h before the Gly administration.

Assessment of locomotor activity

On day 0 and 1 h following the last drug administration, locomotor activity was measured by detecting rat movements using grid floor activity cage (Model no. 7430, Ugo-Basile, Italy). Interruptions of infrared beams were automatically detected during a 10 min test session. Beam interruption information was processed in the activity cage software to provide an index of horizontal movements. Rats were acclimatised for 1 h to the test room, before placing the animal in the activity cage (exposure) [25]. The

basal activity counts of rats were pretested for a 15 min interval the day before the experiment to habituate them to the apparatus; they were adapted for 5 min and the basal activity counts were then recorded for 10 min [26].

Systolic Blood Pressure (SBP) Measurement

Blood pressure was measured non-invasively on day 0 and 1 h following the last drug administration using tail-cuff technique attached to blood pressure recorder **(**UGO BASILE 58000, Italy).

Urine and serum biochemical analysis

On day 2, urine samples were collected from animals of all groups through the housing in individual metabolic cages for 24 h for estimation of urinary total protein (UTP) using commercial reagent kit (Stanbio, USA). Blood samples were withdrawn via the retro-orbital plexus under ether anaesthesia from all rats on day 3, after 1 h of the last drug administration. The serum was isolated for estimation of blood urea nitrogen (BUN), serum creatinine (SCr), potassium (K^+) and sodium (Na^+) levels, using specific commercial kits, (Stanbio, USA), (Quimica Clinica Aplicada S.A., Spain), (Quimica Clinica Aplicada S.A., Spain), and (Teco Diagnostics, USA), respectively.

Renal tissue biochemical and histopathological analysis

Directly after collecting the blood samples, rats were sacrificed by cervical dislocation under ether anaesthesia and both kidneys were isolated. The right kidneys were rinsed in chilled 0.9 % NaCl (pH 7.4) then homogenised. The homogenates were used for estimation of kidney contents of lipid peroxides measured as malondialdehyde (MDA) according to Ruiz-Larrea et al. [27], reduced glutathione (GSH) according to [40] and NOx (nitrite and nitrate, stable metabolites of NO) using commercial reagent kit (Cayman chemical company, Germany).

The left kidneys from all groups were removed and fixed in 10% neutral buffered formal saline for 72 h at least. All the specimens were washed in tap water for half an hour and then dehydrated in ascending grades of alcohol, cleared in xylene and embedded in soft paraffin. Paraffin sections of 5 μm thick were stained with haematoxylin and eosin (H&E) [28]**,** for histopathological examination. Images were captured and processed using Adobe Photoshop version 8.0.

Statistical Analysis

All the values are presented as means ± standard error of the means (SEM). Comparisons between different groups were carried out using one-way analysis of variance (ANOVA) followed by Tukey HSD test for multiple comparisons [29]. Graphpad Prism software, version 5 was used to carry out these statistical tests. For locomotor activity, square root transformed percent was calculated [30], while Statistica version 7 was used for two-way ANOVA followed by Tukey HSD as multiple comparison tests for blood pressure analysis. The difference was considered significant when $p < 0.05$.

Results

Locomotor activity of rats

Gly model of RM-induced ARF markedly decreased the basal locomotor activity on day 3 of Gly administration, compared with normal group. Pretreatment with Carv (2.5 mg/kg) and Nebi (10 mg/kg) led to a significant protection in locomotor activity on day 3 from Gly administration compared to ARF group (Table 1).

Table 1: Locomotor activity

Parameter / Groups	Locomotor activity			
	Count/10 min		Percentage of basal activity	Square-root-transformed % of basal activity
	Day 0	Day 3	Day 3 / Day 0	Day 3
Saline	271.50 ± 8.08	199.33 ± 5.11	73%	0.96^{b} ± 0.02
Gly	172.10 ± 9.23	28.90 ± 2.19	17%	0.41^{a} ± 0.02
Gly-Carv	178.30 ± 8.27	116.90 ± 6.73	65%	0.81^{ab} ± 0.03
Gly-Nebi	187.80 ± 16.57	113.50 ± 7.14	60%	0.80^{ab} ± 0.04

Saline, rats treated with saline and considered as normal rats; Gly, rats treated with glycerol; Gly-Carv, rats treated with glycerol and carvedilol; Gly-Nebi, rats treated with glycerol and nebivolol. Data are presented as mean ± SE, n=10. [a] Significantly different from Saline; $p < 0.05$. [b] Significantly different from Gly; $p < 0.05$.

Systolic blood pressure

Gly markedly increased the basal SBP of rats on day 3. However, pretreatment of rats with Carv and Nebi significantly protected against this Gly-induced elevation of SBP (Fig. 1).

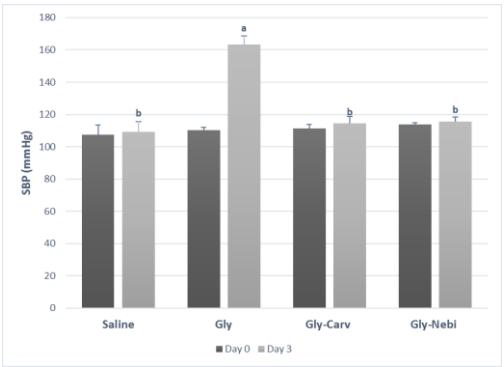

Figure 1: Systolic blood pressure. Saline, rats treated with saline and considered as normal rats; Gly, rats treated with glycerol; Gly-Carv, rats treated with glycerol and carvedilol; Gly-Nebi, rats treated with glycerol and nebivolol. Data are presented as mean ± SE, n=10. a Significantly different from Saline; p < 0.05. b Significantly different from Gly; p < 0.05

Urine and serum biochemical analysis

Induction of ARF in rats by a single dose of Gly markedly increased the normal UTP on day 2 of Gly administration and increased SCr and BUN levels on day 3. A marked decrease in normal Na^+ level and increase in K^+ level were also observed on day 3. Pretreatment of rats with Carv and Nebi preserved the normal levels of UTP, SCr, BUN, Na^+, and K^+ (Table 2).

Table 2: Levels of urine total protein, serum creatinine, blood urea nitrogen, serum sodium and serum potassium

Parameters Groups	UTP (mg/dl)	SCr (mg/dl)	BUN (mg/dl)	Na^+ (mEq/l)	K^+ (mmol/l)
Saline	$30.11^b \pm 2.48$	$0.48^b \pm 0.01$	$21.38^b \pm 0.95$	$147.65^b \pm 1.86$	$3.79^b \pm 0.09$
Gly	$156.08^a \pm 14.34$	$3.75^a \pm 0.25$	$49.10^a \pm 2.03$	$122.03^a \pm 2.36$	$7.17^a \pm 0.24$
Gly-Carv	$53.58^b \pm 3.95$	$0.96^b \pm 0.09$	$21.97^b \pm 1.47$	$140.56^b \pm 2.36$	$4.10^b \pm 0.25$
Gly-Nebi	$53.14^b \pm 3.73$	$1.06^b \pm 0.09$	$22.14^b \pm 1.41$	$140.63^b \pm 2.72$	$4.19^b \pm 0.17$

Saline, rats treated with saline and considered as normal rats; Gly, rats treated with glycerol; Gly-Carv, rats treated with glycerol and carvedilol; Gly-Nebi, rats treated with glycerol and nebivolol; UTP, urine total protein; SCr, serum creatinine; BUN, blood urea nitrogen; Na^+, serum sodium; K^+, serum potassium. Data are presented as mean ± SE, n=10. [a] Significantly different from Saline; $p < 0.05$. [b] Significantly different from Gly; $p < 0.05$.

Renal tissue biochemical analysis

Induction of ARF in rats using Gly markedly increased the normal renal MDA level by 86% and decreased GSH and NOx levels by 83% and 44%, respectively. Pretreatment of rats with Carv or Nebi conserved the normal renal levels of MDA and GSH. Moreover, a marked protection of kidney NOx level was also detected; this protection was more significant in the group treated with Nebi rather than Carv (Fig. 2).

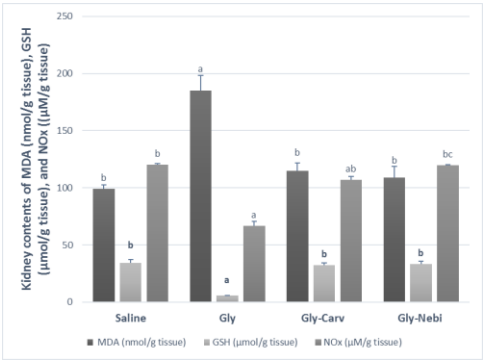

Figure 2: Kidney contents of malondialdehyde, reduced glutathione and nitric oxide. Saline, rats treated with saline and considered as normal rats; Gly, rats treated with glycerol; Gly-Carv, rats treated with glycerol and carvedilol; Gly-Nebi, rats treated with glycerol and nebivolol; MDA, malondialdehyde; GSH, reduced glutathione; NOx, nitrite and nitrate, stable metabolites of NO. Data are presented as mean ± SE, n=10. a Significantly different from Saline; p < 0.05. b Significantly different from Gly; p < 0.05. c Significantly different from Gly-Carv; p < 0.05

Histopathological features of the renal tissues

The renal tissue of the normal rats showed normal glomeruli formed of a tuft of capillaries enclosed in Bowman's capsule and separated from it by the urinary space. Two types of tubules were also observed, proximal convoluted tubules with their brush borders and distal convoluted tubules (Fig. 3. A & B). In the rat sacrificed 72 h following Gly administration, a marked vacuolar degeneration in proximal tubules with discontinuity of the brush border as well as a widening of urinary space of glomeruli were observed (Fig. 3. C). In addition, a marked decrease in the height of the lining epithelium of distal tubules and widening of their lumen are also noticed (Fig. 3. D). On the other hand, the renal tissues of a rat with Gly-induced ARF that were pretreated with Carv showed a significant decrease in vacuolar degeneration induced by Gly in proximal tubules, and the distal tubules showed an increase in the height of their lining epithelium with no signs of vacuolar degeneration. The glomeruli appeared more or less normal (Fig. 3. E). the renal sections of rats treated with Nebi-Gly showed the persistence of the Gly-induced vacuolar degeneration, especially in proximal tubules. However, the lumens of the distal convoluted tubules were less dilated if compared to the Gly-treated group, while the urinary space appeared more or less normal (Fig. 3. F).

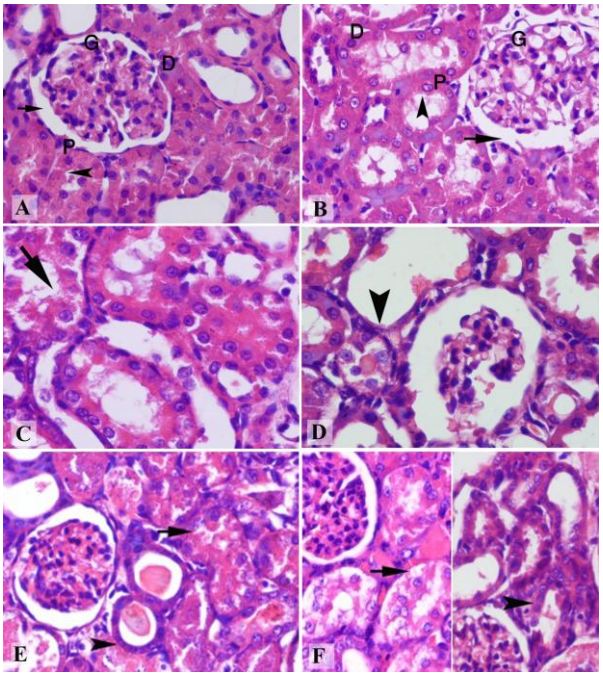

Figure 3: Histopathological features of the renal tissues. Photomicrographs of renal sections from rats treated with the following: Saline (A) & (B) show normal glomeruli (G) enclosed in Bowman's capsule and separated from it by the urinary space (arrow), proximal convoluted tubules (P) with their brush borders (arrowhead), and distal convoluted tubules (D); Gly (C & D) show marked vacuolar degeneration in proximal tubules with discontinuity of the brush border (arrow), widening of urinary space, and a marked decrease in the height of the lining epithelium of distal tubules with widening of their lumen (arrowhead); Gly-Carv (E) shows a significant decrease in vacuolar degeneration in proximal tubules (arrow), an increase in the height of the lining epithelium of the distal tubules with no signs of vacuolar degeneration (arrowhead), and a normal glomeruli; Gly-Nebi (F) shows the persistence of the Gly-induced vacuolar degeneration, especially in proximal tubules, (arrow in the left part of the figure), less dilated lumens of distal convoluted tubules, and a normal urinary space (arrowhead in the right part of the figure). (H & E X 200)

Discussion

Induction of RM-mimicking ARF in rats, in the current study, with Gly that was evidenced by the impairment of the kidney function biomarkers and confirmed by the histopathological findings is in accordance with other studies reported that the acute volume depletion model of Gly-ARF induces a closely related syndrome to the RM-ARF in human beings [31]. Renal vasoconstriction and hypoperfusion have been suspected to play a major role in the pathogenesis of this model [32].

Several potential mechanisms may contribute to this renal vasoconstriction. Muscle necrosis creates a dramatic fluid third spacing, leading to intravascular volume depletion and hypotension; this impairs the renal perfusion and causes a severe renal ischemia and tubular dysfunction [4]. The decreased serum Na^+ level observed in the current Gly model of RM-ARF, and before [33], indicated this tubular dysfunction with a decreased Na^+ reabsorption. However, a significant increase in serum Na^+ levels was reported in other studies [34, 35]. This may be due to acute tubular necrosis that could lead to a decrease in the number of functioning nephrons. This effect may trigger multiple adaptive processes in the hyper-functioning remaining nephrons, most notably the augmented rates of Na^+ reabsorption that lead to hypernatremia. On the other hand, the observed increase in the serum K^+ level in the present Gly model of RM-ARF, which is correspondingly reported previously [33, 36], could be explained by the direct release of the intracellular K^+ from the damaged muscles [37]. Remarkably, this hyperkalemia has not been observed in other studies using different models of ARF rather than RM-ARF [35, 38]. The pathogenesis of Gly-induced RM-ARF can also involve Mb release from the damaged muscles that facilitates the production of reactive oxygen species (ROS) [10]. Oxidative stress has been found to cause renal damage [39]. It promotes the formation of a variety of vasoactive mediators that can affect the renal function directly by causing renal vasoconstriction and thus reduce the GFR [40]. In the present study, induction of renal oxidative stress by Gly was demonstrated clearly by a significant increase in the normal renal MDA and decrease in GSH contents. A similar pattern was recorded by many studies [41, 42]. The increased tissue levels of ROS can also oxidize the locally released NO and diminishes its bioactivity [43]. Correspondingly, a significant decrease in the normal renal tissue content of NOx was demonstrated in the present Gly-ARF model, a result that is in line with other studies [44, 45].

The current Gly-induced RM-ARF was accompanied with a significant decrease in the normal locomotor activity of rats. It has been found that renal failure results in an accumulation of numerous organic substances that possibly act as neurotoxins and result in a development of a case that is known as uremic encephalopathy [46]. Uremic encephalopathy is associated with a generalised decrease in brain energy use, and thus a decrease in the locomotor activity [46].

Moreover, a significant increase in the SBP was also observed in the current Gly-ARF model. A similar finding was observed with gentamicin-induced ARF model [47]. Renal failure reduces the afferent glomerular arteriolar pressure, leading to the activation of the renin-angiotensin system, leading to hypertension [48]. Co-treatment of rats with either Carv or Nebi showed a significant protective effect against the current Gly-induced RM-ARF model. This observed renoprotective effect is in agreement with the findings of studies that used Carv or Nebi as a protective agent against other models of ARF in which restoration of the normal levels of renal function biomarkers was reported [49, 50].

The significant attenuation of Gly-induced oxidative stress in the rats treated with Carv or Nebi indicates that antioxidant pathway played a role in the renoprotective effects of both drugs. Many studies also reported this antioxidant effect of Carv and Nebi [49, 51]. Carv has been found to scavenge oxygen radicals and inhibit their release from activated neutrophils [52, 53]. It was found to accumulate in specific plasma membrane sites allowing it to approach the site of fatty acid side chain unsaturation where lipid peroxidation is thought to occur; this explains its high potency as an antioxidant [54]. On the other hand, Nebi has vasodilating properties mediated by direct stimulation of eNOS, thereby increasing the availability of NO [19]. It has been shown that NO donors can scavenge ROS by the NADPH oxidase [55].

The significant improvement of the serum Na^+ and K^+ levels observed in Gly-Carv and Gly-Nebi as compared to Gly group indicated a protective effect of Carv and Nebi against Gly-induced hyponatremia and hyperkalemia. In addition, adrenergic β-blockade would increase proximal Na^+ reabsorption [56], contributing to the drugs-induced hypernatremia. Correspondingly, in the previous study, Nebi, in combination with hydrochlorothiazide, reduced the Na^+ clearance [57]. On the contrary, Rodriguez-Perez *et al.* [58], and Greven and Gabriels [59] reported that Carv and Nebi, respectively, produced a significant natriuresis followed by hyponatremia in rats with severe nephrosclerosis. This natriuresis was attributed to a compensatory renal mechanism due to an improvement of GFR produced by those drugs, which in turn increased urinary excretion of Na^+ and fluids. On the other hand, the antioxidant activities of Carv and Nebi could explain the reversal of ROS-induced hyperkalemia that resulted from the loss of intracellular K^+ due to the increasing cell membrane permeability by membrane lipids peroxidation [60, 61]. In addition, Carv by having a α_1-adrenergic blocking activity can retain K^+ intracellularly, contributing to

hypokalemia induced by Carv [62]. On the contrary, it has been suggested that β-adrenergic receptor antagonism could suppress the renin-angiotensin aldosterone system (RAAS), by inhibiting renin secretion, hence, predisposing patients to K^+ retention [63].

In addition to those observed renoprotective effects of Carv and Nebi that consequently caused an improvement of the locomotor activity of rats, both have been reported to have a direct neuroprotective effect [64-66]. Carv protected against 3-nitropropionic acid induced behavioural alterations in rats [67], and Nebi improved the neurological status and the hind limb motor function in a spinal cord ischemia/reperfusion injury model in rabbits [65, 66]. Therefore, this improvement in the locomotor activity demonstrated in the current study could be up to a point due to a direct neuroprotective effect against uremic encephalopathy. Similarly, the protective effect of Carv and Nebi against Gly-induced SBP-elevation could be accounted partly for the observed renoprotective effect of those treatments and also to their renowned direct antihypertensive effects [68, 69]

The present data revealed that animals treated with Carv or Nebi showed a significant increase in renal NOx content as compared to Gly group; this protection was more significant in the group treated with Nebi rather than Carv. Correspondingly, previous studies demonstrated that Carv and Nebi increased NO content [70-74].

Carv effects have been found to be blocked by the inhibition of eNOS enzyme using L-NAME [70]. This suggests that Carv's actions are largely NO-mediated. Moreover, this might explain the current observed Carv-induced rise in renal NO content and suggests it to be dependent on stimulation of intact eNOS. The Nebi-induced elevation of NO content was more significant than that of Carv because Nebi can increase NO bioavailability by, at least, two mechanisms: by increasing NOS activity [75], or, under conditions of oxidative stress, by reducing O_2^- generation and inhibiting eNOS uncoupling and, therefore, NO inactivation [76].

NO exerts a protective role against renal damage in several animal models of kidney disease as well as in human chronic renal failure. It promotes the increase of RBF and exerts antigrowth and antiproliferative effects on vascular smooth muscle [77]. It also plays an important role in regulating renal hemodynamic and functions [78]. Interestingly, Maree *et al.* [79] indicated that NOS inhibition worsens Gly-induced ARF model, while NO supplementation protects against it.

In conclusion, the present study revealed that treatment of rats with Carv (2.5 mg/kg/day, po) or Nebi (10 mg/kg, po) protected against renal damage involved in Gly-induced RM-mimicking ARF. The findings demonstrated the involvement of the antioxidant and NO releasing properties of both drugs and suggested their involvement in this renoprotective effect.

References

1. Khan FY. Rhabdomyolysis: a review of the literature. Neth J Med. 2009;67(9): 272-83. PMid:19841484

2. Sani MA, Campana-Salort E, Begu-LeCorroller A, et al., Non-traumatic rhabdomyolysis and diabetes. Diabetes Metab. 2011;37(3): 262-4. http://dx.doi.org/10.1016/j.diabet.2011.03.003 PMid:21550832

3. Tseytlin D, Maynard S. Severe rhabdomyolysis secondary to adenovirus infection: case report and literature review. Clin Nephrol. 2016;85(4): 245-50. http://dx.doi.org/10.5414/CN108649 PMid:26857631

4. Holt SG, Moore KP. Pathogenesis and treatment of renal dysfunction in rhabdomyolysis. Intensive Care Med. 2001;27(5): 803-11. http://dx.doi.org/10.1007/s001340100878

5. Malinoski DJ, Slater MS, Mullins RJ. Crush injury and rhabdomyolysis. Crit Care Clin. 2004;20(1): 171-92. http://dx.doi.org/10.1016/S0749-0704(03)00091-5

6. Gonzalez D. Crush syndrome. Crit Care Med. 2005;33(1 Suppl):S34-41. http://dx.doi.org/10.1097/01.CCM.0000151065.13564.6F PMid:15640677

7. Devarajan P. Cellular and molecular derangements in acute tubular necrosis. Curr Opin Pediatr. 2005;17(2):193-9. http://dx.doi.org/10.1097/01.mop.0000152620.59425.eb PMid:15800411

8. Chatzizisis YS, Misirli G, Hatzitolios AI, Giannoglou GD. The syndrome of rhabdomyolysis: complications and treatment. Eur J Intern Med. 2008;19(8):568-74. http://dx.doi.org/10.1016/j.ejim.2007.06.037 PMid:19046720

9. Nath KA, Norby SM. Reactive oxygen species and acute renal failure. Am J Med. 2000;109(8):665-78. http://dx.doi.org/10.1016/S0002-9343(00)00612-4

10. Moore KP, Holt SG, Patel RP, et al. A causative role for redox cycling of myoglobin and its inhibition by alkalinization in the pathogenesis and treatment of rhabdomyolysis-induced renal failure. J Biol Chem. 1998;273(48):31731-7. http://dx.doi.org/10.1074/jbc.273.48.31731 PMid:9822635

11. Boutaud O, Roberts LJ. 2nd, Mechanism-based therapeutic approaches to rhabdomyolysis-induced renal failure. Free Radic Biol Med. 2011;51(5):1062-7. http://dx.doi.org/10.1016/j.freeradbiomed.2010.10.704 PMid:21034813 PMCid:PMC3116013

12. Bristow MR, Larrabee P, Muller-Beckmann B, et al. Effects of carvedilol on adrenergic receptor pharmacology in human ventricular myocardium and lymphocytes. Clin Investig. 1992;70 (Suppl 1):S105-13. http://dx.doi.org/10.1007/BF00207620 PMid:1350478

13. Li T, Ke W, Sun P, et al. Carvedilol for portal hypertension in cirrhosis: systematic review with meta-analysis. BMJ Open. 2016;6(5):e010902. http://dx.doi.org/10.1136/bmjopen-2015-010902 PMid:27147389 PMCid:PMC4861122

14. Nichols AJ, Gellai M, Ruffolo RR. Studies on the mechanism of arterial vasodilation produced by the novel antihypertensive agent, carvedilol. Fundam Clin Pharmacol. 1991;5(1):25-38. http://dx.doi.org/10.1111/j.1472-8206.1991.tb00698.x PMid:1712335

15. Beattie K, Phadke G, Novakovic J. Carvedilol. Profiles Drug Subst Excip Relat Methodol. 2013;38:113-57. http://dx.doi.org/10.1016/B978-0-12-407691-4.00004-6 PMid:23668404

16. Ruffolo RR, Feuerstein GZ. Pharmacology of carvedilol:

rationale for use in hypertension, coronary artery disease and congestive heart failure. Cardiovasc Drugs Ther. 1997;11 (Suppl 1):247-56. http://dx.doi.org/10.1023/A:1007735729121 PMid:9211017

17. Messerli FH, Grossman E. Beta-blockers in hypertension: is carvedilol different? Am J Cardiol. 2004;93(9) :7-12. http://dx.doi.org/10.1016/j.amjcard.2004.01.020 PMid:15144930

18. Kett MM, Denton KM, Boesen EI, Anderson WP. Effects of early carvedilol treatment and withdrawal on the development of hypertension and renal vascular narrowing. Am J Hypertens. 2004;17(2):161-6. http://dx.doi.org/10.1016/j.amjhyper.2003.09.013 PMid:14751659

19. Munzel T, Gori T. Nebivolol: the somewhat-different beta-adrenergic receptor blocker. J Am Coll Cardiol. 2009;54(16):1491-9. http://dx.doi.org/10.1016/j.jacc.2009.05.066 PMid:19815121

20. Garbin U, Fratta Pasini A, Stranieri C, et al. Effects of nebivolol on endothelial gene expression during oxidative stress in human umbilical vein endothelial cells. Mediators Inflamm. 2008;2008:367590. http://dx.doi.org/10.1155/2008/367590 PMid:18437228 PMCid:PMC2323596

21. Pires MJ, Rodriguez-Pena AB, Arevalo M, et al. Long-term nebivolol administration reduces renal fibrosis and prevents endothelial dysfunction in rats with hypertension induced by renal mass reduction. J Hypertens. 2007;25(12):2486-96. http://dx.doi.org/10.1097/HJH.0b013e3282efeecb PMid:17984671

22. Kumar A, Dogra S. Neuroprotective effect of carvedilol, an adrenergic antagonist against colchicine induced cognitive impairment and oxidative damage in rat. Pharmacol Biochem Behav 2009;92(1):25-31. http://dx.doi.org/10.1016/j.pbb.2008.10.005 PMid:18992766

23. Georgescu A, Popov D, Dragan E, Dragomir E, Badila E. Protective effects of nebivolol and reversal of endothelial dysfunction in diabetes associated with hypertension. Eur J Pharmacol. 2007;570(1-3):149-58. http://dx.doi.org/10.1016/j.ejphar.2007.05.031 PMid:17612521

24. Singh D, Chander V, Chopra K. Protective effect of naringin, a bioflavonoid on glycerol-induced acute renal failure in rat kidney. Toxicology. 2004;201(1-3):143-51. http://dx.doi.org/10.1016/j.tox.2004.04.018 PMid:15297029

25. Kauppila T, Tanila H, Carlson S, Taira T. Effects of atipamezole, a novel alpha 2-adrenoceptor antagonist, in open-field, plus-maze, two compartment exploratory and forced swimming tests in the rat. Eur J Pharmacol. 1991;205(2):177-82. http://dx.doi.org/10.1016/0014-2999(91)90817-A

26. Kelly MA, Rubinstein M, Phillips TJ, et al., Locomotor activity in D2 dopamine receptor-deficient mice is determined by gene dosage, genetic background and developmental adaptations. J Neurosci. 1998;18(9):3470-9. PMid:9547254

27. Ruiz-Larrea MB, Leal AM, Liza M, Lacort M, De Groot H. Antioxidant effects of estradiol and 2-hydroxyestradiol on iron-induced lipid peroxidation of rat liver microsomes. Steroids. 1994;59(6):383-8. http://dx.doi.org/10.1016/0039-128X(94)90006-X

28. Drury R, Wallington E. Carleton's Histology Technique. Oxford University Press: New York, Toronto, 1967:p. 432.

29. Dunn OJ. Multiple comparisons using rank sums. Technometrics. 1964;6(3):241-252. http://dx.doi.org/10.1080/00401706.1964.10490181

30. Jones M, Onslow M, Packman A, Gebski V. Guidelines for statistical analysis of percentage of syllables stuttered data. J Speech Lang Hear Res. 2006;49(4):867-78. http://dx.doi.org/10.1044/1092-4388(2006/062)

31. Singh AP, Singh AJ, Singh N. Pharmacological investigations of Punica granatum in glycerol-induced acute renal failure in rats. Indian J Pharmacol. 2011;43(5):551-6. http://dx.doi.org/10.4103/0253-7613.84971 PMid:22021999 PMCid:PMC3195126

32. Lopez-Farre A, Gomez-Garre D, Bernabeu F, et al. Platelet-activating factor mediates glycerol-induced acute renal failure in rats. Clin Sci (Lond). 1990;79(6):551-8. http://dx.doi.org/10.1042/cs0790551

33. Ayvaz S, Aksu B, Kanter M, et al. Preventive effects of hyperbaric oxygen treatment on glycerol-induced myoglobinuric acute renal failure in rats. J Mol Histol. 2012;43(2):161-70. http://dx.doi.org/10.1007/s10735-012-9391-5 PMid:22311626

34. Ahmed OG, El-Mottaleb NA. Renal function and arterial blood pressure alterations after exposure to acetaminophen with a potential role of Nigella sativa oil in adult male rats. J Physiol Biochem. 2013;69(1):1-13. http://dx.doi.org/10.1007/s13105-012-0182-y PMid:22730078

35. Said MM. The protective effect of eugenol against gentamicin-induced nephrotoxicity and oxidative damage in rat kidney. Fundam Clin Pharmacol. 2011;25(6):708-16. http://dx.doi.org/10.1111/j.1472-8206.2010.00900.x PMid:21105912

36. Ahmed OG, El-Mottaleb NA. Renal function and arterial blood pressure alterations after exposure to acetaminophen with a potential role of Nigella sativa oil in adult male rats. J Physiol Biochem. 2013;69(1):1-13. http://dx.doi.org/10.1007/s13105-012-0182-y PMid:22730078

37. Giannoglou GD, Chatzizisis YS, Misirli G. The syndrome of rhabdomyolysis: pathophysiology and diagnosis. Eur J Intern Med. 2007;18(2):90-100. http://dx.doi.org/10.1016/j.ejim.2006.09.020 PMid:17338959

38. Stojiljkovic N, Stoiljkovic M, Randjelovic P, Veljkovic S, Mihailovic D. Cytoprotective effect of vitamin C against gentamicin-induced acute kidney injury in rats. Exp Toxicol Pathol. 2012;64(1-2):69-74. http://dx.doi.org/10.1016/j.etp.2010.06.008 PMid:20619622

39. Hegazy R, Salama A, Mansour D, Hassan A. Renoprotective Effect of Lactoferrin against Chromium-Induced Acute Kidney Injury in Rats: Involvement of IL-18 and IGF-1 Inhibition. PLoS One. 2016;11(3):e0151486. http://dx.doi.org/10.1371/journal.pone.0151486 PMid:26990190 PMCid:PMC4798745

40. Baud L, Ardaillou R. Involvement of reactive oxygen species in kidney damage. Br Med Bull. 1993;49(3):621-9. PMid:8221027

41. Helmy MM, El-Gowelli HM. Montelukast abrogates rhabdomyolysis-induced acute renal failure via rectifying detrimental changes in antioxidant profile and systemic cytokines and apoptotic factors production. Eur J Pharmacol. 2012;683(1-3):294-300. http://dx.doi.org/10.1016/j.ejphar.2012.03.018 PMid:22449377

42. Yousefipour Z, Oyekan A, Newaz M. Interaction of oxidative stress, nitric oxide and peroxisome proliferator activated receptor gamma in acute renal failure. Pharmacol Ther. 2010;125(3):436-45. http://dx.doi.org/10.1016/j.pharmthera.2009.12.004 PMid:20117134

43. Habibi J, Hayden MR, Sowers JR, et al. Nebivolol attenuates redox-sensitive glomerular and tubular mediated proteinuria in obese rats. Endocrinology. 2011;152(2):659-68. http://dx.doi.org/10.1210/en.2010-1038 PMid:21177830 PMCid:PMC3037162

44. Aydogdu N, Atmaca G, Yalcin O, Batcioglu K, Kaymak K. Effects of caffeic acid phenethyl ester on glycerol-induced acute renal failure in rats. Clin Exp Pharmacol Physiol. 2004;31(9):575-9. http://dx.doi.org/10.1111/j.1440-1681.2004.04050.x PMid:15479163

45. Aydogdu N, Atmaca G, Yalcin O, et al. Protective effects of L-carnitine on myoglobinuric acute renal failure in rats. Clin Exp Pharmacol Physiol. 2006;33(1-2):119-24. http://dx.doi.org/10.1111/j.1440-1681.2006.04336.x PMid:16445710

46. Seifter JL, Samuels MA. Uremic encephalopathy and other brain disorders associated with renal failure. Semin Neurol. 2011;31(2):139-43. http://dx.doi.org/10.1055/s-0031-1277984 PMid:21590619

47. Can C, Sen S, Boztok N, Tuglular I. Protective effect of oral L-arginine administration on gentamicin-induced renal failure in rats.

Eur J Pharmacol. 2000;390(3):327-34. http://dx.doi.org/10.1016/S0014-2999(00)00025-X

48. Kumar P, Clark M. Renal disease in Textbook of Clinical Medicine. Saunders: London, 2005:p. 605-648. PMid:15826618

49. Morsy MA, Heeba GH. Nebivolol Ameliorates Cisplatin-Induced Nephrotoxicity in Rats. Basic Clin Pharmacol Toxicol. 2016;118(6):449-55. http://dx.doi.org/10.1111/bcpt.12538 PMid:26617394

50. Rodrigues MA, Rodrigues JL, Martins NM, et al. Carvedilol protects against cisplatin-induced oxidative stress, redox state unbalance and apoptosis in rat kidney mitochondria. Chem Biol Interact. 2011;189(1-2):45-51. http://dx.doi.org/10.1016/j.cbi.2010.10.014 PMid:21044617

51. Carvalho Rodrigues MA, dos Santos NA, da Silva Faria MC, et al. Carvedilol protects the kidneys of tumor-bearing mice without impairing the biodistribution or the genotoxicity of cisplatin. Chem Biol Interact. 2016;245:59-65. http://dx.doi.org/10.1016/j.cbi.2015.12.020 PMid:26751708

52. Dulin B, Abraham WT. Pharmacology of carvedilol. Am J Cardiol. 2004:93(9):3-6. http://dx.doi.org/10.1016/j.amjcard.2004.01.003 PMid:15144929

53. Lysko PG, Feuerstein GZ, Ruffolo RR. Carvedilol: a novel multiple action antihypertensive drug. Pharm News. 1995;2:12-16.

54. Feuerstein GZ, Yue TL, Cheng HY, Ruffolo RR. Myocardial protection by the novel vasodilating beta-blocker, carvedilol: potential relevance of anti-oxidant activity. J Hypertens Suppl. 1993;11(4):S41-8. http://dx.doi.org/10.1097/00004872-199306003-00010

55. Afzal MZ, Reiter M, Gastonguay C, et al. Nicorandil, a Nitric Oxide Donor and ATP-Sensitive Potassium Channel Opener, Protects Against Dystrophin-Deficient Cardiomyopathy. J Cardiovasc Pharmacol Ther. 2016. http://dx.doi.org/10.1177/1074248416636477 PMid:26940570

56. Sealey JE, Laragh JH. A proposed cybernetic system for sodium and potassium homeostasis: coordination of aldosterone and intrarenal physical factors. Kidney Int. 1974;6(5):281-90. http://dx.doi.org/10.1038/ki.1974.114

57. Sacco G, Evangelista S, Manzini S, Parlani M, Bigioni M. Combined antihypertensive and cardioprotective effects of nebivolol and hydrochlorothiazide in spontaneous hypertensive rats. Future Cardiol. 2011;7(6):757-63. http://dx.doi.org/10.2217/fca.11.70 PMid:22050062

58. Rodriguez-Perez JC, Losada A, Anabitarte A, et al. Effects of the novel multiple-action agent carvedilol on severe nephrosclerosis in renal ablated rats. J Pharmacol Exp Ther. 1997;283(1):336-44. PMid:9336341

59. Greven J, Gabriels G. Effect of nebivolol, a novel beta 1-selective adrenoceptor antagonist with vasodilating properties, on kidney function. Arzneimittelforschung. 2000;50(11):973-9. PMid:11148863

60. Halliwell B, Gutteridge JM. The importance of free radicals and catalytic metal ions in human diseases. Mol Aspects Med. 1985;8(2):89-193. http://dx.doi.org/10.1016/0098-2997(85)90001-9

61. Labrid C. Cellular disorders induced by ischemia. The effect of trimetazidine. Presse Med. 1986;15(35):1754-7. PMid:3024147

62. Clausen T, Flatman JA. Beta 2-adrenoceptors mediate the stimulating effect of adrenaline on active electrogenic Na-K-transport in rat soleus muscle. Br J Pharmacol. 1980;68(4):749-55. http://dx.doi.org/10.1111/j.1476-5381.1980.tb10868.x PMid:6247002 PMCid:PMC2044229

63. Shah KB, Rao K, Sawyer R, Gottlieb SS. The adequacy of laboratory monitoring in patients treated with spironolactone for congestive heart failure. J Am Coll Cardiol. 2005;46(5):845-9. http://dx.doi.org/10.1016/j.jacc.2005.06.010 PMid:16139135

64. Lysko PG, Lysko KA, Webb CL, Feuerstein G. Neuroprotective effects of carvedilol, a new antihypertensive, at the N-methyl-D-aspartate receptor. Neurosci Lett. 1992;148(1-2):34-8. http://dx.doi.org/10.1016/0304-3940(92)90798-C

65. Ilhan A, Yilmaz HR, Armutcu F, Gurel A, Akyol O. The protective effect of nebivolol on ischemia/reperfusion injury in rabbit spinal cord. Prog Neuropsychopharmacol Biol Psychiatry. 2004;28(7):1153-60. http://dx.doi.org/10.1016/j.pnpbp.2004.06.023 PMid:15610928

66. Seren M, Budak B, Turan N, et al. Collaborative therapy with nebivalol and L-NAME for spinal cord ischemia/reperfusion injury. Ann Vasc Surg. 2008;22(3):425-31. http://dx.doi.org/10.1016/j.avsg.2007.12.024 PMid:18466820

67. Kumar P, Kumar A. Prolonged pretreatment with carvedilol prevents 3-nitropropionic acid-induced behavioral alterations and oxidative stress in rats. Pharmacol Rep. 2008;60(5):706-15. PMid:19066418

68. Jovanovic D, Jovovic D, Mihailovic-Stanojevic N, et al. Influence of carvedilol on chronic renal failure progression in spontaneously hypertensive rats with adriamycin nephropathy. Clin Nephrol. 2005;63(6):446-53. http://dx.doi.org/10.5414/CNP63446 PMid:15960146

69. Guerrero E, Voces F, Ardanaz N, et al. Long-term treatment with nebivolol improves arterial reactivity and reduces ventricular hypertrophy in spontaneously hypertensive rats. J Cardiovasc Pharmacol. 2003;42(3):348-55. http://dx.doi.org/10.1097/00005344-200309000-00005 PMid:12960679

70. Afonso RA, Patarrao RS, Macedo MP, Carmo MM. Carvedilol's actions are largely mediated by endogenous nitric oxide. Rev Port Cardiol. 2006;25(10):911-7. PMid:17190240

71. Afonso RA, Patarrao RS, Macedo MP, Carmo MM. Carvedilol action is dependent on endogenous production of nitric oxide. Am J Hypertens. 2006;19(4):419-25. http://dx.doi.org/10.1016/j.amjhyper.2005.11.011 PMid:16580580

72. Kalinowski L, Dobrucki LW, Szczepanska-Konkel M, et al. Third-generation beta-blockers stimulate nitric oxide release from endothelial cells through ATP efflux: a novel mechanism for antihypertensive action. Circulation. 2003;107(21):2747-52. http://dx.doi.org/10.1161/01.CIR.0000066912.58385.DE PMid:12742996

73. Gandhi C, Zalawadia R, Balaraman R. Nebivolol reduces experimentally induced warm renal ischemia reperfusion injury in rats. Ren Fail. 2008;30(9):921-30. http://dx.doi.org/10.1080/08860220802353900 PMid:18925533

74. Uzar E, Acar A, Evliyaoglu O, et al. The anti-oxidant and anti-apoptotic effects of nebivolol and zofenopril in a model of cerebral ischemia/reperfusion in rats. Prog Neuropsychopharmacol Biol Psychiatry. 2012;36(1):22-8. http://dx.doi.org/10.1016/j.pnpbp.2011.08.011 PMid:21888941

75. Evangelista S, Garbin U, Pasini AF, et al. Effect of DL-nebivolol, its enantiomers and metabolites on the intracellular production of superoxide and nitric oxide in human endothelial cells. Pharmacol Res. 2007;55(4):303-9. http://dx.doi.org/10.1016/j.phrs.2006.12.008 PMid:17280840

76. Ignarro LJ. Different pharmacological properties of two enantiomers in a unique beta-blocker, nebivolol. Cardiovasc Ther. 2008;26(2):115-34. http://dx.doi.org/10.1111/j.1527-3466.2008.00044.x PMid:18485134

77. Varagic J, Ahmad S, Brosnihan KB, et al. Salt-induced renal injury in spontaneously hypertensive rats: effects of nebivolol. Am J Nephrol. 2010;32(6):557-66. http://dx.doi.org/10.1159/000321471 PMid:21042014 PMCid:PMC2992650

78. Ruilope LM, Lahera V, Rodicio JL, Romero JC. Participation of nitric oxide in the regulation of renal function: possible role in the genesis of arterial hypertension. J Hypertens. 1994;12(6):625-31. http://dx.doi.org/10.1097/00004872-199406000-00002 PMid:7963487

79. Maree A, Peer G, Schwartz D, et al. Role of nitric oxide in glycerol-induced acute renal failure in rats. Nephrol Dial Transplant. 1994;9 (Suppl 4):78-81. PMid:7528365

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):337-341.
http://dx.doi.org/10.3889/oamjms.2016.061
eISSN: 1857-9655
Basic Science

Curative Effect of Aqueous Leaf Extract of *Crinum Giganteum* on NMDA-Receptor Antagonist-Induced Schizophrenic Wistar Rat Model

Elizabeth Finbarrs-Bello[1,2*], Emmanuel Nebeuwa Obikili[1], Esom Emmanuel Anayochukwu[1], Anyanwu Emeka Godson[1]

[1]Department of Anatomy, College of Medicine, University of Nigeria, UNEC, Enugu, Nigeria; [2]Department of Anatomy, Ebonyi State University, Abakaliki Ebonyi State, Nigeria

Citation: Finbarrs-Bello E, Obikili EN, Anayochukwu EE, Godson AE. Curative Effect of Aqueous Leaf Extract of *Crinum Giganteum* on NMDA-Receptor Antagonist-Induced Schizophrenic Wistar Rat Model. Open Access Maced J Med Sci. 2016 Sep 15; 4(3):337-341. http://dx.doi.org/10.3889/oamjms.2016.061

Keywords: Amygdala; *Crinum giganteum*; Amy Chlorpromazine; Schizophrenia; NMDA; NSE.

***Correspondence:** Finbarrs-Bello, E. Department of Anatomy, College of Medicine, University of Nigeria, UNEC, Enugu, Nigeria. Tel: +2348064113179. E-mail: finbello@yahoo.com

Received: 16-Mar-2016; **Revised:** 10-May-2016; **Accepted:** 11-Apr-2016; **Online first:** 06-Aug-2016

Competing Interests: The authors have declared that no competing interests exist.

Abstract

AIM: This study evaluated the curative potential of *Crinum giganteum* in the treatment of schizophrenia using an NMDA-receptor antagonist-induced schizophrenic Wistar rat model.

METHODS: Twenty-five adult Wistar rats of both sexes of average weights 180 g were divided into two groups: control and schizophrenic rat models. The controls received 0.1 ml of 0. 9% saline, while schizophrenia was induced in models using 25 mg/kg of ketamine hydrochloride (i.p.) for 7 days. On the 8 day models were divided into group's k1, k2, k3 and k4 of 5 rats each. K1 and the controls were sacrificed then, groups k2 and k3 were treated with 5 mg/kg and 10 mg/kg aqueous leaf extract of *Crinum giganteum* while, k4 (standard) received 25 mg/kg of chlorpromazine orally for 28 days. Amygdala were harvested, processed and stained with Haematoxylin and Eosin (H &E) stain, Neuron-specific enolase (NSE) marker was also used to monitor the curative effect on the amygdala.

RESULTS: Degenerative changes and increased NSE immunoreactivity were observed in the untreated models. Extract-treated models showed normal amygdala and negative NSE immunoreactivity while chlorpromazine treated models revealed decreased NSE immunoreactivity.

CONCLUSION: *Crinum giganteum* extracts exhibits better curative effect than the standard antipsychotic agent.

Introduction

In the last three decades, herbal agents have gained popularity and increased patronage due to their folklore use in the management of some disease conditions. The world health organisation (WHO) indices showed that 1% of the world population depend on herbal agents in the treatment of diseases including mental disorders such as schizophrenia [1].

Schizophrenia disturbs thought processes, emotions and regulatory mechanisms involved in the secretion of neurotransmitters: dopamine, serotonin, acetylcholine and glutamate in the limbic areas [2, 3].

The most widely researched are the dopamine hypotheses which implicated diminished secretion of dopamine neurotransmitter in the neurochemistry of schizophrenia [4-8]. However, the glutamate hypothesis of schizophrenia posits dysfunction of the N-methyl-D-aspartate (NMDA) glutamate receptor in schizophrenia. The NMDA receptors are a major subtype of glutamate receptors and mediate slow excitatory postsynaptic potentials (EPSPs). These slow EPSPs are considered critical for the proper expression of complex behaviours, such as associative learning, working memory, behavioural

flexibility and attention which are impaired in schizophrenia [4, 5]. In early development, it aids the development of neural pathways whose malfunction may lead to susceptibility to schizophrenia [1].

Evidence from basic and clinical researchers show that genes associated with the risk for schizophrenia influence the modulatory sites on the NMDA receptor or intracellular receptor interacting proteins that link glutamate receptors to signal transduction pathways [9]. A postmortem study reported changes in glutamate receptor binding, transcription, and subunit protein expression in the prefrontal cortex, thalamus, and hippocampus of subjects with schizophrenia [10]. Antagonists of the NMDA receptor elicit schizophrenic symptoms in recreational use or administration of a single low dose of such agents [11, 12]

Phencyclidine (PCP) or ketamine produces "schizophrenia-like" symptoms that resemble positive (delusion and hallucination), negative (avolition, apathy, and blunted affect), and cognitive deficits in healthy individuals and rodents [4, 5, 13-16]. In addition, the NMDA glutamate receptor regulates the function of other neurotransmitter systems implicated in the pathophysiology of schizophrenia [17].

In pharmacotherapy, dopamine transmitter is the target of most antipsychotic drugs for schizophrenia and NMDA receptor antagonist-induced schizophrenia [7]. Generally, antipsychotics are able to manage symptoms like delusion, hallucination and aggression [18-20]. They are still best described as control measures as they do not totally cure the mental disorders. Thence, agents that modulate glutamate via the NMDA receptors promise to be a treatment entity towards the discovery of better pharmacological target and agents that could treat schizophrenia besides dopamine.

Crinum giganteum is a major herb used in the treatment of mental illnesses in some parts of Africa like Cameroun, Niger Republic and Nigeria. In Nigeria, it is predominantly used in northern where it called gadalli, Albacce Buru or Albacce Dawaddi [21]. Traditional medicine practitioners in the region have claimed that *Crinum giganteum* could cure schizophrenia and other mental condition [22]. It's been known that herbal agents could have a toxic effect, lacks standard formulation and adequate dosing regimen [22]. To authenticate this claim, scientific evaluation of the plant is necessary; this study evaluates the curative potential of aqueous leaf extract of *Crinum giganteum* on the amygdala using a NMDA receptor antagonist to induce schizophrenia system in Wistar rats.

This was aimed at ascertaining the curative effect using neuron-specific enolase (NSE) marker and comparing this property with a standard antipsychotic (chlorpromazine) agent.

Materials and Methods

Collection and authentication of plant materials

The leaves were procured from the open market and identified by the curator Prof. Mrs M.O Nwosu of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka as *Crinum giganteum*. Herbarium sheet was prepared and a voucher specimen (UNH/13/401) was deposited at the herbarium of same Department.

Preparation of plant extract

The leaves were washed with distilled water and air-dried under shade for seven days. Thereafter, the leaves were pulverised into a fine powder, 100 grammes of the dried leaf powder was placed in a beaker containing 500ml of distilled water. The mixture was heated on a hot plate with continuous stirring at 30 ºC -40ºC for 20 minutes. It was then allowed to cool and then filtered through mesh cloth. The filtrate (aqueous extract) was evaporated to a paste using a vacuum evaporator. This was transferred into a suitable container and kept in the refrigerator at low temperature (4 ºC) for the experiment.

Animals and ethical concern

Twenty-five (25) adult Wistar rats of both sexes of average weights 180 g were purchased from the animal house of the College of Medicine. The university of Nigeria, and housed at the animal facility of the same college. The animals were housed in netted iron cages in groups of five, fed with grower's mash and provided water *ad libitum.* The rats were maintained under laboratory conditions (temperature 24±2 ^{o}C with relative humidity 60-70%, and 12-hour light-dark cycle). They were acclimatised for two weeks before the experiment. The experimental protocols and techniques used in the study were in accordance with accepted principles for laboratory animal use and care. The study was reviewed and approved by the University Health Research Ethics Committee with certificate number NKREC/05/01/2008B-FWA00002458-1RB00002323.

Induction of schizophrenia in rat models

Twenty (20) rats were induced using 25 mg/kg ketamine hydrochloride (a NMDA receptor antagonist) per body weight, intraperitoneal (i.p), for 7 days. The control (group A) had 5 rats which received 0.1ml 0.9% saline. The animals exhibited side to side head rocking and continuous staggering locomotion.

Treatment of animals and tissue processing

On the 8 days, the ketamine group (n = 20) was divided into four groups (k1-k4). The control (group A) and group k1 (untreated model) were sacrificed same day. Groups k2 and k3 received 5 mg/kg and 10 mg/kg of aqueous leaf extract of gadalli orally respectively, while group k4 received 25 mg/kg of chlorpromazine orally for 28 days. The rats were anaesthetized with 50 mg/kg thiopental sodium and aortic perfusion fixation with 4% paraformaldehyde was carried out. The brains were dissected out and further fixed in 4% paraformaldehyde overnight, amygdala was harvested. Fixed tissues were dehydrated in ascending grades of ethanol (50%, 70%, 90% and 100%), cleared in xylene and embedded in paraffin wax. Serial sections of 10µm thick were obtained using a rotatory microtome. Part of the paraffinized sections was stained using haematoxylin and eosin (H &E) and the rest were used for the immunohistochemical study.

Immunohistochemical demonstration of NSE

The Avidin-Biotin Complex (ABC) method also referred to the Avidin-biotin Immunoperoxidase method was used. Paraffin processed tissues were sectioned at 2 microns on the rotary microtome and placed on the hot plate at 70 degrees for at least 1hour. Sections were brought down to water by passing the on 2 changes of xylene, then 3 changes of descending grades of alcohol (100%, 90%, 70% and 50%) and finally to water. Antigen retrieval was performed on the sections by heating them in a citric acid solution of PH 6.0 using the microwave at power 100v for 15 minutes. The sections were equilibrated gradually with cool water to displace the hot citric acid for at least 5 minutes for the section to cool. Peroxidase blocking was done on the sections by simply covering sections with 3% hydrogen peroxide for 15 mins. Sections were then washed with phosphate-buffered saline (PBS) and protein blocking was performed using avidin for 15 mins. Sections were washed again with PBS and endogenous biotin in tissues was blocked using biotin for 15 mins.

After washing with PBS sections were incubated with the respective diluted primary antibody NSE antibody (diluted 1:100) for 60 mins. Excess antibodies were washed off with PBS and a secondary antibody (LINK) was applied on the section for 15 min. Sections were washed and the (LABEL) which is the horseradish peroxidase (HRP) was applied on all sections for 15mins. A working DAB (3, 3'-diaminobenzidine) solution was made up by mixing 1 drop (20 microns) of the DAB chromogen to 1ml of the DAB substrate. This working solution was applied on sections after washing off the HRP with PBS for at least 5mins. The brown reactions begin to appear at this moment especially for positive targets. Excess DAB solution and precipitate were washed off with water. Sections were counterstained with Haematoxylin solution for at least 2 mins and blued briefly. Sections were dehydrated in alcohol, cleared in xylene and mounted in DPX.

Results

We can see from the Figure 1 that sections of amygdala of rats (a) control (0.1ml saline) shows normal neurons, (b) untreated schizophrenic model (25 mg/kg ketamine) shows cytoplasmic vacuolations, (c) and (d) schizophrenic models treated with 5 mg/kg and 10 mg/kg of ethanolic leaf extract of *Crinum giganteum* shows normal neurons respectively, and (e) schizophrenic model treated with 25 mg/kg of chlorpromazine shows relatively normal neuron.

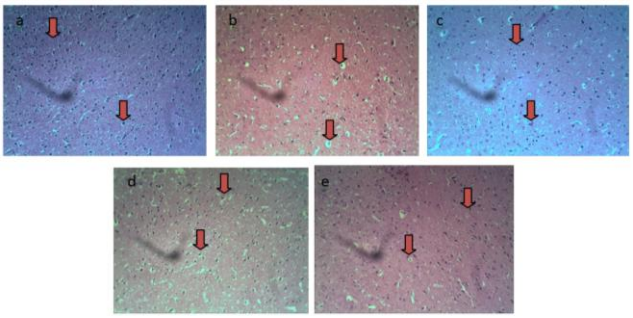

Figure 1: Sections of amygdala of rats (a) control (0.1ml saline), (b) untreated schizophrenic model (25 mg/kg ketamine), (c) and (d) schizophrenic models treated with 5 mg/kg and 10 mg/kg of ethanolic leaf extract of Crinum giganteum, and (e) schizophrenic model treated with 25 mg/kg of chlorpromazine.Arrows. (H&E) x 200

Sections of amygdala of rats (a) control (0.1ml saline) show negative immunoreactivity, (b) untreated schizophrenic model (25 mg/kg ketamine) shows positive NSE immunoreactivity, (c) and (d) schizophrenic models treated with 5 mg/kg and 10 mg/kg of ethanolic leaf extract of *Crinum giganteum* shows NSE negative immunoreactivity respectively, and (e) schizophrenic model treated with 25 mg/kg of chlorpromazine shows positive NSE immunoreactivity (Figure 2).

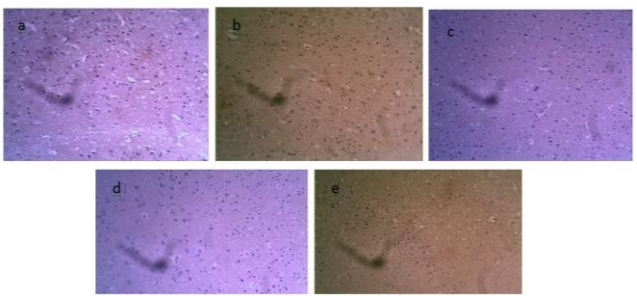

Figure 2: Sections of amygdala of rats (a) control (0.1ml saline), (b) untreated schizophrenic model (25 mg/kg ketamine), (c) and (d) schizophrenic models treated with 5 mg/kg and 10 mg/kg of ethanolic leaf extract of Crinum giganteum, and (e) schizophrenic model treated with 25 mg/kg of chlorpromazine. NSE x 200

Discussion

In this study, ketamine-induced neuronal damage was characterised by cytoplasmic vacuolation and eccentric nuclei. Ketamine induction of vacuolation and neuronal cell death in rodents has been established [7, 23, 24]. This is possible considering the mechanisms propounded from previous studies via inhibition or antagonism of N-Methyl-D- Aspartate (NMDA) receptors [23, 24].

Compensatory upregulation of NMDA receptor expression, which is tied to the toxic influx of calcium and elevated reactive oxygen species (ROS) generation and neuronal cell death [25, 26]. Another mechanism involves blockade of an excitatory NMDA glutamate receptor on the GABA neurones, which could trigger decrease GABA release and activate compensatory increase blood flow and metabolism [27]. Ketamine neurotoxicity also triggers induction of heat shock proteins (Hsp70) and denaturation of intracellular proteins in pyramidal neurones [27-29]

The neuronal damage (ketamine neurotoxicity) observed in the untreated schizophrenic models was attenuated by treatment with the varying doses of the extract of *crinum giganteum*. Meanwhile, the standard antipsychotic treatment showed relatively normal neurone which was less prominent compared to the extract treatment. Their potential or degree of the effects can further be deduced from the NSE immunoreactivities.

Neuron-specific enolase (NSE) is expressed in all neuronal cell types, its detection has been used to identify neuronal cells and monitor disease progress in the CNS [30]. The untreated schizophrenic rat models showed positive and increased expression of NSE which confirms the neuronal damage earlier reported in the group. This was consistent with increased NSE levels seen in acute neuronal injury in the area of the brain [31]. The negative NSE immunoreactivity in the extract treated groups and the verisimilitude with the control attest to that fact that ketamine effect was reversed and the amygdala integrity was restored. However, curative effect of the standard antipsychotic treatment (chlorpromazine) was less compared to the extract treatment going by the positive but decreased NSE immunoreactivity.

We attributed the effect of the extract to the phytochemicals present in the aqueous leaf extract of *crinum giganteum* such as alkaloids, saponins and glycosides. Saponins generally exhibit antioxidant activity and ginsenosides saponins are known to foster neurogenesis [23, 32]. Similarly, glycosides possess neuroprotective effect [33, 34], which must have played a role in the reversal effect of the extract. The activities of these phytochemicals in the extract have conferred neuroprotective effects by attenuating effect of the NMDA receptor antagonist (ketamine) in the treated schizophrenic rat models.

References

1. Joseph TC, Glen TK. The neurochemistry of schizophrenia. Basic Neurochemistry. 8th ed. Elsevier Inc. Toronto, 2012: 1000-1011.

2. Osaretin A, Taiwo E, Olumuyiwa A. Extracts of Cnestisferruginea and Rauwolfia vomitoria affect blood chemistry and GABAergic neurotransmission in ketamine – induced psychotic rats. FASEB Journal. 2011;25:764-774.

3. Ezequiel U, Jose L, Richard W, Antonio E. Memantine reverses social withdrawal induced by ketamine in rats. Experimental Neurobiology. 2013; 22(1):18-22. http://dx.doi.org/10.5607/en.2013.22.1.18 PMid:23585718 PMCid:PMC3620454

4. Corsen GY. Domino E. Dissociative anaesthesia further pharmacologic studies and first clinical experience with the PCP derivatives (C1-581). Anesth Analag . 1966;45:129 -140.

5. Krystal H, Karper LP, Seibyl JP. Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. Arch Gen Psychiatry.1994;51(3):199-214. http://dx.doi.org/10.1001/archpsyc.1994.03950030035004 PMid:8122957

6. Silbersweig DA, Stein E, Froth C, Cahill C, Holmes A, Grootwuk S, Seaward J, Mckenna P, Chue SE, Schuor L. A functional new anatomy of hallucination in schizophrenia. Nature. 1995; 378(6553): 176-179. http://dx.doi.org/10.1038/378176a0

7. Bertram GK, Serzan BM, Anthony TT. Sedative-Hypnotic drugs: Basic and Clinical pharmacology.11th International edition, McGraw Hill Companies inc., 2009: 371-509.

8. Sharry KT, Wai C. Pharmacological models of psychosis: Amphetamine and Ketamine. Medical Bulletin. 2011;5:17 -19.

9. Harrison P, Law A, Eastwood S. Glutamate receptors and transporters in the hippocampus in schizophrenia. Annals of the New York Academy of Sciences. 2003;1003: 94-101. http://dx.doi.org/10.1196/annals.1300.006 PMid:14684437

10. Clinton S, Haroutunian V, Davis K, Meador-Woodruff J. Altered transcript expression of NMDA receptor-associated postsynaptic proteins in the thalamus of subjects with schizophrenia. American Journal of Psychiatry. 2003;160:1100-1109. http://dx.doi.org/10.1176/appi.ajp.160.6.1100 PMid:12777268

11. Alder CM, Malhotra AK, Elman I, Goldberg. T, Egan M. Pickar D, Brevier A. Comparison of ketamine –induced thought disorder in health volunteer and thought disorder in schizophrenia. Am J Psychiatry. 1999;156:1646-1649. http://dx.doi.org/10.1176/ajp.156.10.1646 PMid:10518181

12. Glen MA, Maamo M. Haloperidol for the treatment of ketamine-induced emergence delirium. J Anesth Clin. 2007;23(1):65-67.

13. Luby ED, Cohen BD, Rosen B, Gothleb J, Kelly R. Study on a new schizophrenomemitic drug serryl. Arch Neural Psychiatry. 1959;81:363-369. http://dx.doi.org/10.1001/archneurpsyc.1959.02340150095011

14. Javitt D. Negative schizophrenic symptomatology and the PCP (phencyclidine)model of schizophrenia. Hillside Journal of Clinical Psychiatry. 1987;9:12-35. PMid:2820854

15. Lahti AC, Koffel B, Laporte B, Tamminga CA. Subanesthetic doses of ketamine stimulate psychosis in schizophrenia, Neuropsychopharmacology. 1995; 17(3):141-150. http://dx.doi.org/10.1016/0893-133x(94)00131-i

16. Frank RS, Robert LH. Psychosis: atypical limbic epilepsy versus limbic hyperexicitability with onset at puberty. Epilepsy Behav. 2007;10(4):515-520.

http://dx.doi.org/10.1016/j.yebeh.2007.02.014 PMid:17416210 PMCid:PMC2680611

17. Gerburg K, Hans-Gert B, Axel B, Gisela G, Gerald W. Increased neurogenesis in a rat ketamine model of schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry. 2012;38(2):310-316.

18. Raji Y, Ifabunmi S, Akinsomisoye OS, Morakinyo AO, Oloyo AK. Gonadal Responses to Antipsychotic Drugs: Chlorpromazine and Thioridazine Reversibly Suppress Testicular Functions in Albino Rats. International Journal of Pharmacology. 2005;1(3): 287-292. http://dx.doi.org/10.3923/ijp.2005.287.292

19. Loga P. Chlorpromazine in Migraine. Emergency Medicine Journal. 2007;24(4): 297-300. http://dx.doi.org/10.1136/emj.2007.047860 PMid:17384391 PMCid:PMC2658244

20. Turner T. Chlorpromazine: unlocking psychosis. British medical journal. 2007;334(Suppl 1): 7-9. http://dx.doi.org/10.1136/bmj.39034.609074.94 PMid:17204765

21. Keay RW. Trees of Nigeria. Oxford University Press. USA, 1989:1-6.

22. Tyler VE. Herbs affecting the central nervous system: Perspectives on newcrops and new uses. ASHS Press, Alexandria, V A. In: J. Janick (ed.), 1999:442-449.

23. Malhotra AK, Pinals DA, Adler CM, Elman I, Clifton A, Pickar D, Breier A. Ketamine-induced exacerbation of psychotic symptoms and cognitive impairment in neuroleptic-free schizophrenics. Neuropsychopharmacology. 1997;17(3):141-50. http://dx.doi.org/10.1016/S0893-133X(97)00036-5

24. Chatterjee M, Verma R, Ganguly S, Palit G. Neurochemical and molecular characterization of ketamine-induced experimental psychosis model in mice. Biological Psychiatry. 2004;56(5): 317–322.

25. Sharp FR, Butman M, Aardalen K, Nickolenko J, Nakki R, Massa SM, Swanson RA, Sagar SM. Neuronal injury produced by NMDA antagonists can be detected using heat shock proteins and can be blocked with antipsychotics. Psychopharmacol Bull. 1999;30:555-560.

26. Liu ZL, Xu RX, Yang ZJ, Dai YW, Luo CY, Du MX, Zou YX, Jiang XD. [Responses of neurons and astrocytes in rat hippocampus to kainic acid-induced seizures]. Di Yi Jun Yi Da Xue Xue Bao. 2003;23(11):1151-5.

27. Olney JW, Faber NB. Efficacy of clozapine compared with other antipsychotics in preventing NMDA-antagonist Neurotoxicity. J Clin Psychiatry. 1994;55(Suppl B): 43-46. PMid:7961572

28. Sharp FR, Butman M, Wang S, Koistinaho J, Graham SH, Sagar SM, Noble L, Berger P, Longo FM. Haloperidol prevents induction of the hsp70 heat shock gene in neurons injured by phencyclidine (PCP), MK801, and ketamine. J Neurosci Res. 1992; 33:605-616. http://dx.doi.org/10.1002/jnr.490330413 PMid:1484394

29. Sharp FR, Butman M, Koistinaho J, Aardalen K, Nakki R, Massa SM, Swanson RA, Sagar SM. Phencyclidine induction of the hsp 70 stress gene in injured pyramidal neurons is mediated via multiple receptors and voltage gated calcium channels. Neuroscience. 1999;62:1079-1092. http://dx.doi.org/10.1016/0306-4522(94)90345-X

30. Haimoto H, Takahashi Y, Koshikawa T et al. Immunohistochemical localization of gamma-enolase in normal human tissues other than nervous and neuroendocrine tissues. Lab Invest. 1985;52(3): 257-263. PMid:3974199

31. Craig SP, Day IN, Thompson RJ, Craig IW. Localization of neuron-specific enolase (ENO2). Cytogenet. 1991;12:13.

32. Radad K, Rudolf M, Wolf-Dieter R. Ginsenosides and their CNS targets. CNS neuroscience and therapeutics. 2011;17(6): 761-768. http://dx.doi.org/10.1111/j.1755-5949.2010.00208.x PMid:21143430

33. David OK, Emma LW. Herbal extract and phytochemicals; plant secondary metabolites and the enhancement of human brain function. Adv Nutri J. 2011;2:32-50. http://dx.doi.org/10.3945/an.110.000117 PMid:22211188 PMCid:PMC3042794

34. Kumar GP, Khanum F. Neuroprotective potential of phytochemicals. Phcog. 2012;6(12): 81-89. http://dx.doi.org/10.4103/0973-7847.99898 PMid:23055633 PMCid:PMC3459459

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):342-347.
http://dx.doi.org/10.3889/oamjms.2016.088
eISSN: 1857-9655
Basic Science

Influence of Parental and Some Demographic Characteristics on Overweight/Obesity Status among a Sample of Egyptian Children

Nayera E. Hassan[1], Sahar A. El-Masry[1*], Tarek Farid[2], Aya Khalil[1]

[1]Biological Anthropology Department, Cairo, Egypt; [2]Pediatric Department, Medical Research Division, National Research Centre, Cairo, Egypt (Affiliation ID 60014618)

Citation: Hassan NE, El-Masry SA, Farid T, Khalil A. Influence of Parental and Some Demographic Characteristics on Overweight/Obesity Status among a Sample of Egyptian Children. Open Access Maced J Med Sci. 2016 Sep 15; 4(3):342-347. http://dx.doi.org/10.3889/oamjms.2016.088

Keywords: Obesity; overweight; children; parental factors; residence.

***Correspondence:** Sahar Abd El-Raufe El-Masry. Biological Anthropology Dept., National Research Centre, El-Bohooth St., Dokki, Giza, Cairo 12622, Egypt. Tel: +2 0106606640. E- mail: masrysa@yahoo.com

Received: 28-Jun-2016; **Revised:** 27-Jul-2016; **Accepted:** 28-Jul-2016; **Online first:** 27-Aug-2016

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: Overweight/obesity is a multi-factorial problem, which results from rapidly changing social, economic, and physical environments that have led to an energy imbalance.

AIM: To identify the association between childhood overweight/obesity and some socio-demographic risk factors, as parental age, body mass index (BMI), education and occupation, family size and residence (urban/rural).

SUBJECTS AND METHODS: Cross-sectional study included 154 children of both sexes; aged 5-18 years; with their parents; one of them was working at the National Research Centre and from their relatives and neighbours. Data was collected about the child birth weight, family size, parental ages, education, occupation and place of residence. Anthropometric measurements including weight, height, and body mass index (BMI) of children and their parents were conducted.

RESULTS: Obesity was detected among 19.5% of children (BMI > 95th percentile), 75.3% of their mothers and 49.6% of their fathers (BMI > 30 Kg/m^2). While overweight was present in 11.0% of the children (BMI > 85th- <95 percentile), 16.9% of their mothers and 36.5% of their fathers (BMI > 25-29.9 Kg/m^2). Child obesity was more prominent in urban than rural areas (21.3% versus 12.5%) and among housewives (22.8%) than among working mothers (16%, $p < 0.016$). Child overweight was more common in rural than urban areas (12.5% versus 10.7%) and among children with high father education (20%). Child BMI had significant positive correlations only with the child age, parental ages and BMIs, and family size. In spite of that, parental BMIs had significant positive correlations with each other and with family size, and significant negative correlations with maternal education and occupation and paternal education.

CONCLUSION: Childhood obesity and overweight were more prominent in urban than rural areas, among children with non-working housewives mothers and highly educated fathers (college or above). Parental education and occupation had an indirect significant effect on child BMI through their significant effect on parental BMIs.

Introduction

An important goal in addressing the global obesity epidemic is childhood obesity prevention [1]. In recent decades, the worldwide obesity epidemic is increasing at an alarming rate in childhood and can be observed in developing countries, which have shown an increase in the prevalence of childhood obesity [2].

Obesity is an established "risk factor" for several chronic diseases [3-5]. The World Health Organization [6] recognised obesity as a major public health epidemic.

Obesity is a multi-factorial problem, because of rapidly changing social, economic, and physical environments that have led to an energy imbalance in the population through a dramatic reduction in physical activity [7-8] and changes in dietary patterns [9].

Parental and familial characteristics are presumed to have an impact on offspring's obesity status not only through shared genes but also through

shared environments that determine nutrition and physical activity patterns early in life, as well as through the interaction of both [10].

Chaparroand Koupil [11] through their study on three generations of Swedish men and women; to investigate the impact of parental educational trajectories on their adult offspring's overweight/obesity status; concluded that "Socioeconomic inequalities can have long-term consequences and impact the health of future generations. For overweight/obesity in their concurrent young cohorts, this inequality was not fully offset by an upward educational trajectory in their parent's generation".

In Egypt, as in other parts of the world, the obesity epidemic affects a growing number of children and adolescents. A study among female adolescents showed that 35 percent of the girls were overweight and 13 percent were obese. Overweight was more prevalent in urban than in rural girls [12]. In the report of diet, nutrition and prevention of chronic non-communicable diseases in Egyptian adolescents [13], it was found that about 20.5% of the adolescents were either overweight or obese with higher prevalence among urban than rural and females compared to males. In our previous study for assessment of the prevalence of metabolic syndrome among Egyptian school students suffering from obesity [14], the prevalence of obesity is 8.0% among 5798 Egyptian school students (26.4% were in the pre-pubertal period and 73.6% were in the pubertal period); however, the prevalence of overweight is 11%.

So, the purpose of current research is to estimate overweight and obesity prevalence and to identify factors, particularly parental risk factors as age, BMI, education, occupation, and demographic factors as family size and residence (urban/rural), that might be associated with overweight and obesity in a sample of children and adolescents aged 5–18 y to propose adapted action.

Subjects and Methods

Sample

This study was derived from a cross-sectional survey through a project funded by National Research Centre (NRC) Egypt, 2013-2016: entitled "Familiar Overweight and Obesity in Children and Adolescents: Diagnostic Clinical, Behavioral, Genetic and Biochemical Markers and Intervention" (10th Research Plan of the NRC), after taking approval from Ethical Committee of NRC (Registration Number is 13/168).

A total number of 154 child of both sexes (77 males and 77 females) with their parents; one of them (mainly the mother) was working at the National Research Centre and from their relatives and neighbors; were chosen randomly from all categories of the workers to participate in the study after signing a written consent form of the Medical Ethical Committee of NRC. The age range of the children was between 5 and 18 years with a mean age was 10.83 $\pm$ 3.82. Participants were informed about the purpose of the study and their permission in the form of written consent was obtained.

Methods

Trained interviewers collected detailed questionnaire from the mothers. Anthropometric measurements including weight, height, and body mass index (BMI) of the children and their parents were conducted.

Detailed questionnaire

Data was collected by trained doctors about the child age in years; his/her birth weight in Kg., place of residence (urban/rural), family size (No. of persons in the family), parental (mothers and fathers) age, education and occupation. Parental education was classified into 3 grades: illiterate, precollege and college. Maternal occupation was graded as housewives or working mothers, while father´s occupation was graded as manual and non-manual workers.

Anthropometric measurements

Weight was measured using a commercial scale (Seca Scale, Germany) with accuracy up to nearest 100 g. The subjects were asked to remove their footwear and wear minimal clothes before weighing them. Standing body height was measured, to the nearest 0.1 cm by using Holtain Stadiometer with the shoulder in a relaxed position and arms hanging freely and without shoes. The scales were recalibrated after each measurement following the recommendations of the International Biological Program [15]. Body Mass Index (BMI) was calculated as body weight in kilogrammes/height in metere^2. Children BMI percentile was calculated specific for age and sex based on the Egyptian Growth Reference Charts [16]. A child with BMI below 85th percentile was considered healthy weight, with BMI between 85th and95th percentile overweight and those with BMI ≥ 95th percentile obese. While mothers or fathers with BMI below 25 Kg/m^2 were considered healthy weight, with BMI ≥ 25-29.9 Kg/m^2 overweight and with BMI ≥ 30 Kg/m^2 were considered obese.

Statistical analysis

Data were analysed using the Statistical Package for Social Sciences (SPSS/Windows Version

16, SPSS Inc., Chicago, IL, USA). Statistical significance was set at $P < 0.05$. Parametric data were expressed as mean ± SD, while the non-parametric data (qualitative) were expressed as frequency distribution: numbers and percentage of the total. Comparisons between the different non-parametric variables were analysed using Chi- square test. Spearman´s correlation test was used to examine the association between child and parental BMI with the different variables under study.

Results

The distribution of the children and their parental BMI and some selected demographic variables was shown in Table 1. Obesity was detected among 19.5% of the children (their BMI ≥ 95th percentile), 75.3% of their mothers and 49.6% of their fathers (BMI > 30 Kg/m^2). While overweight was present in 11.0% of the children (their BMI ≥85th-<95th percentile), 16.9% of their mothers and 36.5% of their fathers (BMI ≥ 25-29.9 Kg/m^2).

The majority of the children (79.2%) were living in urban areas. The family size ranged between 2 up to 8 members in the family with a mean of 5.1 ± 1.12. Most of the mothers (65.1%) were non-working housewives, and 48.9% of the fathers were manual workers. College education or above was present in 40.1% of the mothers and 45.9% of the fathers.

Table 1: Descriptive characteristics of the study sample

Weight Status		
Child BMI [N (%)]	<85th percentile	107 (69.5%)
(N = 154)	85th to <95th percentile	17 (11.0%)
	.> 95th percentile	30 (19.5%)
Mother BMI [N (%)]	BMI < 25 Kg/m^2	12 (7.8%)
(N = 154)	BMI 25-29.9 Kg/m^2	26 (16.9%)
	BMI > 30 Kg/m^2	116 (75.3%)
Father BMI [N (%)]	BMI < 25 Kg/m^2	19 (13.9%)
(N = 137)	BMI 25-30 Kg/m^2	50 (36.5%)
	BMI > 30 Kg/m^2	68 (49.6%)
Child individual factors		
Child age years (Mean ± SD[Range])		10.83 ± 3.82 [5.1-18]
Child sex	Boys [N(%)]	77 (50%)
	Girls (%)	77 (50%)
Birth weight (mean ± SD)		2.94 ± 0.57
Family factors		
Maternal age years (mean ± SD)		38.92 ± 8.48 [24-59]
Maternal Education	Illiterate	33 (21.7%
	Precollege	58 (38.2%)
	Collage	61 (40.1%)
Maternal Occupation	Housewife	95 (65.1%)
	Working	51 (34.9%)
Father age years (mean ± SD)		45.42 ± 7.81 [30-60]
Father Education	Illiterate	23 (17.3%)
	Precollege	49 (36.8%)
	Collage	61 (45.9%)
Father Occupation135	Manual	66 (48.9%)
	Non-Manual	69 (51.1%)
Family size (N=153)		5.1 ± 1.12 [2-8]
Residence	Rural	32 (20.8%)
	Urban	122 (79.2%)

The residence whether urban or rural was significantly related to the prevalence of obesity and overweight (Table 2), where obesity was more prominent in the urban areas (21.3% versus 12.5%) and overweight was more in rural areas (12.5% versus 10.7%). The maternal occupation had a significant effect on their childhood obesity, as obesity was more common among housewives (22.8%) than among working mothers (16%, $p < 0.016$).

Table 2: Frequency of child obesity and overweight (%) by age, sex and socioeconomic characteristics

Characteristics		N	Obesity (29)	Overweight (14)
Age	5-11	97	17 (17.5%)	10 (10.3%)
	12-18	57	13 (22.8%)	7 (12.3%)
	P value		0.456	0.467
Sex	Boys (%)	77	11 (14.3%)	10 (13%)
	Girls (%)	77	19 (24.7%)	7 (9.1%)
	P value		0.144	0.467
Maternal Education	Illiterate	33	9 (27.3%)	3 (9.1%)
	Precollege	54	12 (22.2%)	5 (9.3%)
	Collage	61	9 (14.8%)	8 (13.1%)
	P value		0.741	0.305
Maternal Occupation	Housewife	92	21 (22.8%)	7 (7.6%)
	Working	50	8 (16%)	8 (16%)
	P value		0.016*	0.796
Father Education	Illiterate	22	(40.9%)	1 (4.5%)
	Precollege	47	9 (19.1%)	4 (8.5%)
	Collage	60	8 (13.3%)	12 (20%)
	P value		0.962	0.003*
Father Occupation	Manual	62	13 (21%)	8 (12.9%)
	Non-Manual	69	14 (20.3%)	9 (13%)
	P value		0.847	0.808
Residence	Rural	32	4 (12.5%)	4 (12.5%)
	Urban	122	26 (21.3%)	13 (10.7%)
	P value		0.000**	0.029*

Father education had also a significant effect on their childhood overweight, which was the most prominent among children with father education of college or above (20%), and the least percentage was detected among children with illiterate fathers (4.5%, $p < 0.003$). However, there was the insignificant effect of the child age or sex, maternal education or father occupation on the prevalence of their children obesity and overweight.

Table 3: Correlation between child BMI and the other factors

Variables	Child BMI		Number
	r	p	
Child age	0.535**	0.000	145
Birth weight	0.125	0.136	143
Mother age	0.359**	0.000	145
Mother BMI	0.302**	0.000	148
Maternal Education	-0.136	0.099	148
Maternal Occupation	0.035	0.680	142
Father age	0.291**	0.001	120
Father BMI	0.227*	0.007	139
Father Education	-0.164	0.064	129
Father Occupation	-0.015	0.869	131
Residence U/R	-0.115	0.160	150
Family size	0.200*	0.015*	149

Child BMI had significant positive correlations with the child age, parental ages and BMIs, and family size (Table 3). In addition, the parental education and occupation and the place of residence had insignificant correlations with child BMI.

In spite of that, parental BMIs had significant positive correlations with each other and with family size and had significant negative correlations with maternal education and occupation and paternal education (Table 4). Moreover, Father BMI had significant negative correlations with father occupation and place of residence.

Table 4: Correlation between parental BMI and the other factors

	Mother BMI		Father BMI	
	r	p	r	p
Child age	0.089	0.281	0.095	0.267
Birth weight	0.137	0.105	-0.043	0.630
Mother age	0.063	0.443	0.075	0.382
Mother BMI	-	-	0.359	0.000**
Maternal Education	-0.262	0.001**	-0.254	0.002**
Maternal Occupation	-0.208	0.012*	-0.206	0.016
Father age	0.152	0.095	0.122	0.199
Father BMI	0.359	0.000**	-	-
Father Education	- 0.388	0.000**	-0.244	0.007**
Father Occupation	-0.089	0.304	-0.207	0.021*
Residence U/R	0.094	0.248	-0.260	0.002**
Family size	0.179	0.027*	0.196	0.020*

Discussion

Several factors may play an important role in explaining child obesity, including parental influence, food prices, access to fast food, environment, and opportunities for physical activities, school nutrition policies, and advertising. Yet, such ''root causes'' cannot always explain excess variance within regions or racial groups [17]. In recent years, one additional explanation for the persistent increase in obesity levels – and one that has received considerable attention– is the effect of parental risk factors as age, BMI, education, employment, and demographic factors as family size and residence (urban/rural).

Current research aimed to investigate overweight and obesity prevalence and to identify factors, particularly parental risk factors and demographic factors as family size and residence (urban/rural), that might be associated with overweight and obesity in a sample of 154 child aged 5–18 y of both sexes, with their parents. Obesity was detected among 19.5% of the children, 75.3% of their mothers and 49.6% of their fathers. While overweight was present in 11.0 % of the children, 16.9% of their mothers and 36.5% of their fathers. The prevalence of overweight and obesity in this study was higher than that reported in previous Egyptian study conducted by Hafez et al. [18] who showed that the prevalence of overweight and obesity was 11% and 3.8%, respectively, among children attending government schools in Cairo. Hassan et al. [14]; in Giza; and El-Shafie and his colleagues [19]; in Dakahlia; found that the prevalence of overweight and obesity was 11.5 and 8.5%, respectively in primary school children.

This variation might be partially attributed to the difference in standard curves used for BMI Z-score classes. Nevertheless, this prevalence was higher than that reported in similar studies in UK and Brazil (8.7% and 4%) respectively [20].

Childhood obesity and overweight in the present study were more prominent in urban than rural areas, among children with non-working housewife mothers and highly educated fathers (college or above). In addition, they were positively correlated with child age, parental ages, BMIs and family size. Maternal and paternal BMIs recorded negative correlation with maternal education, occupation and paternal education; and positive correlation with each partner BMI and family size. Therefore, parental education and occupation had an indirect negative significant effect on child BMI through their significant effect on their parental BMIs.

Reviewing literatures for current results: as regards BMI-Z score classes, Bahbah et al. [21] in their study of obesity and overweight in primary school children living in Menoufia governorate; Menouf district; revealed that the incidence of obesity was higher among urban than among rural children; which coincides with our results; and children attending private schools and of high socioeconomic levels were more obese. This variation could be attributed to dietary variation between urban and rural children.

On the other hand, Koiralaa et al. [22] in their study on prevalence and factors associated with childhood overweight/obesity of private school children in Nepal found that children from families, having ≤ 2 siblings, upper-class family and advantaged ethnic group and children who were of larger birth weight (> 4.0 kg) had a greater likelihood of being overweight/obese.

Talat and El Shahat [23]; in Urban Sharkia Governorate; Egypt; concluded that risk factors for overweight and obesity were high in low level of parent education. The students of illiterate fathers and mothers had the highest incidence of obesity while the students of university fathers and mothers had the lowest incidence of obesity. The relation between the level of father's & mother's education and obesity was found to be significant. However, childhood obesity and overweight in the present study were more prominent in highly educated fathers (college or above).

Our study showed that parental education and occupation had an indirect negative significant effect on child BMI through their significant effect on their parental BMIs except father ´s education. As the highest prevalence of obesity was among children with low educated parents, since they are responsible for food selection for their children as well as their lifestyle activities. This agrees with several studies carried out in the developed countries which explain this association by the belief of low educated parents that overweight children are healthier than normal weight children. Therefore, they prefer high calories food which causes obesity in their children (Güven et al [24] in Turkey; Thibault et al [25] in France). Like our study, most studies carried out in developing countries revealed that the highest prevalence of obesity was among children with high educated parents due to parental style with low energy expenditure [(Matijasevich et al [20] in Brazil; Andegiorgish et al [26] in China).

In addition, there was indirect significant association between obesity and *fathers' occupation*

since the highest prevalence of obesity was among students of unskilled workers fathers while the lowest percentage was among students of professional fathers. This agrees with several Turkish and Australian studies which showed that "fathers' socioeconomic status has an impact on the stable household habits, dietary values and physical activity" [27-28].

Results revealed an insignificant association between obesity and *working status of the mother* where obesity is significantly related to non-working housewife mothers; as obesity occurs due to unhealthy eating habits and sedentary lifestyle rather than working status of the mother. This goes parallel with Güven et al. [24], in Turkey. However, other studies in developing countries found a significant association between obesity and working status of the mother as the child is more likely to be overweight if his mother works more hours per week during childhood that impede young children's access to healthy foods and physical activity [25, 29].

In conclusion, childhood obesity and overweight were more prominent in rural than urban areas, among children with non-working housewives mothers and highly educated fathers (college or above). Parental education and occupation had an indirect significant effect on child BMI through their significant effect on their parental BMIs.

References

1. Caballero B. The global epidemic of obesity: an overview. Epidemiol Rev. 2007;29 (1):1-5. http://dx.doi.org/10.1093/epirev/mxm012 PMid:17569676

2. Gupta N, Goel K, Shah P, Misra A. Childhood obesity in developing countries: epidemiology, determinants and prevention. Endocr Rev. 2012;33:48-70. http://dx.doi.org/10.1210/er.2010-0028 PMid:22240243

3. Eckel RH, Krauss RM. American Heart Association call to action: obesity as a major risk factor for coronary heart disease. AHA Nutrition Committee. Circulation. 1998;97:2099-100. http://dx.doi.org/10.1161/01.CIR.97.21.2099 PMid:9626167

4. Murphy TK, Calle EE, Rodriguez C, Kahn HS, Thun MJ. Body mass index and colon cancer mortality in a large prospective study. Am J Epidemiol. 2000;152:847–54. http://dx.doi.org/10.1093/aje/152.9.847 PMid:11085396

5. Field AE, Coakley EH, Must A, Spadano JL, Laird N, Dietz WH, et al. Impact of overweight on the risk of developing common chronic diseases during a 10-year period. Arch Intern Med. 2001;161:1581–6. http://dx.doi.org/10.1001/archinte.161.13.1581 PMid:11434789

6. World Health Organization. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. Report no. 0512- 3054. Geneva: World Health Organization, 2000.

7. Steinbeck KS. The importance of physical activity in the prevention ofoverweight and obesity in childhood: a review and an opinion. Obes Rev. 2001;2:117–30. http://dx.doi.org/10.1046/j.1467-789x.2001.00033.x PMid:12119663

8. Riddoch CJ, Bo Andersen L, Wedderkopp N, Harro M, Klasson-Heggebo L, Sardinha LB, et al. Physical activity levels and patterns of 9- and 15-yr-old European children. Med Sci Sports Exerc. 2004; 36:86-92. http://dx.doi.org/10.1249/01.MSS.0000106174.43932.92 PMid:14707773

9. Maffeis C. Aetiology of overweight and obesity in children and adolescents. Eur J Pediatr. 2000;159(suppl 1):S35–44. http://dx.doi.org/10.1007/PL00014361 PMid:11011954

10. Martin MA. The intergenerational correlation in weight: how genetic resemblance reveals the social role of families. Am J Sociol. 2008;114 (Suppl. l), S67-S105. http://dx.doi.org/10.1086/592203 PMid:19569401 PMCid:PMC2764266

11. Chaparro MP, Koupil I. The impact of parental educational trajectories on their adult offspring's overweight/obesity status: A study of three generations of Swedish men and women. Social Science & Medicine. 2014;120:199-207. http://dx.doi.org/10.1016/j.socscimed.2014.09.024 PMid:25259658

12. Jackson RT, Rashed M, Saad-Eldin R. Rural urban differences in weight, body image, and dietingbehavior among adolescent Egyptian schoolgirls. Int J Food Sci Nutr. 2003;54: 1-11. http://dx.doi.org/10.1080/0963748031000062047 PMid:12701232

13. DNPCNCD. Diet, 2008. Nutrition and prevention of chronic non communicable diseases in Egyptian adolescents. Final Report, 2008.

14. Hassan NE, El-masry SA, Fouad WA, Sherif L, Elwakkad A, Mona Anwar M, Zaki ST. Prevalence of metabolic syndrome among obese School Students. e-SPEN Journal. 2011;6:248- 252, 2011. http://dx.doi.org/10.1016/j.eclnm.2011.09.005

15. Hiernaux J, Tanner JM. Growth and physical studies. In:Human Biology: A guide to Field Methods. Eds. Weiner JS, Lourie SA, IBP. London, Blackwell Scientific Publications. Oxford UK, 1969:315-340.

16. Ghalli I, Salah N, Hussien F, Erfan M, El- Ruby M, Mazen I, Sabry M, Abd El-Razik M, Saad M, Hossney L, Ismaail S and Abd El-Dayem S et al: Egyptian growth curves 2002 forinfants, children and adolescents. published in: Sartorio A,Buckler JMH and Marazzi N. Crescere nel mondo. Ferringpublisher, 2008.

17. Gwozdz W, Sousa-Poza A, Reisch LA, Bammann K, Eiben G, Kourides Y, Kovács É, Lauria F, Konstabel K, Santaliestra-Pasias AM, Vyncke K, Pigeot I. Peer effects on obesity in a sample of European children. Econ Hum Biol. 2015;18:139-52. http://dx.doi.org/10.1016/j.ehb.2015.05.002 PMid:26115518

18. Hafez AS, El-Awady MY, Hassan NE. Obesity profile among primary school children in Cairo. Egypt J Commun Med. 2000;18 :99-119.

19. El-ShafieAM, Sleem AA. Prevalence of obesity in primary school children living in Dakahlia governorate (Mit-Ghamr State) [Master thesis in Pediatrics]. Menoufia: Faculty of Medicine, Menoufia University, 2013.

20. Matijasevich A, Victora C, Golding J, Barros F, Menezes AM, Araujo CL, et al. Socioeconomic position and overweight among adolescents: data from birth cohort studies in Brazil and UK. BMC Public Health. 2009;9:105. http://dx.doi.org/10.1186/1471-2458-9-105 PMid:19368733 PMCid:PMC2673220

21. Bahbah MH, Slama ESI, Ramadan AEM, Abo Zeed MA. Prevalence of obesity and overweight in primary school children living in Menoufia overnorate, Menouf district. Benha Med J [serial online] 2015 [cited 2016 Apr 1];32:73-7. Available from:http://www.bmfj.eg.net/ text.asp?2015/32/1/73/170572

22. Koiralaa M, Khatrib RB, Khanalc V, Amatyaa A. Prevalence and factors associated with childhood overweight/obesity of private school children in Nepal. Obesity Research & Clinical Practice. 2015;9(3):220-227. http://dx.doi.org/10.1016/j.orcp.2014.10.219 PMid:25434691

23. Talat MA, El Shahat E. Prevalence of overweight and obesity among preparatory school adolescents in Urban Sharkia Governorate, Egypt. Egyptian Pediatric Association Gazette. 2016;64(1):20-5. http://dx.doi.org/10.1016/j.epag.2015.11.004

24. Güven A, Odaci H, Ozgen IT, Bek Y. Effects of individual

factors on adolescent obesity: study in Turkey. Pediatr Int. 2008;50(3):356-62. http://dx.doi.org/10.1111/j.1442-200X.2008.02585.x PMid:18533952

25. Thibault H, Contrand B, Saubusse E, Baine M, Maurice-Tison S. Risk factors for overweight and obesity in French adolescents: Physicalactivity, sedentary behavior and parental characteristics. Nutrition. 2010;26:192-200. http://dx.doi.org/10.1016/j.nut.2009.03.015 PMid:19577429

26. Andegiorgish AK, Wang J, Zhang X, Liu X, Zhu H. Prevalence of overweight, obesity, and associated risk factors among school children and adolescents in Tianjin, China. Eur J Pediatr. 2012;171(4):697-703. http://dx.doi.org/10.1007/s00431-011-1636-x PMid:22160003

27. Ball K, Mishra G. Whose socioeconomic statusinfluences a woman's obesity risk; her mother's, her father's, or her own? Int J Epidemiol. 2006; 35:131-138. http://dx.doi.org/10.1093/ije/dyi216 PMid:16284404

28. EL-bayoumyI, Shady I, Lotfy H. Prevalence of obesity among adolescents (10-14 years) in Kuwait. Asia Pac J Public Health. 2009;21:153-159. http://dx.doi.org/10.1177/1010539509331786 PMid:19190003

29. Hawkins T, Cole C. Law, Millennium Cohort Study Child Health Group. Maternal employment and early childhood overweight: findings from UK Millennium Cohort Study. Int J Obes (Lond). 2008; 32(1):30-38. http://dx.doi.org/10.1038/sj.ijo.0803682 PMid:17637703 PMCid:PMC2679151

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):348-352.
http://dx.doi.org/10.3889/oamjms.2016.092
eISSN: 1857-9655
Basic Science

New Era for Usage of Serum Liver Enzymes as A Promising Horizon for the Prediction of Non-Alcoholic Fatty Liver Disease

Ahmed Abd Allah Salman*, Soheir Abd Elfattah Aboelfadl, Mona Abd Elmenem Heagzy

Cairo University, Faculty of Medicine - Internal Medicine, Cairo, Egypt

Citation: Salman AAA, Aboelfadl SAE, Heagzy MAE. New Era for Usage of Serum Liver Enzymes as A Promising Horizon for the Prediction of Non-Alcoholic Fatty Liver Disease. Open Access Maced J Med Sci. 2016 Sep 15; 4(3):348-352. http://dx.doi.org/10.3889/oamjms.2016.092

Keywords: Liver; enzymes; NASH; prediction; Horizon.

***Correspondence:** Ahmed Abd Allah Salman. Cairo University, Faculty of Medicine - Internal Medicine, Cairo, Egypt. Phone: +201000468664. Fax:+20223646394. E-mail: awea84@hotmail.com

Received: 17-Jun-2016; **Revised:** 36-Jul-2016; **Accepted:** 30-Jul-2016; **Online first:** 01-Sep-2016

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: Liver histology remains the gold standard for assessing non-alcoholic fatty liver disease (NAFLD). Noninvasive serological markers and radiological methods have been developed to evaluate steatosis to avoid biopsy.

AIM: To put cutoff value for liver enzymes that could predict non-alcoholic steatohepatitis (NASH).

PATIENTS AND METHODS: This study was conducted on 54 patients (with NAFLD diagnosed by the US). Patients were subjected to history, physical, anthropometric measurements, investigations including liver enzymes, abdominal US, and liver biopsy. According to biopsy results, patients were subdivided according to NASH development. Also, biopsy results were correlated to the levels of liver enzymes.

RESULTS: Forty-seven patients who were suspected to have NAFLD by sonar were confirmed by biopsy. There was a significant correlation between steatosis degree in biopsy and sonar. Correlation study between steatosis in biopsy and ALT level showed highly significant positive correlation. Correlation study between steatosis in biopsy on one side & AST and GGT on the other side showed significant positive correlation. Cutoff value for detection of NASH using ALT & AST & and GGT were 50.5, 56, 60.5 respectively with sensitivity = 95.5, 90.5, 86.4 % and specificity = 93.8, 100, 87.5%.

CONCLUSION: Cut off values of liver enzymes can be combined with abdominal sonar to predict NASH.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is one of the manifestations of metabolic syndrome, that include hypertension, diabetes, excess body fat around the waist or abnormal cholesterol levels that can predispose to heart disease, diabetes, and stroke [1].

The prevalence of NAFLD is very high in obese and overweight individuals. NAFLD spectrum ranges from simple fatty infiltration to more severe manifestations such as hepatocyte inflammation, hepatic fibrosis, and cirrhosis [2].

Liver biopsy is considered the best method for determining disease stage in NAFLD. A biopsy is not convenient for very large studies and especially for studying hepatic fibrosis progression as it is invasive. Moreover, histological assessment of NAFLD may cause sampling error and can result in misinterpretation of the fibrosis score especially in cases of insufficient specimen [3].

Ultrasound (US) is now the most widely used investigation for screening asymptomatic people with abnormal liver functions and suspected fatty liver [4]. Noninvasive serological markers may be used to assess steatosis and inflammation to avoid the need for biopsy [5]. In patients with NAFLD, serum Gamma-glutamyltransferase (GGT) is often raised and it has been linked to increased death rate [4]. Alanine aminotransferase (ALT) can be used as an indicator for liver injury. It is an important issue to differentiate simple steatosis from steatohepatitis because steatohepatitis has a progressive course and can cause liver failure [6].

The aim of this study was to evaluate liver enzymes and ultrasonic staging of hepatic steatosis as predictors for the severity of NAFLD, and trying to

put cutoff values for liver enzymes that could predict the development of non-alcoholic steatohepatitis (NASH).

Patients and Methods

This study was performed in Kasr El-Ainy hospital, Internal Medicine outpatient clinic over a period of 11 months on 54 obese subjects (BMI "body mass index" < 30 kg/m^2). They came complaining of dyspepsia, 17 patients (31.5 %), osteoarthritis, 15 patients (27.8 %), back pain, 12 patients (22.2 %) and obesity, 10 patients (18.5 %).

The selection of participants in this study was based on the following Inclusion criteria:

1. All participants aged above 18 years.

2. BMI above 30 kg/m^2.

3. All patients showed a picture of bright liver ± hepatomegaly on abdominal ultrasound.

Exclusion criteria:

1. Patients are known to be alcoholic.

2. Patients are known to be positive for hepatitis C or B.

3. Patients are known to be diabetic.

Each patient was subjected to full medical history, full clinical examination, and anthropometric measurements (weight and height of each participant were measured while the participant was clothed only in a light gown, and the BMI was calculated as body weight in kilogram (kg) divided by height squared in meters "m" (Kg/m^2), also waist circumference `was measured midway between rib margin and the iliac crest in a standing position by the same examiner.

Serum biochemistry profile (including serum levels of ALT, AST "aspartate transferase" and GGT) was done for every participant.

Abdominal US was performed by the same operator using a Toshiba Apilo xv scanner equipped with a broadband 5.3 megahertz curved array probe to assess the presence of liver steatosis (bright liver). Liver biopsy was taken for all patients who were suspected to have NAFLD by the abdominal US. Liver biopsy was fixed in ten percent neutral buffered formalin then embedded in paraffin blocks. Five micrometre thick sections were cut and stained with hematoxylin and eosin and examined under light microscope for histopathological diagnosis and scoring using NAS "NAFLD activity score" scoring system according to Histological Scoring System for Nonalcoholic Fatty Liver Disease [7]. This scoring system addresses the full spectrum of lesions of NAFLD and allows a diagnostic categorization into NASH, borderline NASH or not NASH. Fibrosis staging was evaluated (separately from NASH) from 0 to 4 scales [8]. As regarding statistical analysis, we considered borderline NASH and NASH as one group to facilitate comparison between the 2 studied groups (NASH and non-NASH).

Ethics

The study protocol conformed to ethical guidelines of the 1975 declaration of Helsinki, approved by Cairo University Research Ethics Committee (REC) (No.n-7-2015 in 28-5-2015).

A written informed consent was obtained from all patients participating in the study. Data were statistically described in terms of mean ± standard deviation (± SD), median and range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using Kruskal-Wallis test with posthoc multiple 2-group comparisons. For comparing categorical data, Chi-square ($\chi 2$) test was performed. The exact test was used instead when the expected frequency is less than 5. Agreement between ultrasound and biopsy results was tested using kappa statistic. P values less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

Receiver operator characteristic (ROC) curves were derived and area under the curve (AUC) analysis performed to get the best cutoff values for predicting NASH.

Results

The present study included 54 patients, suspected to have NAFLD (clinically & by abdominal ultrasound); and selected from the outpatient clinic. There were 50 females (92.6%) and 4 males (7.4%). The age of the participants ranged from 18- 60 years with a mean ± SD = 43.17 ± 7.371. BMI of the participants ranged from 30.2kg/m ^2 to 42.1 kg/m^2 with a mean ± SD = 34.774 ± 3.7613. Abdominal US showed a high sensitivity in detection of NAFLD as 87% of the patients who were suspected to have NAFLD by sonar were confirmed to have NAFLD by liver biopsy (47 patients out of 54 patients). To facilitate statistical data, we considered fibrofatty and grade 1 steatosis in sonar as one group (group 1), also we considered grade 3 and 4 steatosis as one group (group 3). As to liver biopsy, we considered grade 0 and 1 steatosis as one group

(group 1), then we correlate grading of steatosis by the abdominal US to grading of steatosis by liver biopsy.

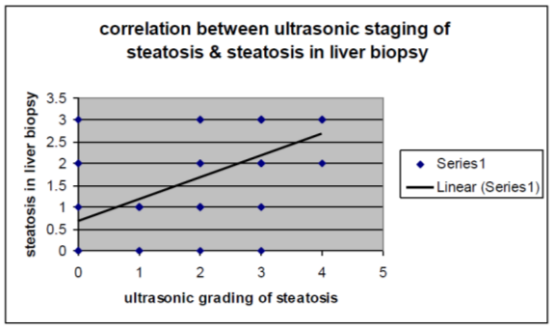

Figure 1: Correlation between ultrasonic staging of steatosis & steatosis in liver biopsy

We found that there was a significant correlation between both parameters. The sensitivity of sonar in detecting steatosis grade compared to biopsy was: 61% in grade 1, 25% in grade 2, and 75% in grade 3 (Figure 1).

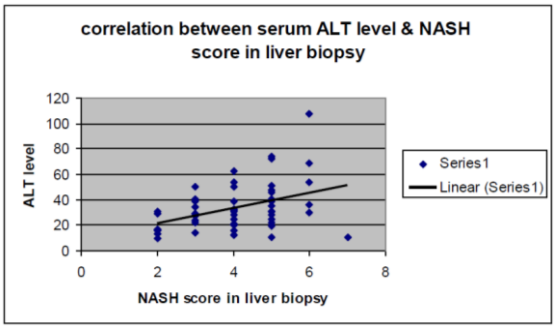

Figure 2: Correlation between serum alanine transferase (ALT) & non-alcoholic steatohepatitis (NASH) score in liver biopsy

Correlation study between steatosis in biopsy & other parameters revealed: 1-Highly significant positive correlation with ALT (Figure 2) (r = 0.8, P = 0.0001); 2- Positive correlation with AST (r = 0.2, P =0.05); 3- Positive correlation with GGT (R = 0.4, P = 0.05).

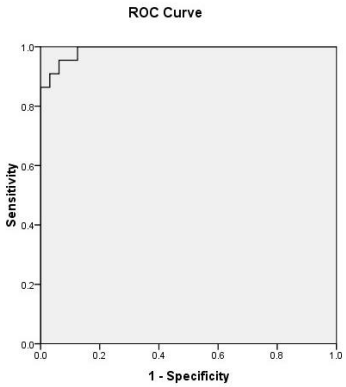

Figure 3: ROC curve to detect a cut off value of serum ALT that could predict the development of NASH

According to the results of the liver biopsy, patients were divided into 2 groups: Group 1included patients who have NASH (NASH and borderline NASH by biopsy). They were 34 patients out of the 54 patients. Group 2 includes patients who do not have NASH. They were 20 patients out of the 54 patients. Comparison between NASH & non-NASH groups as regards laboratory data revealed the significantly higher level of ALT, AST and GGT in NASH group than non- NASH group (P value < 0.001).

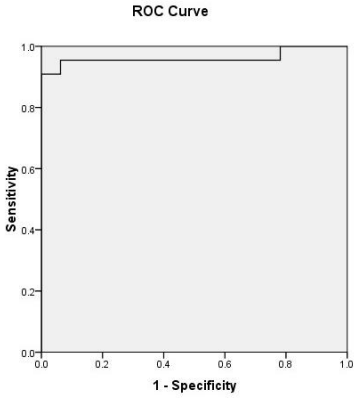

Figure 4: ROC curve to detect a cutoff value of serum aspartate transferase (AST) that could predict the development of NASH

Cutoff value for detection of NASH using ALT= 50.5 IU/L with sensitivity= 95.5% and specificity= 93.8% (Figure 3). Cutoff value for detection of NASH using AST= 56 IU/L with sensitivity= 90.5% and specificity= 100% (Figure 4). Cutoff value for detection of NASH using GGT= 60.5 IU/L with sensitivity= 86.4% and specificity= 87.5% (Figure 5).

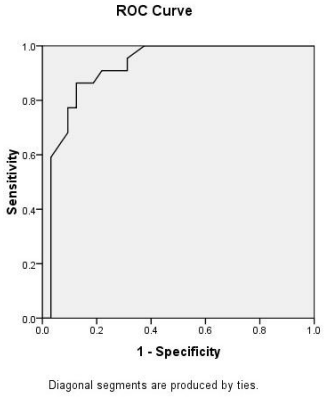

Figure 5: ROC curve to detect a cut off value of serum gamma-glutamyl-transferase (GGT) that could predict the development of NASH

Discussion

NAFLD includes a spectrum of disorders defined essentially by macrovesicular hepatic fat

infiltration occurring in people who do not consume alcohol in amounts considered harmful to the liver [9].

According to the results of the liver biopsy, patients were divided into 2 groups the first one included patients who were proven to have steatohepatitis and the second group included patients who do not have steatohepatitis. Although the present study cannot differentiate between both studied groups as regards sex, we cannot rely on this result because of the unequal distribution of sex in the study.

ALT and AST are important markers of liver injury. Their levels can be raised in many liver diseases. ALT is considered to be more accurate for hepatocyte injury and this is mainly attributed to its presence in high concentrations in hepatocyte cytosol. As regards AST, it has two types, cytosolic and mitochondrial and is found in the hepatocytes, cardiomyocytes, leukocytes and erythrocytes and other tissues [10].

In the present study, it was found that ALT, AST, and GGT were higher in patients with NASH in comparison to NAFLD patients, and the difference was highly significant. This agrees with Shi and colleagues, (2009) [10] who studied patients proven to have NAFLD by biopsy and found that ALT and AST levels of NASH group were higher than those of non-NASH group and found also that ALT is a reliable indicator of the severity of inflammation. Similarly, Fracanzani and colleagues, (2011) (studied patients with NAFLD and found that ALT levels were higher in steatohepatitis [11].

In contrast to this, Wong and colleagues. (2009) found that patients with ALT less than half the upper limit of normal may have the possibility of NASH [12]. Also, Oh and colleagues, (2006) found that raised ALT above normal values did not differentiate between patients who have steatohepatitis and patients who do not have steatohepatitis by liver biopsy [13].

As to AST, Ong and colleagues, (2005) found that AST was a reliable predictor of NASH [14]. The study done by Pulzi and colleagues, (2011) used a cutoff value of 30 IU/L for GGT that has a sensitivity 70% of and a specificity of 88.6% to distinguish NASH from non-NASH groups [15]. Tahan and colleagues, (2008) studied biopsy-proven NAFLD patients and divided them into normal and high GGT groups and found that there were no major differences regarding the degree of steatosis and inflammation [16].

As to ultrasonography, it is the most widely used and less invasive method used for NAFLD diagnosis. It can detect steatosis, which appears as hyperechogenic parenchyma, termed “bright liver”, and “blurring of the vascular margins” [17].

Regarding liver US, which was done to all patients with the same operator prior to biopsy, it was found that the prevalent steatotic grade in NASH group was grade 3 (37.5%), in comparison to the non-NASH group (most of them have grade 1 steatosis (75%). This may reflect that the degree of steatosis increases when patients develop steatohepatitis. The sensitivity of sonar in detecting steatosis compared to liver biopsy is 61% in grade 1, 25% of grade 2 and 75% in grade 3.

The results of the current study agree with the results of Fracanzani et al, (2011) who found that advanced steatosis independently predicts NASH [11]. Liver histology is the best diagnostic method for grading liver steatosis, inflammation and fibrosis, but it is not routinely used in patients with non-progressive fatty liver [18].

In the present study, the correlation between liver ultrasound and liver biopsy was highly significant in detecting the degree of hepatic steatosis and subsequently the development of NASH. Cutoff value for detection of NASH using ALT= 50.5 IU/L with sensitivity = 95.5% and specificity = 93.8%. In contrast to our results, Verma et al., 2013 found that there is no ideal ALT value that can predict NASH and advanced fibrosis [19]. The study was done by Somaye et al., 2011 found that using the cut-off value of 35 IU/L for serum ALT level, did not have a major contribution to stage NAFLD [20].

The present study showed that the cutoff value for detection of NASH using AST = 56 IU/L (international unit per litre) with sensitivity = 90.5% and specificity = 100%, and for GGT = 60.5 IU/L with sensitivity = 86.4% and specificity = 87.5%. In regard to this, Mikako and Hirofumi, 2012 stated that serum GGT value above 96.5 IU/L, predicted the advanced stage of fibrosis with 83% sensitivity and 69% specificity [21].

Although the present study tried to highlight the important role of US in NAFLD diagnosis and also the good correlation between US and biopsy results “if done by an experienced operator”, the quite novel role of this study is to elucidate also the promising role of liver enzymes as surrogate markers for NASH development. If we used these parameter levels, we can predict the possibility of NASH development and so careful follow-up of patients with a diet and lifestyle interventions to decrease the development of complications such as fibrosis, cirrhosis and hepatocellular carcinoma. This approach may avoid the need for a liver biopsy which may be refused by most patients and so we could miss patients who need lifestyle interventions and regular follow-up. The combination of all of the three parameters may suggest a higher possibility of NASH development and so more attention to patient follow-up. The importance of this study lies in the presentation of an alternative practical tool that could help in the diagnosis of NASH without the need for invasive tools such as liver biopsy.

It is worth to mention that there were some

limitations in this study such as a discrepancy of a number of patients in both groups (NASH patients vs. non-NASH patients), and relatively small number of the studied patients (it was not easy to persuade patients to undergo liver biopsy).

In the present study, we concluded that liver enzymes level can be combined with abdominal sonar to predict the presence of NASH as a non-invasive method for diagnosis. Larger studies are needed to verify the level of liver enzymes that could predict accurately NASH development.

References

1. Desai P, Tamarapu Parthasarathy P, Galam L et al. A new role for inflammasomes: sensing the disturbances in non-alcoholic fatty liver disease. Front Physiol. 2013; 4: 156. http://dx.doi.org/10.3389/fphys.2013.00156 PMid:23847540 PMCid:PMC3696836

2. Baranova A, Tran TP, Afendy A et al. Molecular signature of adipose tissue in patients with both Non-Alcoholic Fatty Liver Disease (NAFLD) and Polycystic Ovarian Syndrome (PCOS). J Transl Med. 2013; 11: 133. http://dx.doi.org/10.1186/1479-5876-11-133 PMid:23721173 PMCid:PMC3681627

3. Parkash O, Hamid S. Are we ready for a new epidemic of under recognized liver disease in South Asia especially in Pakistan? Non -alcoholic fatty liver disease. J Pak Med Assoc. 2013; 63(1):95-9. PMid:23865141

4. Obika M, and Noguchi H. Diagnosis and Evaluation of Nonalcoholic Fatty Liver Disease. Experimental Diabetes Research. 2012; 2012:145754. http://dx.doi.org/10.1155/2012/145754 PMid:22110476 PMCid:PMC3205741

5. Fierbinteanu-Braticevici C, Dina I, Petrisor A, et al. Noninvasive investigations for non-alcoholic fatty liver disease and liver fibrosis. World J Gastroenterol. 2010; 16(38):4784–4791. http://dx.doi.org/10.3748/wjg.v16.i38.4784 PMid:20939106 PMCid:PMC2955247

6. Steven G Pearce, Nirav C Thosani, and Jen-Jung Pan. Noninvasive biomarkers for the diagnosis of steatohepatitis and advanced fibrosis in NAFLD. Biomarker Research. 2013; 1:7. http://dx.doi.org/10.1186/2050-7771-1-7 PMid:24252302 PMCid:PMC4177607

7. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histologic scoring system for NAFLD. Hepatology. 2005; 41: 1313-1321. http://dx.doi.org/10.1002/hep.20701 PMid:15915461

8. Brunt EM, Kleiner DE, Wilson LA, et al. Portal chronic inflammation in non-alcoholic fatty liver disease (NAFLD): a histologic marker of advanced NAFLD-Clinicopathologic correlations from the non-alcoholic steatohepatitis clinical research network. Hepatology. 2009; 49: 809-820. http://dx.doi.org/10.1002/hep.22724 PMid:19142989 PMCid:PMC2928479

9. Marchesini G, Moscatiello S, Agostini F, Villanova N, Festi D. Treatment of non- alcoholic fatty liver disease with focus on emerging drugs. Expert Opin Emerg Drugs. 2011; 1: 121–136. http://dx.doi.org/10.1517/14728214.2011.531700 PMid:21352073

10. Shi JP, Xun YH, Hu CB, et al. Clinical and histological features of non-alcoholic fatty liver disease. 2009; 17 (11): 812-816.

11. Fracanzani AL, Valenti L, Bugianesi E, et al. Risk of nonalcoholic steatohepatitis and fibrosis in patients with non-alcoholic fatty liver disease and low visceral adiposity. J Hepatol. 2011; 54(6): 1244-1249. http://dx.doi.org/10.1016/j.jhep.2010.09.037 PMid:21145841

12. Wong VS, Wong GH, Tsang SC, Hui AY, Chan AH, Choi PI, Chim AI, Chu S, Chan FI, Sung JY, Chan HY. Metabolic and histological features of non-alcoholic fatty liver disease patients with different serum alanine aminotransferase levels. Alimentary pharmacology & therapeutics. 2009;29(4):387-96. http://dx.doi.org/10.1111/j.1365-2036.2008.03896.x PMid:19035982

13. Oh SY, Cho YK, Kang MS, et al. The association between increased alanine aminotransferase activity and metabolic factors in nonalcoholic fatty liver disease. Metabolism. 2006; 55(12):1604-1609. http://dx.doi.org/10.1016/j.metabol.2006.07.021 PMid:17142131

14. Ong JP, Elariny H, Collantes R et al. Predictors of non-alcoholic steatohepatitis and advanced fibrosis in morbidly obese patients. Obes Surg. 2005; 15(3): 310-315. http://dx.doi.org/10.1381/0960892053576820 PMid:15826462

15. Pulzi F, Cisternas R, Melo MR, et al. New clinical score to diagnose non-alcoholic steatohepatitis in obese patients. Diabetology & Metabolic Syndrome. 2011; 3: 3. http://dx.doi.org/10.1186/1758-5996-3-3 PMid:21345221 PMCid:PMC3055806

16. Tahan V, Canbakan B, Balci H, et al. Serum gamma glutamyltranspeptidase distinguishes non-alcoholic fatty liver disease at high risk. Hepatogastroenterology. 2008;55(85): 1433-1438. PMid:18795706

17. Webb M, Hanny YH, Zelber-Sagie S, et al. A practical index for ultrasonographic quantification of liver steatosis. The 58th Annual Meeting of the American Association for the Study of Liver Diseases. Boston, MA. Totowa, NJ: The Humana Press, 2007.

18. Angelico F, Del Ben M, Conti R, et al. Non alcoholic fatty liver syndrome: a hepatic consequence of common metabolic disease. J Gastroenterology Hepatol. 2003; 18: 588-594. http://dx.doi.org/10.1046/j.1440-1746.2003.02958.x

19. Verma S, Jensen D, Hart J, Mohanty SR. Predictive value of ALT levels for non-alcoholic steatohepatitis (NASH) and advanced fibrosis in non-alcoholic fatty liver disease (NAFLD). Liver Int. 2013; 33(9): 1398-405. http://dx.doi.org/10.1111/liv.12226 PMid:23763360

20. Somaye K, Seyed M A, Ali Z. Non-alcoholic fatty liver disease and correlation of serum alanine aminotransferase level with histopathologic findings. Hepat Mon. 2011;11 (6):452-458.

21. Mikako O, Hirofumi N. Diagnosis and Evaluation of Nonalcoholic Fatty Liver Disease. Experimental Diabetes Research. 2012;2012:145754.

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):353-358.
http://dx.doi.org/10.3889/oamjms.2016.095
eISSN: 1857-9655
Basic Science

Dietary Behaviour Pattern and Physical Activity in Overweight and Obese Egyptian Mothers: Relationships with Their Children's Body Mass Index

Nayera E. Hassan[1], Saneya Wahba[2], Inas R. El-Alameey[2*], Sahar A. El-Masry[1], Mones M. AbuShady[2], Enas R. Abdel Hameed[2], Tarek S. Ibrahim[2], Samia Boseila[2] (Affiliation ID: 60014618)

[1]Biological Anthropology Department, Medical Division, National Research Centre, Giza, Egypt; [2]Child Health Department, Medical Division, National Research Centre, Giza, Egypt

Citation: Hassan NE, Wahba S, El-Alameey IR, El-Masry SA, AbuShady MM, Abdel Hameed ER, Ibrahim TS, Boseila S. Dietary Behaviour Pattern and Physical Activity in Overweight and Obese Egyptian Mothers: Relationships with Their Children's Body Mass Index. Open Access Maced J Med Sci. 2016 Sep 15; 4(3):353-358. http://dx.doi.org/10.3889/oamjms.2016.095

Keywords: Overweight and Obese; mothers; Body Mass Index; dietary Behaviour; physical Activity.

***Correspondence:** Inas R. El Alameey, PhD. Child Health Department, National Research Centre, Giza, Egypt. E-mail: inasno@hotmail.com

Received: 30-Jul-2016; **Revised:** 12-Aug-2016; **Accepted:** 14-Aug-2016; **Online first:** 01-Sep-2016

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: Obesity and related morbidity increase in Egyptian women and their children. A better understanding of dietary and activity patterns is needed to reduce obesity prevalence.

AIM: The present study aimed to assess dietary patterns and physical activity in Egyptian overweight and obese mothers and to explore its relationships with their children's body mass index (BMI).

SUBJECTS AND METHODS: This descriptive case-control study was conducted at the National Research Center. The study included a sample of 64 overweight and obese mothers and 75 children, compared with apparently healthy non-obese mothers and their children of matched age and social class. Tested questionnaires were used to collect information of the studied subjects.

RESULTS: A statistically significantly higher incidence of unemployment, large family size was observed in overweight & obese women compared to controls ($P < 0.05$). Those women who consumed vegetables more than 3 times a week were less likely to be overweight or obese ($P < 0.05$). No significant association were detected between mothers' physical activity, dietary behaviour variables and children's BMI except for consuming beverages with added sugar (95%CI = 0.074-0.985, $P<0.05$).

CONCLUSION: Improper dietary patterns, nonworking mothers and big family size are associated with obesity among Egyptian women. Emphasis should be given to increasing physical activity and encourage healthier diets among Egyptian mothers and their children.

Introduction

Obesity has become an epidemic worldwide problem affecting both developed and developing countries [1]. The prevalence of overweight and obesity in the Arab countries has reached an alarming level ranging between 50% and 70% for women in the 21st century [2] which necessitates urgent action. In Egypt, the prevalence of obesity has increased markedly with the changing food habits and the increasingly sedentary lifestyles, with nearly 70 percent of the Egyptian adult population being obese [3]. According to the most recent World Health Organization statistics, Egypt occupies the 14th place in obesity worldwide [4]. Childhood obesity is currently around one in every ten is obese. According to CDC, the percentage of childhood obesity in the 1980's jumped from 7 percent to 17 percent (12.5 million) in 2010 [5].

Several risk factors have been cited as potential causes for obesity in mothers' and young children, including socioeconomic status, lack of physical activity [6], changes in eating habits such as skipping breakfast [7], consumption of fast foods rich in fat, sugar and salt, with a decrease in the intake of whole grains, legumes, fruits and vegetables or excessive eating [8, 9]. Obesity has led to an increase

in the prevalence of chronic non-communicable diseases as type 2 Diabetes, and cardiovascular disease [10]. All of these factors are being the main causes of morbidity, mortality, and disability in Arab countries, and causing more than 50% of total deaths [11]. Hence, our study aim was to identify the relationships of specific behaviors (dietary patterns and physical activity) on body mass index (BMI) of a sample of Egyptian overweight and obese mothers compared to controls and to study the effect of these behaviors, maternal BMI, socioeconomic factors, family structure on their children's BMI.

Subjects and Methods

Design and Sample

This descriptive case-control study was conducted at the National Research Centre (NRC), Giza, during the period from April 2014 to March 2015. The study included 64 overweight & obese mothers and 75 children compared with apparently healthy non-obese mothers and their children of matched age and social class. The mother's age was ranging from 24-57 years (mean age, 39.38 ± 8.51 years). All studied women provided signed written consent form of the Medical Ethical Committee of NRC to participate in assessments. A thorough history and general examination of mothers and their children were done to exclude forms of obesity other than exogenous dietetic obesity.

Methods

Data Collection

Data were collected through the pretested questionnaire after modification of the same items to fit the Egyptian setting. Data collectors were conducted and supervised by a senior dietitian. Dietary patterns and physical activity were estimated as times per week according to Giovannucci et al, [12].

Anthropometric measurements

Anthropometric measurements including weight and height were assessed for all the studied subjects at the National Research Centre. Body weight was measured to the nearest 0.1kg by a standard clinical balance. Standing body height was measured, to the nearest 0.1 cm by using Holtain Stadiometer. The scales were recalibrated after each measurement following the recommendations of the International Biological Program [13]. Body mass index (BMI) was calculated by dividing weight by height squared (kg/m^2), then we applied the cutoff points recommended by World Health Organization (WHO) reference as follows: Overweight (BMI = 25.00 - 29.99), obesity (BMI = ≥ 30.00) while those with BMI > 18-24.9 Kg/m^2 were considered normal [14].

Data statistical analyses

Statistical analyses were performed using the SPSS statistical package software for Windows version 21 (SSPS Inc, Chicago, USA). Results were expressed as the mean ± SD, frequencies and percentages. Multiple logistic regression analysis was done to detect predictors of obesity in children. Odds ratios and 95% confidence intervals were calculated for the association between different variables and obesity in mothers and children. A p-value < 0.05 was considered significant and p < 0.005 was considered highly significant.

Results

The study included 64 overweight and obese women (BMI > 25), with age ranging from 24-57 years (mean age, 39.38 ± 8.51 years), and their mean BMI was 35.18 ± 5.40. The age of 75 children ranged from 6-19 years and their mean BMI was 17.62 ± 8.77. Table 1 shows age and anthropometric measures of the all studied sample.

Table 1: Age and anthropometric measures of overweight and obese mothers and their children

Variables		Age (yrs)	Weight (kgs)	Height (cm)	BMI
Mothers	Mean ± SD	39.38 ± 8.51	88.14 ± 15.78	158.03 ± 8.06	35.18 ± 5.40
	Range	(24-57)	(55-159)	(148-195)	(25.2-50.4)
Children	Mean ± SD	10.46 ± 4.17	36.70 ± 28.61	136.67 ± 19.24	17.62 ± 8.77
	Range	(6-19)	(12.2-95.4)	(121-169)	(8.2-33.40)

Regarding the education levels of the participants, almost 83.3% have low educational levels; (20%) were illiterate, (63.3%) were up to secondary educated, while (16.7%) were university educated. About 73.3% of overweight & obese women in the studied sample are not working, while 26.7% are working. More than half of the overweight & obese sample (58.8%) had a large family size between 4 and 10 family members (adults and children) in the home. As regards unemployment, large family size, there was a statistically significantly higher incidence among overweight & obese women compared to controls (P < 0.05), while no significant relationship with their levels of education (p > 0.05), as shown in Table 2.

Consumption of fast fried food*:* The frequency of consuming of fast fried food prepared outside the home was a common practice in 53.9 % of

the studied women, at least 1-3 times per week by (40.1%) versus 4-7 times per week by (13.8%), and 44.6% did not consume any fast fried food.

Table 2: Comparison of socio-demographic characteristics of overweight and obese women and control groups

Variables		Control group	Overweight & Obese group	P- value
Women occupation	- Non working	14.3%	73.3%	0.002**
	- Worker, employee	85.7%	26.7%	
Women education levels	- Illiterate	0%	20.0%	0.387
	- Up to secondary	85.7%	63.3%	
	- University educated	14.4%	16.7%	
Family size	4 or less members	83.3%	41.2%	0.048*
	>4 members	16.7%	58.8%	

*Significant difference at *P* < 0.05; **highly significant difference at P < 0.005.

Sugary Beverages: The intake of sugar-sweetened beverages in the form of canned fruit juice, carbonated beverages, and hot drinks (tea, coffee, and herbal tea) with added sugar was high among the studied women (95.9 %).

Vegetables and fruits were consumed at least 1-3 times weekly by 64.6% and 44.6% of the sample and 4-7 times weekly by 32.3%% and 50.9%of sample respectively.

The breakfast meal was skipped by 16.9% of the studied women, while 77 % used to have breakfast; the majority of them (58.5 %) had breakfast 4-7 times weekly and the remaining 18.5 % had their breakfast at least, 1-3 times per week.

Table 3: Dietary patterns and physical activity per week in the studied overweight and obese women

Variables	Dietary patterns and physical activity per week		
	0- time	1-3 times	4-7 times
1-Fast food	44.6%	40.1%	13.8%
2-Beverage with sugar	3.2%	42.9%	53.0%
3-Vegetables	0%	64.6%	32.3%
4-Fruits	0%	44.6%	50.9%
5-Breakfast meal	16.9%	18.5%	58.5%
6-Physical activity	76.9%	9.2%	9.2%

Concerning physical activity: Most (76.9%) of mothers stated that, they did not practice any sort of physical activity. Walking was the main physical activity performed where 9.2% used to walk 1- 3 weekly and the same percentage 9.2% walked 4-7 times/ week (Table 3).

Table 4: Comparison between cases and controls as regard to practising physical activity and some diet consumption

Variables	Behavior during last week	Control group	Overweight & Obese group	P- value
Physical activity / week	No	85.7%	81.4%	0.77
	Yes	14.3%	18.6%	
Dietary patterns				
- Fast food / week	No	57.1%	48.3%	0.659
	Yes	42.9%	51.7%	
- Beverage with added sugar / week	No	0%	3.4%	0.62
	Yes	100%	96.6%	
- Breakfast / week	No	14.3%	17.2%	0.844
	Yes	85.7%	82.8%	

*Significant difference at *P* < 0.05.

Comparing the weekly dietary behaviour between the studied samples revealed a higher percentage of fast food consumption in overweight & obese mothers (15.7% vs. 42.9%) respectively with no significant differences). Having breakfast and consumption of sugar-sweetened beverages was a common practice in the overweight & obese group, with no significant difference when compared to the control group (P > 0.05). There was no statistical difference between overweight & obese mothers as regards physical activity, both groups being mostly inactive (Table 4).

Table 5 showed that women who consumed vegetables more than 3 times per week were less likely to be overweight or obese (OR = 0.176, 95% CI 0.031-0.991, P < 0.05).

Table 5: Association between frequency of vegetables and fruits consumption and mother's overweight and obesity women

Variables		Non-obese women	Overweight & Obese women	Pearson Chi-Square	Odds ratio	95% CI	P value
Vegetables consumption per week	1-3 times	28.6%	69.5%	4.61	00.176	0.031-0.991	0.032*
	4-7 times	71.4%	30.5%				
Fruits Consumption per week	1-3 times	57.1%	49.2%	0.160	10.39	0.284-6.707	0.690
	4-7 times	42.9%	50.8%				

*Significant difference at P < 0.05.

The regression analysis showed a statistically significant positive association between children's BMI and consumption of beverages with added sugar by their mothers (95% CI 0.074-0.985, P < 0.05). The other variables as maternal BMI, education, occupation, family size, room number, physical activity, and other dietary behaviour variables were not significant predictors of children's BMI as shown in Table 6.

Table 6: Association between children BMI category and mother's BMI, socio-demographic characteristics, dietary behaviour, and physical activity (Multiple regression analysis)

Variables	B	Odds ratio	95% CI	P- value
Maternal education	-0.209	0.811	0.219-3.0	0.755
Maternal occupation	-0.076	0.927	0.125-6.8	0.941
Family size	0.1	1.1	0.256-4.7	0.893
Number of rooms	-0.105	0.9	0.219-3.6	0.884
Maternal BMI	-0.804	0.448	0.37-5.3	0.526
Beverages/ week	1.331	1.259	0.074-0.985	0.047*
Vegetables / week	-0.135	0.873	0.602-1.267	0.476
Fruits/ week	-0.214	0.807	0.514-1.267	0.352
Breakfast / week	0.075	1.078	0.77-1.51	0.660
Physical activity	-0.439	0.645	0.25-1.60	0.346

*Significant difference at P < 0.05.

Discussion

Obesity is one of the most prevalent nutrition disorders with increasing trend among people of all ages all over the world creating a health and economic burden on their government services [1]. Most of the Arab countries had a major change in their lifestyle pattern including food consumption and nutrient intake of high-caloric during last decades [15]. A sedentary lifestyle has lead to the significant

prevalence of obesity among Egyptian populations [16]. Hence, the aim of this study was to identify the relationship of specific behaviors (dietary patterns and physical activity) that are known to affect energy intake with the BMI among a sample of overweight and obese Egyptian mothers and to explore the possible association between maternal BMI, socioeconomic factors, family structure and the BMI of their children.

Our study included 64 overweight and obese women, with an age ranging from 24-57 years (mean age, 39.38 ± 8.51 years) with a mean BMI of 33.98 ± 6.28. Seventy-five children with an age ranged from 6-19 years and their mean BMI was 17.62 ± 8.77.

Education plays a role in obesity prevalence, where illiteracy was associated with obesity in the Gulf countries [17]. In the present study, economic status was measured by the education level, occupation, and family size of overweight and obese women compared to that of the controls. A large family size and low occupation percentages were common among overweight and obese women. About 73.3% of overweight and obese women were not working while 85.7% of the control group were employed ($p = 0.002$). As regards education of the overweight and obese women, (20%) were illiterate, (63.3%) were educated up to secondary school level, while only (16.7%) had a university degree, the difference with the control group was statistically not significant although none of the mothers of the control group was illiterate. Our findings are in agreement with studies from Gulf countries, and Colorado that has shown a strong relationship between obesity prevalence and low educational level, unemployment, poverty, and low-income [17, 18].

Maternal behaviours influence their children's behaviours directly and the shared home environment also provides access to foods among them, such as eating fast food meals together [19]. In our study, more than 50% (53.9%) of overweight and obese women consumed fast food 1-7 times per week. Fast foods are considered a high source of total energy, total fat, saturated fat, and cholesterol. Higher frequency of fast-food consumption with added sugars has been reported in persons of lower socioeconomic status among obese women in African Americans, and Arab countries [20-22].

Consuming a diet high in fruits and vegetables is associated with lower risks of numerous chronic diseases. Fruits and vegetables in their natural state have high water and fibre content and thus are low in calories and energy density which may help people feel full with a greater quantity of food at a meal while consuming fewer calories [23].

Vegetables were consumed almost on a daily basis by 71.4% of the non-obese mothers with a significantly higher percentage than the rate of consumption of the obese and overweight mothers ($p < 0.005$). In our study, vegetable consumption was the only strong protector associated with controlling body mass index against obesity with a significance of less than 0.05. No significant difference in the frequency of fruit consumption was detected between the 2 groups ($P > 0.05$). Our results were similar to some other studies in Australia and Spain that have reported low consumption of vegetables in overweight and obese women [23, 24]. Data from WHO (Regional Office in Cairo) indicate that 79% to 96% of adults in 6 Arab countries (Egypt, Jordan, Iraq, Kuwait, Saudi Arabia, and Syria) eat less fruit and vegetables per day [11].

Skipping breakfast may be related to obesity, as individuals who do not eat early in the morning, may feel hungry later on and then may consume a greater number of calories during the evening hours than individuals who eat consistently throughout the day [25, 26]. In our study, 77 % of the overweight and obese women had their breakfast meal and it was skipped by only 16.9%, but most of the Egyptian housewives tend to have late breakfast. The majority of the participants had irregular meals with two main meals per day.

Regular consumption of sugar-sweetened beverages, particularly carbonated soft drinks, contributes to the epidemic of overweight and obesity [27, 28]. In our study, there was no significant difference between overweight & obese and non-obese women concerning the intake of sugar-sweetened beverages in the form of canned fruit juice, carbonated beverages, and tea with added sugar. This is not in agreement with Rasheed et al., [29] who found that regular drinking of soft drinks was significantly more common among Saudi obese women than non-obese women. On the other hand, consumption of beverages with added sugar by the mothers in our study was the only significant predictor for children's BMI, most probably due to easy access to these beverages kept in the house.

Lack of physical activity in adults of 7 Arab countries (Egypt, Iraq, Jordan, Kuwait, Saudi Arabia, Sudan, and Syria) ranged from 33% to 86% according to WHO statistics [11]. The increase in the number of hours spent watching television and dependence on modern means of transport and the decreasing physical activity leads to the sedentary lifestyle in Arab societies [30].

Analysis of physical activity pattern in our study has shown that more than 3/4 of the mothers (76.9%) had a sedentary life pattern. Walking and housework were almost the only activities that the participants were engaged in. Walking was practised by 9.2% of the participants for 1-3 times/ week while the same percentage (9.2%) walked 4-7 times weekly.

The problem of childhood obesity is not only what the children eat, but also their consumption patterns which mean where, when, and how much (amount) of food does a child eat [31, 32]. Our results

showed a statistically positive association between children's BMI and consumption of beverages with added sugar by the mothers (95%CI 0.074-0.985, P < 0.05), other variables as physical activity, dietary behaviour were not significant predictors of children's BMI. Since parents are influential in the development of health behaviours of their children; efforts to reduce obesity should take into consideration the child's behaviours and weight status within the family [33].

In conclusion, nonworking mothers, large family size, and higher frequency of fast-food consumption with added sugars are associated with obesity among the studied Egyptian mothers. We recommend the encouragement of consuming healthy diets and the promotion of physical activity among Egyptian mothers, and their children.

Limitations: The present study had several notable limitations including the small sample size, in addition to the wide age range of children which made it difficult for more statistics.

References

1. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of obesity among adults: United States, 2011–2012. NCHS Data Brief No. 131. Hyattsville (MD): National Center for Health Statistics, 2013.

2. Sweileh WM, Zyoud SH, Al-Jabi SW, Sawalha AF. Quantity and quality of obesity-related research in Arab countries: assessment and comparative analysis. Health Res Policy Syst. 2014; 12(33): 1-10. http://dx.doi.org/10.1186/1478-4505-12-33

3. Shaheen F, Hathout M, Tawfik A. Prevalence of obesity in Egypt. National survey, final report. Cairo National Nutrition Institute, 2004.

4. WHO: Action plan for the global strategy for the prevention and control of non-communicable diseases, 2008–2013. Geneva, World Health Organization (WHO), 2013.

5. Centers for Disease Control and Prevention. Overweight and obesity: Health consequences. Retrieved from http://www.cdc.gov/obesity/causes/health.html,2011.

6. Musaiger AO, Al Hazzaa HM, Al-Qahtani A, Elati J, Ramadan J, AboulElla NA, Mokhtar N, Kilani HA. Strategy to combat obesity and to promote physical activity in Arab countries. Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy. 2011:489–97.

7. Kant AK, Andon M B, Angelopoulos TJ, Rippe J M. Association of breakfast energy density with diet quality and body mass index in American adults: National Health and Nutrition Examination Surveys, 1999–2004. Am J Clin Nutr. 88 (5):1396-404. PMid:18996877

8. Bleich S, Cutler D, Murray C, Adams A. Why is the developed world obese? Annu Rev Public Health. 2008; 29:273–295. http://dx.doi.org/10.1146/annurev.publhealth.29.020907.090954 PMid:18173389

9. Paradis AM, Godin G, Perusse L, Vohl MC. Associations between dietary patterns and obesity phenotypes. Int J Obes (Lond). 33(12):1419-26. http://dx.doi.org/10.1038/ijo.2009.179 PMid:19736556

10. Nguyen NT, Magno CP, Lane KT, Hinojosa MW, Lane JS. Association of Hypertension, Diabetes, Dyslipidemia, and Metabolic Syndrome with Obesity: Findings from the National Health and Nutrition Examination Survey, 1999 to 2004. J Am Coll Surg. 2008; 207(6):928-934. http://dx.doi.org/10.1016/j.jamcollsurg.2008.08.022 PMid:19183541

11. WHO/EMRO. Regional data on non-communicable diseases risk factors. World Health organization, Regional Office of East Mediterranean. Non-communicable diseases. Website: http//www.emr.who.int.ncd. Accessed April 10, 2010.

12. Giovannucci E, Colditz G, Stampfer MJ, Rimm EB, Litin L, Sampson L, Willett WC. The assessment of alcohol consumption by a simple self-administered questionnaire. Am J Epidemiol. 1992;133(8):810-7.

13. Tanner JM, Hiernaux J, Jarman S. Growth and physique studies, in Human Biology: A Guide to Field Methods, Weiner JS and Lourie JA, Eds., pp. 315–340, IBP. London Blackwell Publications, Oxford, UK, 1969.

14. WHO, Anthro Plus for personal computers. Manual Software for assessing growth of the world's children and adolescents. Geneva, 2009. (http://www.who.int/growthref/tools/en).

15. Al-Hazza H, Musaiger AO, ATLS Research Group. Physical activity patterns and eating habits of adolescents living in major Arab Cities: The Arab Teens Lifestyle Study. Saudi Med J. 2010; 31(20):210-211.

16. Hassan NE, El-Masry SA, Elshebini SM, Al-Tohamy M, Ahmed NH, Abdel Rasheed E, El-Saeed GS, Hassan NM, Zikri EN, El Hussieny MS. Comparison of Three Protocols: Dietary Therapy and Physical Activity, Acupuncture, or Laser Acupuncture in Management of Obese Females. OA Maced J Med Sci. 2014; 2(2):191-197. http://dx.doi.org/10.3889/oamjms.2014.030

17. Alnohair S. Obesity in Gulf Countries. International Journal of Health Sciences, Qassim University. 2014; 8 (1): 79–83. http://dx.doi.org/10.12816/0006074

18. Akil L, Ahmad AH. Effects of Socioeconomic Factors on Obesity Rates in Four Southern States and Colorado. Ethn Dis. 2011; 21(1): 58–62. PMid:21462731 PMCid:PMC3101796

19. Birch LL, Davison KK. Family environmental factors influencing the developing behavioral controls of food intake and childhood overweight. Pediatr Clin North Am. 2001; 48:893–907. http://dx.doi.org/10.1016/S0031-3955(05)70347-3

20. Larson NI, Story MT, Nelson MC. Neighborhood environments: disparities in access to healthy foods in the U. S. Am J Prev Med. 2009; 36(1):74-81 http://dx.doi.org/10.1016/j.amepre.2008.09.025 PMid:18977112

21. Pereira MA, Kartashov AI, Ebbeling CB, Van Horn L, Slattery ML, Jacobs DR, Ludwig DS. Fast-food habits, weight gain, and insulin resistance (the CARDIA study): 15-year prospective analysis. Lancet. 2005; 365(9453):36-42. http://dx.doi.org/10.1016/S0140-6736(04)17663-0

22. Greenwood JL, Stanford JB. Preventing or improving obesity by addressing specific eating patterns. J Am Board Fam Med. 2008;21(2):135-40. http://dx.doi.org/10.3122/jabfm.2008.02.070034 PMid:18343861

23. Charlton K, Kowal P, Soriano MM, Williams S, Banks E, Byles J. Fruit and Vegetable Intake and Body Mass Index in a Large Sample of Middle-Aged Australian Women. Nutrients. 2014; 6(6):2305-19. http://dx.doi.org/10.3390/nu6062305 PMid:24941447 PMCid:PMC4073152

24. Vioque J, Weinbrenner T, Castelló A, Asensio L, Garcia de la Hera M. Intake of Fruits and Vegetables in Relation to 10-year Weight Gain Among Spanish Adults. Obesity (Silver Spring). 2008; 16(3):664-70. http://dx.doi.org/10.1038/oby.2007.121 PMid:18239583

25. Ma Y, Bertone ER, Stanek EJ, Reed GW, Hebert JR, Cohen NL, Merriam PA, Ockene IS. Association between eating patterns and obesity in a free-living US adult population. Am J Epidemiol. 2003;158(1):85-92. http://dx.doi.org/10.1093/aje/kwg117 PMid:12835290

26. Schulze MB, Teresa TF, Manson JE, Willett WC, Hu FB. Dietary Patterns and Changes in Body Weight in Women. Obesity. 2006; 14 (8) :1444–1453. http://dx.doi.org/10.1038/oby.2006.164 PMid:16988088

27. Hu FB. Sugar-sweetened soft drinks consumption and risk of type 2 diabetes and cardiovascular risk. CMR Journal. 2009; 2 (2): 15–18.

28. Bray GA. Soft drinks and obesity: the evidence. CMR Journal. 2009; 2 (2) : 10–14.

29. Rasheed LJ, Greenwood JL, Stanford JB. Preventing or improving obesity by addressing specific eating patterns. J Am Board Fam Med. 2008; 21(2):135-40. http://dx.doi.org/10.3122/jabfm.2008.02.070034 PMid:18343861

30. Musaiger AO, Al-Ahdal E. Social and dietary factors associated with obesity among women in Saudi Arabia. Obesity in the Arab World, Ed., Arab Center for Nutrition, Bahrain, 2010. PMid:20174744

31. Spear B, Barlow S, Ervin C, Ludwig D, Saelens B & Schetzina K. Recommendations for treatment of child and adolescent overweight and obesity. Pediatrics. 2007; 120 (4) 254-288. http://dx.doi.org/10.1542/peds.2007-2329F PMid:18055654

32. Hediger M, Overpeck M, Kuczmarski R, Ruan J. Association between infant breastfeeding and overweight in young children. The Journal of the American Medical Association. 2001; 285(19):2453-60. http://dx.doi.org/10.1001/jama.285.19.2453 PMid:11368697

33. Sonneville KR, Rifas-Shiman SL, Kleinman KP, Gortmaker SL, Gillman MW, Taveras EM. Associations of obesogenic behaviors in mothers and obese children participating in a randomized trial. Obesity (Silver Spring). 2012; 20(7):1449-54. http://dx.doi.org/10.1038/oby.2012.43 PMid:22349735 PMCid:PMC3835375

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):359-363.
http://dx.doi.org/10.3889/oamjms.2016.099
eISSN: 1857-9655
Basic Science

Serum IL-10, MMP-7, MMP-9 Levels in *Helicobacter pylori* Infection and Correlation with Degree of Gastritis

Gontar Siregar[1*], Sahat Halim[2], Ricky Sitepu[2]

[1]University of Sumatera Utara, Gastroentero-Hepatology, Komp. Tasbi YY No 203, Medan, Sumatera Utara 20122, Indonesia; [2]University of Sumatera Utara, Internal Medicine, Medan, Sumatera Utara, Indonesia

Citation: Siregar G, Halim S, Sitepu R. Serum IL-10, MMP-7, MMP-9 Levels in Helicobacter pylori Infection and Correlation with Degree of Gastritis. Open Access Maced J Med Sci. 2016 Sep 15; 4(3):359-363. http://dx.doi.org/10.3889/oamjms.2016.099

Keywords: *Helicobacter pylori*; gastritis; IL-10; MMP-7; MMP-9.

***Correspondence:** Prof. Gontar Alamsyah Siregar. University of Sumatera Utara, Gastroentero-Hepatology, Komp. Tasbi YY No 203, Medan, Sumatera Utara 20122, Indonesia. E-mail: gontarsir@gmail.com

Received: 19-Jul-2016; **Revised:** 22-Aug-2016; **Accepted:** 24-Aug-2016; **Online first:** 05-Sep-2016

Competing Interests: The authors have declared that no competing interests exist.

Abstract

AIM: *Helicobacter pylori* causes gastric mucosal inflammation and immune reaction. However, the increase of IL-10, MMP-7, and MMP-7 levels in the serum is still controversial. The objective of this study was to investigate the serum levels of IL-10, MMP-7 & MMP-9 in gastritis patients with *H. pylori* infection.

MATERIALS AND METHODS: A cross-sectional study was done on seventy gastritis patients that consecutive admitted to endoscopy units. The diagnosis of gastritis was made based on histopathology and diagnosis of *H. pylori* infection was based on rapid urease test. Serum samples were obtained to determine to circulate IL-10, MMP-7, and MMP-9 level. Univariate and bivariate analysis were done by SPSS version 22.

RESULTS: Forthy percentages of the patients were infected with *H. pylori*. The IL-10 level was significantly higher in *H. pylori*-infected patients compared to non-infected patients. However, there were no differences between serum levels of MMP-7 and MMP-9 in infected and non-infected *H. pylori* patients.

CONCLUSIONS: The immune response to *H. pylori* promotes systemic inflammation, which was reflected by the increased levels of serum IL-10. However, there were no significant differences in MMP-7 and MMP-9 serum levels between positive and negative infected *H. pylori* patients.

Introduction

Helicobacter pylori are bacteria that specifically infect the epithelial cells of the human gastric. *Helicobacter pylori* are found in the gastric of more than 50% of humans and frequently is the major cause of gastritis throughout the world [1].

The increase in cytokine production caused by *Helicobacter pylori* has been studied before, including the anti-inflammatory cytokine. This cytokine is responsible for the decrease in immune response. IL-10 is an anti-inflammatory cytokine that is capable of decreasing the inflammatory responses caused by *H. pylori*, which will cause the rise in bacterial numbers and the effects mediated by the epithelial cells of the gaster [2]. Patients with chronic gastritis caused by *H. pylori* infection often develops peptic ulcers, as well as gastric carcinoma and lymphoma [3].

Matrix metalloproteinases (MMPs) are believed to have an essential role in the inflammation process and carcinogenesis, by causing degradation and remodelling of the extracellular matrix and basal membrane. MMP are secreted through transmembrane endoproteinases. MMP has a catalytic zinc domain, which is required for proteolytic activity. MMP are able to degrade at least one extracellular matrix component. MMP-7 and MMP-9 are members of the MMP family, which increase in *H. pylori* gastritis and in early gastric carcinoma [4].

There have been many studies on the immune response based on gastric mucosal cytokine and MMP expression in *H. pylori* infection, however, the data on levels of anti-inflammatory cytokines and

MMP in the serum.

In this study, the objective is to evaluate the increase of IL-10, MMP-7 and MMP-9 in patients with *H. pylori* infection without any systemic disease and the correlation with the histopathologic degree of gastritis.

Patients and Methods

Patient selection

A cross-sectional study was done on seventy consecutive gastritis patients that were admitted to endoscopy units at Adam Malik General Hospital and Permata Bunda Hospital, Medan, Indonesia from May-October 2014. All patients gave informed consent and the study was approved by the local ethical committee. None of the patients had received antibiotics, bismuth compounds, H2 antagonists, proton pump inhibitors or immune modulating drugs within the last four weeks before endoscopy. Patients with evidence of malignancy, immunosuppression, metabolic disorders, or gastrointestinal haemorrhage and patients who had a history of gastric surgery were excluded [2, 5-7].

Diagnosis of gastritis

Gastritis degree was evaluated from a biopsy of the mucosa of gastric antrum and body. The biopsy specimens were fixed in 10% formalin and embedded in paraffin. The samples were stained using Hematoxylin-Eosin and were evaluated by the pathologist of anatomic pathology referring to the visual analogue scale of the updated Sydney System. The higher degree was used if differences of degree were found between the body and antrum. The degree of chronic inflammation, neutrophil infiltration, atrophy, and intestinal metaplasia were scored 0 to 3, i.e., normal (0), mild (1), moderate (2), and severe (3) [5].

Diagnosis of Helicobacter pylori

H. pylori were considered positive based on the positive results of the rapid urease test. In this study, we used Pronto Dry®. We used gastric antral biopsy specimen that was taken within 2 cm from the pylorus for Pronto Dry®. The results of the rapid urease test were read within 24 hours. The Pronto Dry® was considered to be positive if the colour changed from amber to pink-red [6].

Serum levels of IL-10, MMP-7, and MMP-9

Venous blood was drawn using a serum separator tube and allowed to clot for 30-45 minutes at room temperature before centrifugation for 15 minutes at approximately 1,000g. Serum was immediately stored frozen in aliquots at -20°C until assays for IL-10, MMP-7, and MMP-9 were performed. IL-10 was assayed by the high sensitive EBioscience technique, Bender MedSystems GmbH 1030 Vienna, Austria. Circulating MMP-7 and MMP-9 levels were examined in serum using the Quantikine Human MMP-7-ELISA and Quantikine Human MMP-9-ELISA (Quantikine, R&D System, Inc., Minneapolis). Serum levels were expressed as pg/ml. Levels above the mean were categorised as the high level, and levels below the mean were categorised as the low level.

Statistical analysis

SPSS version 22 (SPSS Inc., Chicago) was used for analysis. The data were analysed using univariate and bivariate analysis with 95% confidence intervals. The results were expressed as the mean $\pm$ standard deviation. Bivariate analysis was carried out using the independent t-test, Mann-Whitney test, and Chi-square test with a p-value < 0.05 was considered statistically significant.

Results

Demographics of Respondents

There were 70 subjects, consisting of 35 males (50%) and 35 females (50%) subjects. The average age of the subjects was 49.9 $\pm$ 13.04 (SD) years. Most subjects were from the age group of 46-60 years old. 40% patients were infected with *H. pylori*. The occupation of most of the respondents was housewives (38.57%). The majority of subjects had an overweight or underweight nutritional status (58.57%) (Table 1).

Table 1: Characteristics of the subjects

Variables	*H. pylori* positive	*H. pylori* negative	Frequency
Sex			
Males	17 (24.28%)	18 (25.71%)	35 (50%)
Females	11 (15.71%)	24 (34.29%)	35 (50%)
Age (years)			
<30	4 (5.71%)	3 (4.29%)	7 (10%)
30-45	9 (12.86%)	12 (17.14%)	21 (30%)
46-60	9 (12.86%)	18 (25.71%)	27 (38.57%)
>60	6 (8.57%)	9 (12.86%)	15 (21.43%)
Job			
Self employed	7 (10%)	15 (21.43%)	22 (31.43%)
Employee	4 (5.71%)	5 (7.14%)	9 (12.85%)
Farmer	3 (4.29%)	2 (2.86%)	5 (7.15%)
Housewife	9 (12.86%)	18 (25.71%)	27 (38.57%)
Other	5 (7.14%)	2 (2.86%)	7 (10%)
Nutritional status			
Normal	9 (12.86%)	20 (28.57%)	29 (41.43%)
Underweight and Overweight	19 (27.14%)	22 (31.43%)	41 (58.57%)
Total	28 (40%)	42 (60%)	70 (100%)

Serum IL-10, MMP-7 and MMP-9 levels in Helicobacter pylori infection

The circulatory IL-10 levels were significantly higher in *H. pylori*-infected patients compared to non-infected *H. pylori* patients ($p < 0.005$). However, there were no significant differences between serum levels of MMP-7 and MMP-9 in both groups (Table 2).

Table 2: Serum level of IL-10, MMP-7 & MMP-9 in HP (+) and HP (-)

Serum Level (pg/ml)	*H.pylori* positive (mean ± SD)	*H.pylori* negative (mean ± SD)	Independent t-test
IL-10	1.62 ± 1.56	1.39 ± 2.32	0.043*
MMP-7	8.69 ± 6.82	8.03 ± 6.20	0.886
MMP-9	951.86 ± 522.07	889.91 ± 478.78	0.640

*p < 0.05.

Association serum level of IL-10, MMP-7, MMP-9 and degree of gastritis

There was no correlation found between IL-10 serum levels with the degree of chronic inflammation or neutrophil infiltration. There was no correlation found between MMP-7 serum levels with the degree of chronic inflammation or neutrophil infiltration. The same results were found for MMP-9 serum levels. There was no correlation found between MMP-9 serum levels with the degree of chronic inflammation or neutrophil infiltration (Table 3).

Table 3: Association serum levels of IL-10, MMP-7, MMP-9 and degree of chronic inflammation and neutrophil infiltration

Cytokines		Chronic Inflammation				Neutrophil Infiltration			
		Normal + Mild N (%)	Moderate + Severe N (%)	OR (95% CI)	P	Normal + Mild N (%)	Moderate + Severe N (%)	OR (95% CI)	p
IL-10	High	15 (21.43)	19 (27.14)	1.01 (0.59-1.70)	0.978	26 (37.14)	8 (11.43)	0.872 (0.65-1.17)	0.364
	Low	16 (22.86)	20 (28.57)			24 (34.29)	12 (17.14)		
MMP-7	High	12 (17.14)	23 (32.86)	1.58 (0.91-2.75)	0.092	23 (32.86)	12 (17.14)	1.17 (0.87-1.58)	0.290
	Low	19(27.14)	16 (22.86)			27 (38.57)	8 (11.43)		
MMP-9	High	14 (20)	21 (30)	1.21 (0.72-2.06)	0.470	24 (34.29)	11 (15.71)	1.08 (0.81-1.46)	0.597
	Low	17 (24.29)	18 (25.71)			26 (37.14)	9 (12.86)		

There was no correlation found between IL-10 serum levels with the degree of atrophy or intestinal metaplasia. There was no correlation found between MMP-7 serum levels and the degree of atrophy or intestinal metaplasia.

Table 4: Association serum levels of IL-10, MMP-7, MMP-9 and degree of atrophy and intestinal metaplasia

Cytokines		Atrophy				Intestinal Metaplasia			
		Normal + Mild N (%)	Moderate + Severe N (%)	OR (95% CI)	P	Normal + Mild N (%)	Moderate + Severe N (%)	OR (95% CI)	p
IL-10	High	27 (38.57)	7 (10)	1.13 (0.88-1.45)	0.318	25 (35.71)	9 (12.86)	1.12 (0.91-1.38)	0.276
	Low	32 (45.71)	4 (5.71)			30 (42.86)	6 (8.57)		
MMP-7	High	30 (42.85)	5 (7.14)	0.83 (0.65-1.07)	0.145	30 (42.86)	5 (7.14)	0.97 (0.79-1.18)	0.743
	Low	25 (35.71)	10 (14.28)			29 (41.43)	6 (8.57)		
MMP-9	High	29 (41.43%)	6 (8.57%)	1.04 (0.810-1.33)	0.771	27 (38.57)	8 (11.43)	1.03 (0.85-1.27)	0.743
	Low	30 (42.85)	5 (7.14)			28 (40)	7 (10)		

The same results were found for MMP-9 serum levels. There was no correlation found between MMP-9 serum levels and the degree of atrophy or intestinal metaplasia (Table 4).

Discussion

The average age of the subjects was 49.9 ± 13.04 (SD) years, which concludes that most of the gastritis patients are at their productive ages. The results of this study are similar to a study conducted by Garg B, et al, which reported that the average age of gastritis patients was 47 years old and the study reported by Mustapha SK, et al with an average age of 47 years old [7, 8].

In this study, a group of gastritis patients without major gastric disease (example: peptic ulcer and gastric carcinoma) were studied with the objective to examine the presence of *H. pylori* infection in gastritis patients. It is known that *H. pylori* are correlated with increased expressions of IL-10, MMP-7, and MMP-9 in the mucosa [9-12].

However, studies on the levels of IL-10, MMP-7 and MMP-9 in the serum are still limited and controversial. Owing to that, this study was conducted with the objective to find the differences between the serum levels of IL-10, MMP-7 and MMP-9 in patients with positive and negative *H. pylori,* and to analyse the correlations between the serum levels and the degree of gastritis based on histopathology appearance.

IL-10 as an anti-inflammatory cytokine has a role in the initial immune response which is mediated by B cell. Cytokine IL-10 is involved in the decrease of the inflammatory response [13]. IL-10 inhibits the synthesis of IFN-c, IL-1, IL-6, IL-8 and TNF-α, and also acts as a feedback mechanism in reducing these cytokines [13, 14]. IL-10 might contribute to the failure of the immune response to clear *H. pylori* infection, and in a previous study we found the increased secretion of IL-10 in biopsy specimens in *H. pylori* infection, with the secretion of the cytokine correlating with the degree of chronic inflammation. Yamaoka *et al* have reported increased expression of IL-10 mRNA in biopsies from infected patients [15].

IL-10 helps to maintain bacterial colonisation and exerts its effects directly on gastric epithelial cells [2, 10, 11]. Goll et al reported that samples from *H. pylori*-positive patients showed increased production of IL-10 as much as 6.7 times the production of patients with negative *H. pylori.*

In this study, the serum levels of IL-10 increased significantly in positive *H. pylori* compared to negative *H. pylori* patients. This result was similar to that reported by Dlugovittzky et al, where the IL-10

serum levels of the positive *H. pylori* subjects were higher compared to negative *H. pylori* subjects ($p<0.01$) [16]. However, other studies reported different conclusions, In the studies performed by Russo et al and Bayraktaroglu et al, they reported that there were no significant correlations between the positive *H. pylori* subjects and negative subjects, which might be caused by inhibition of further inflammation [17, 18].

H. pylori is able to trigger the expression of MMP, which is a proteolytic enzyme that has a role in maintaining and remodelling the interaction between epithelial cells and the basal membrane [19, 20]. A study, conducted by Bebb JR *et al*, found that gastric biopsy specimens from a positive *H. pylori* subject will show in a higher level of MMP-7 protein in the antrum and corpus [19]. A similar result is found from the study conducted by Wroblewski *et al*, which concluded that the MMP-7 expression in the antrum and corpus increased with the presence of *H. pylori* [21].

In this study, there were no significant differences in MMP-7 serum levels between positive and negative *H. pylori* subjects. The same results were found in the study by Rautelin et al, which concluded that there were no significant differences in MMP-7 serum levels between positive and negative *H. pylori* gastritis subjects [22].

Studies by Rautelin HI *et al* and Li SL *et al* found that MMP-9 level increased significantly in the gastric mucosa of gastritis patients with positive *H. pylori* compared to the negative [22, 23]. In a study conducted by Oliviera, it was found that *H. pylori* through a T4SS (Type IV Secretion System) pathway increases the activity of MMP-9 as a response to the invasion of *H. pylori* on the gastric epithelium [24].

In this study, there was no difference in the serum levels of MMP-9 between the positive and negative *H. pylori* patients. Similar results were also found in the study reported by Rautelin et al and Ettehad et al, where it was concluded that there were no significant differences in the MMP-9 levels of the gastritis patients between the positive and negative *H. pylori* subjects [25, 26].

In this study, it was found that there were no significant correlations found between serum levels of IL-10, MMP-7, MMP-9 and the degree of chronic inflammation, neutrophil infiltration, atrophy, or intestinal metaplasia ($p>0.05$).

In conclusion, the IL-10 serum levels increased significantly in positive *H. pylori* subjects. There was no significant difference in the average serum levels of MMP-7 and MMP-9 between positive and negative infected *H. pylori* patients. There were no correlations found between serum levels of IL-10, MMP-7, MMP-9 with the degree of gastritis based on histopathology.

References

1. Suzana MK, Skender T, Emine DS, Halil A, Vjollca SM, Agron K, et al. Helicobacter pylori gastritis updated sydney classification applied in our material. Sec Biol Med Sci. 2009;30(1):45–60.

2. Zakaria Z. The role of interleukin-10 in Helicobacter pylori infection. Master [Tesis]. Nottingham,UK: University of Nottingham, 2010:108-31.

3. Maeda S, Mentis AF. Pathogenesis of Helicobacter pylori infection. Helicobacter. 2007; 12(Suppl 1):10-14. http://dx.doi.org/10.1111/j.1523-5378.2007.00529.x PMid:17727454

4. Lu P, Takai K, Weaver VM, Werb Z. Extracellular Matrix Degradation and Remodelling in Development and Disease. Cold Spring Harb Perspect Biol. 2011:3;1-24. http://dx.doi.org/10.1101/cshperspect.a005058 PMid:21917992 PMCid:PMC3225943

5. Rugge M, Pennelli G, Pilozzi E, et al. Gastritis: the histology report. Dig Liver Dis. 2011;43S:S373–84. http://dx.doi.org/10.1016/S1590-8658(11)60593-8

6. Rojborwonwitaya J, Vijitjunykul N. Comparison of the Accuracy of Two Commercial Rapid Urase Tests, CLOtest® and Pronto Dry®, in detecting Helicobacter pylori Infection.

7. Garg B, Sandhu V, Sood N, et al. Histopathological analysis of chronic gastritis and correlation of pathological features with each other and with endoscopic findings. Pol J Pathol. 2012;3:172-8. http://dx.doi.org/10.5114/pjp.2012.31501

8. Mustapha SK, Bolori MT, Ajayi N, et al. Endoscopic findings and the frequency of Helicobacter pylori among dyspeptic patients in North-Eastern Nigeria. Internet J Gastroenterol. 2006; 6(1). http://www.ispub.com (accessed 10 May 2014)

9. Cheng HC, Yang HB, Chang WL, Chen WY, Yeh YC, Sheu BS. Expressions of MMPs and TIMP-1 in Gastric Ulcers May Differentiate H. pylori-Infected from NSAID-Related Ulcers. Sci World J. 2012:2012;1-9.

10. Goll R, Gruber F, Olsen T, Cui G, RascHpichler G, Buset M, et al. Helicobacter pylori stimulate a mixed adaptive immune response with a strong T-regulatory component in human gastric mucosa. Helicobacter. 2007;12:185-92. http://dx.doi.org/10.1111/j.1523-5378.2007.00495.x PMid:17492997

11. Lundin BS, Enarsson K, Kindlund B, Lundgren A, Johnsson E, Quiding-Jarbrink M, et al. The local and systemic T-cell response to Helicobacter pylori in gastric cancer patients is characterised by production of interleukin-10. Clin Immunol. 2007;125: 205-13. http://dx.doi.org/10.1016/j.clim.2007.07.011 PMid:17826353

12. Yanagisawa N, Geironson L, Al-Soud WA, and Ljungh A. Expression of Matrix Metalloproteinase-2, -7, and -9 on the human colon, liver and bile duct cell lines by enteric and gastric Helicobacter species. FEMS Immunol Med Microbiol. 2005; 44(2):197-204. http://dx.doi.org/10.1016/j.femsim.2004.11.009 PMid:15866216

13. Achyut BR, Tripathi P, Ghoshal UC, Moorchung N, Mittal B. Interleukin-10 (-819 C/T) and Tumor Necrosis Factor-a (-308 G/A) Gene Variants Influence Gastritis and Lymphoid Follicle Development. Dig Dis Sci. 2008;53:622–9. http://dx.doi.org/10.1007/s10620-007-9925-y PMid:17717744

14. Andersen LP, Holck S, Janulaityte-Gu¨nther D, Kupcinskas L, Kiudelis G, Jonaitis L, et al. Gastric inflammatory markers and interleukins in patients with functional dyspepsia, with and without Helicobacter pylori infection. FEMS Immunology and Medical Microbiology. 2005;44:233–8. http://dx.doi.org/10.1016/j.femsim.2004.10.022 PMid:15866221

15. Yamaoka Y, Kodama T, Kita M, Imanishi J, Kashima K, Graham DY. Relation between cytokines and Helicobacter pylori in gastric cancer. Helicobacter. 2001;6:116-24. http://dx.doi.org/10.1046/j.1523-5378.2001.00017.x PMid:11422466

16. Dlugovitzky DG, Nogueras MG, Fiorenza G, Raynoldi J, Proske

A, Badano A, et al. Changes in Cytokine levels related to the Immunopathogenesis of Helicobacter pylori disease. Immunological and histological effects of triple treatment (omeprazol, azytromycin, and amoxycillin). Inmunología. 2005; 24(1): 11-6

17. Russo F, Jirillo E, Clemente C, Messa C, Chiloiro M, Riezzo G, et al. Circulating cytokines and gastrin levels in asymptomatic subjects infected by Helicobacter pylori (H. pylori). Immunopharmacol Immunotoxicol. 2001;23(1):13-24. http://dx.doi.org/10.1081/IPH-100102563 PMid:11322645

18. Bayraktaroglu T, Aras AS, Aydemir S, Davutoglu C, Ustundag Y, Atmaca H, et al. Serum levels of tumor necrosis factor-a, interleukin-6 and interleukin-8 are not increased in dyspeptic patients with Helicobacter pylori-associated gastritis. Mediators of Inflammation. 2004;13(1):25-8 http://dx.doi.org/10.1080/09629350410001664789 PMid:15203561 PMCid:PMC1781536

19. Bebb JR, Letley DP, Thomas RJ, Aviles F, Collins HM, Watson SA, et al. Helicobacter pylori upregulates matrilysin (MMP-7) in Epithelial Cells In Vivo and In Vitro in A Cag Dependent Manner. Gut. 2003:52; 1408-13. http://dx.doi.org/10.1136/gut.52.10.1408 PMid:12970131 PMCid:PMC1773843

20. Cheng HC, Yang HB, Chang WL, Chen WY, Yeh YC, and Sheu BS. Expressions of MMPs and TIMP-1 in Gastric Ulcers May Differentiate H. pylori-Infected from NSAID-Related Ulcers. Sci World J. 2012:2012; 1-9.

21. Wroblewski LE, Noble PJM, Pagliocca A, Pritchard DM, Hart CA, Campbell F, et al. Stimulation of MMP-7 (matrilysin) by Helicobacter pylori in human gastric epithelial cells: role in epithelial cell migration. J Cell Sci. 2003:116; 3017-26. http://dx.doi.org/10.1242/jcs.00518 PMid:12808021

22. Rautelin HI, Oksanen AM, Veijola LI, Sipponen PI, Tervahartiala TI, Sorsa TA, Lauhio A. Enhanced systemic matrix metalloproteinase response in Helicobacter pylori gastritis. Ann Med. 2009:41; 208-15. http://dx.doi.org/10.1080/07853890802482452 PMid:18979291

23. Li SL, Zhao JR, Ren XY, Xie JP, Ma QZ, Rong QH. Increased Expression of Matrix Metalloproteinase-9 Associated With Gastric Ulcer Recurrence. World J Gastroenterol. 2013 ;19: 4590-5. http://dx.doi.org/10.3748/wjg.v19.i28.4590 PMid:23901237 PMCid:PMC3725386

24. Oliveira MJ, Costa AC, Costa AM, Henriques L, Suriano G, Atherton JC, et al. Helicobacter pylori Induces Gastric Epithelial Cell Invasion in a c-Met and Type IV Secretion System-dependent Manner. J Biol Chem. 2006:281; 34888-96. http://dx.doi.org/10.1074/jbc.M607067200 PMid:16990273

25. Rautelin H, Tervahartiala T, Lauhio A, Sorsa T, Kolho KL. Assessment of systemic matrix metalloproteinase and their regulator response in children with Helicobacter pylori gastritis. Scand J Clin Lab Invest. 2010:70; 492-6. http://dx.doi.org/10.3109/00365513.2010.520732 PMid:20854182

26. Ettehad GH, Parastar N, Pahlavan Y, Amani M. Serum Level of Metalloproteinase in Patients Infected with Helicobacter pylori in Ardabil. J Ardabil Univ Med Sci. 2012:12(3); 230-8.

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):364-373.
http://dx.doi.org/10.3889/oamjms.2016.032
eISSN: 1857-9655
Basic Science

Immunomodulatory Effect of *H. Pylori CagA* Genotype and Gastric Hormones On Gastric Versus Inflammatory Cells Fas Gene Expression in Iraqi Patients with Gastroduodenal Disorders

Ali Ibrahim Ali AL-Ezzy*

Department of Pathology, College of Veterinary Medicine, Diyala University, Baquba, Diyala Province, Iraq

Citation: AL-Ezzy AIA. Immunomodulatory Effect of *H. Pylori CagA* Genotype and Gastric Hormones On Gastric Versus Inflammatory Cells Fas Gene Expression in Iraqi Patients with Gastroduodenal Disorders. Open Access Maced J Med Sci. 2016 Sep 15; 4(3):364-373. http://dx.doi.org/10.3889/oamjms.2016.032

Key words: Fas gene; gastric disorders; pepsinogens; gastrin -17; *H. pylori, CagA*.

***Correspondence:** Ali Ibrahim Ali Al-Ezzy. Department of Pathology, College of Veterinary Medicine, Diyala University, Baquba, Diyala Province, Iraq. E-mail: alizziibrahim@gmail.com

Received: 23-Jan-2016; **Revised:** 19-Feb-2016; **Accepted:** 20-Feb-2016; **Online first:** 15-Mar-2016

Competing Interests: The authors have declared that no competing interests exist.

Abstract

AIM: To evaluate the Immunomodulatory effects of *CagA* expression; pepsinogen I, II & gastrin-17 on PMNs and lymphocytes Fas expression in inflammatory and gastric cells; demographic distribution of Fas molecule in gastric tissue and inflammatory cells.

METHODS: Gastroduodenal biopsies were taken from 80 patients for histopathology and *H. pylori* diagnosis. Serum samples were used for evaluation of pepsinogen I (PGI); (PGII); gastrin-17 (G-17).

RESULTS: Significant difference ($p < 0.001$) in lymphocytes & PMNs Fas expression; epithelial & lamina propria Fas localization among *H. pylori* associated gastric disorders. No correlation between grade of lymphocytes & PMNs Fas expression in gastric epithelia; lamina propria and types of gastric disorder. Significant difference ($p < 0.001$) in total gastric Fas expression, epithelial Fas; lamina propria and gastric gland Fas expression according to *CagA*, PGI; PGII; PGI/PGII; Gastrin-17. Total gastric Fas expression has significant correlation with *CagA*, PGII levels. Gastric epithelial and gastric lamina propria Fas expression have significant correlation with *CagA*, PGI; PGII levels. Significant difference ($p < 0.001$) was found in lymphocytes & PMNs Fas expression; epithelial & lamina propria localization of lymphocytes & PMNs Fas expression according to *CagA*, PGI; PGII; PGI/PGII; Gastrin-17. Lymphocytes Fas expression have correlation with PGI, PGII, PGI/PGII. PMNs Fas expression have correlation with PGI, PGII.

CONCLUSION: Fas gene expression and localization on gastric and inflammatory cells affected directly by *H. pylori CagA* and indirectly by gastric hormones. This contributes to progression of various gastric disorders according to severity of *CagA* induced gastric pathology and gastric hormones disturbance throughout the course of infection and disease.

Introduction

Helicobacter pylori infection is associated with several benign and malignant human diseases. Most infected individuals remain asymptomatic. If untreated, infection lasts for decades .The prevalence of infection ranging between 50% in developed countries and 90% in developing countries [1].

H. pylori occupies a unique niche, extremely acidic environment [2]. The urease of *H. pylori* is essential for its colonization and survival at extremely low pH, to ensure cytoplasmic homeostasis during large pH changes that occur during feeding. *H. pylori* can use molecular hydrogen as energy source; thus, its growth depends to some extent on the hydrogen excreted [3]. *H. pylori* infected gastric mucosa evolves through stages of chronic gastritis, intestinal metaplasia, glandular atrophy, and dysplasia before carcinoma develops.

H. pylori Infection is associated mostly with chronic antral gastritis, characterized by a mucosal infiltration of polymorphonuclear (PMNs) and mononuclear leukocytes [4]. Some studies have

reported that *H. pylori* infection is suggested by the presence of active inflammation [3]. Neutrophil infiltration is a hallmark of active inflammation [4]. It is unknown whether neutrophil infiltration may be a marker of H. pylori infection.

It is now established that *Helicobacter pylori* causes more than 80% of duodenal ulcers and up to 60% of gastric ulcers [3]. The link between *Helicobacter pylori* infection and subsequent gastritis and peptic ulcer disease has been established through studies of human volunteers, antibiotic treatment studies and epidemiological studies. In some individuals, *Helicobacter pylori* also infect the corpus region of the stomach. This results in a more widespread inflammation that predisposes not only to ulcer in the corpus region, but also to stomach cancer [3].

Because of extremely low pH, the stomach is a hostile environment to most other microorganisms. The ability of *H. pylori* to flourish in the stomach has been attributed to protective mechanisms such as its production of urease, protecting the bacterium from gastric acidity by creating a basic microenvironment [5]. However, we reasoned that *H. pylori* might have evolved away to gain growth advantage in this niche, possibly by exploiting a gastric factor. A logical candidate would be one up regulated by *H. pylori* infection.

One such factor is the gastric hormone gastrin. Gastrin is produced as a prohormone by G cells located within the gastric antrum. The prohormone processed to shorter peptides, the most abundant of which is 17 amino acids long, termed gastrin-17 (G17). The major role attributed to gastrin within gastric tissue is the regulation of acid secretion [6]. After infection, gastrin levels are found to be consistently elevated and normal physiological negative feedback control of secretion is lost. Furthermore, after *H. pylori* eradication, gastrin levels are reduced and normal feedback control of gastrin secretion is restored [6, 7].

A number of studies have investigated the pathogenicity of *H. pylori* in relation to cytotoxic products, including urease, Cag, and vacuolating toxin (VacA) [8]. Potential apoptosis-inducing activity was reported in VacA [8] and urease [9]. Apoptosis in *H. pylori*-associated gastritis accompanies the activation of Fas and the Fas ligand system [10] in epithelial cells. Fas is a member of the tumor necrosis factor receptor family, which, when bound by its ligand, activates caspase-8, an initiator of the downstream apoptotic process that includes the cleavage of other death substrates, cellular and nuclear morphological changes and, ultimately, cell death [11]. Variations in host responses might cause the *H. pylori* mediated pathogenesis to result in a variety of clinical outcomes.

The aim of the present study is to evaluate the Immunomodulatory effects of *CagA* gene expression and gastric secretions (pepsinogen I, -II, Gastrin17) on inflammatory response mainly PMNs and lymphocytes infiltrations as well as expression of Fas gene in inflammatory and gastric cells under influence of *CagA* and gastric hormones and demographic distribution of Fas molecule in gastric tissue and inflammatory cells.

Materials and Methods

Patients

In this cross sectional study, (80) patients, age range 16-80 years, mean (47.24 ± 18.82) years, with clinical indications for upper gastrointestinal tract endoscopy during June 2013 to January 2015 were studied. Males represent 44 (55%) versus 36 (45%) females. This study was conducted according to the principles of Helsinki declaration. Before endoscopy, a full explanation about the purpose of this study to all patients was done. Dully-filled consent form obtained from all patients that agree to participate in the study. Approval of ethical review Committee of College of medicine – Diyala University - Iraq, was taken prior to initiation of the work at gastroenterology department of Baqubah teaching hospital in Diyala province - Iraq. Any patient under antibiotics or colloidal bismuth compounds for past one month treatment; having a history of previous gastric surgery and recent or active gastrointestinal bleeding was excluded from this study.

Methods

After topical pharyngeal anesthesia for overnight fasted Patients, A sterile flexible endoscope was introduced for full investigation of Stomach and duodenum. Six biopsy samples from congested, inflamed or erosive lesions were picked via sterile biopsy forceps. Samples were placed in Serim® PyloriTek® Test Kit for detection of urease activity. Each PyloriTek strip has a built-in positive analyte control and a negative control, which run concurrently with the test specimen. The PyloriTek positive control automatically appears with every test within the normal 1-hour time. With competitive tests the positive control is run after waiting 24 hours then inserting a urease positive control material [12].

Biopsy sample was placed in sterile glass slide with a drop of normal saline and teased with sterile scalpel to make smaller fragments of tissue then another sterile glass slide was placed over the teased first tissue and the tissue was crushed between the two glasses then stain by Gram's staining. Existence of Gram negative spiral bacteria embedded in the tissue cells was diagnostic for *H. pylori* [13].

True positive results were considered if a combination of urease test and Gram stain give positive results for a single biopsy specimen [14].

Human Fas gene and *H. pylori CagA* gene expression detected by insitu hybridization procedure in 5μm thickness serial gastric mucosal sections fixed on positively charged slides using biotinylated long DNA probe for Human Fas gene; Cat. No. IH-60047 (fas-6001-B); *H. pylori*l *CagA* gene, Cat. No. IH-60061 (HPY-6001-B) (Maxim biotech-USA) and the DNA Probe hybridization/Detection System – In Situ Kit (Maxim biotech-USA), according to Maxim biotech instruction manual [15].The examination and scoring were done under light microscope by pathologists at power X 400 according to the scoring system [16]. For serological assay blood was drawn from each patient during the visit to the endoscopy unit. Separated serum samples were stored at 27˚C until analyses. Serum pepsinogen I(PGI) and II(PGII) and gastrin-17 (G-17) were assayed with ELISA using monoclonal antibodies to pepsinogen I and II and gastrin-17 (Biohit Diagnostics, Biohit, Devon, UK). All procedures were carried out according to the manufacturer's instructions and results of pepsinogen I and II reported in μg/l and pmol/l for gastrin-17. The pepsinogen I: II ratio was calculated and reported in fraction [17].

Statistical analysis

Frequency of variables express as percentage. PG I, II and G-17 values express as mean ± standard deviation (Mean ± SD). Pearson test for correlation was used for non-categorical data. Chi-test used to compare the PG I, PGII, and G17 according to *CagA* gene expression.

The level of significance was 0.05 (two-tail) in all statistical testing; significant of correlations (Pearson, spearman) include also 0.01 (two-tail). Statistical analysis was performed using SPSS for windows TM version 17.0, and Microsoft Excel for windows 2010.

Results

As shown in Table 1 gastric infiltrated lymphocytes have mild Fas expression in (61.25%) versus (31.25%) moderate expression (Figure1). Mild and moderate lymphocytes Fas expression reported mainly in gastritis (25%) and lastly in, duodenitis (3.75%) and prepyloric ulcer (2.5%). Significant difference reported ($p < 0.001$) in lymphocytes Fas expression without correlation between grade of Fas expression and types of gastric disorder ($p = 0.266$). Gastric infiltrated PMNs have mild Fas gene expression in (46.25%), mainly in gastritis (12.5%) and lastly in prepyloric ulcer (2.5%) (Figure1). Moderate PMNS Fas expression reported in (3.75%) in gastric ulcer and gastritis. Significant difference reported ($p < 0.001$) in PMNs Fas expression without correlation between grade of Fas expression and types of gastric disorder ($p = 0.643$).

Table 1: Lymphocytes and PMNs Fas gene expression and localization in gastroduodenal disorders

Presentation	Lymphocyte Fas gene					χ^2	P value	R	P value
	<5%	5-25%	26-50%	50% >	Total				
Gastric ulcer	1 (1.25%)	10 (12.5%)	4 (5%)	0 (0%)	15 (18.75%)				
Du	0 (0%)	6 (7.5%)	6 (7.5%)	0 (0%)	12 (15%)				
Gastropathy	3 (3.75%)	10 (12.5%)	2 (2.5%)	0 (0%)	15 (18.75%)				
Gastritis	2 (2.5%)	20 (25%)	8 (10%)	0 (0%)	30 (37.5%)	197.467	0.000	0.126	0.266
Duodenitis	0 (0%)	3 (3.75%)	3 (3.75%)	0 (0%)	6 (7.5%)				
Prepyloric ulcer	0 (0%)	0 (0%)	2 (2.5%)	0 (0%)	2 (2.5%)				
Total	6 (7.5%)	49 (61.25%)	25 (31.25%)	0 (0%)	80 (100%)				
Presentation	*PMNs Fas gene*								
Gastric ulcer	6 (7.5%)	7 (8.75%)	2 (2.5%)	0 (0%)	15 (18.75%)				
Du	5 (6.25%)	7 (8.75%)	0 (0%)	0 (0%)	12 (15%)				
Gastropathy	9 (11.25%)	6 (7.5%)	0 (0%)	0 (0%)	15 (18.75%)				
Gastritis	19 (23.75%)	10 (12.5%)	1 (1.25%)	0 (0%)	30 (37.5%)	147.179	0.000	-0.053	0.643
Duodenitis	1 (1.25%)	5 (6.25%)	0 (0%)	0 (0%)	6 (7.5%)				
Prepyloric ulcer	0 (0%)	2 (2.5%)	0 (0%)	0 (0%)	2 (2.5%)				
Total	40 (50%)	37 (46.25%)	3 (3.75%)	0 (0%)	80 (100%)				
Presentation	Cellular Fas gene expressed in epithelial lining					χ^2	P value	R	P value
	<5%	5-25%	26-50%	50% >	Total				
Gastric ulcer	0 (0%)	11 (13.75%)	4 (5%)	0 (0%)	15 (18.75%)				
Du	0 (0%)	4 (5%)	8 (10%)	0 (0%)	12 (15%)				
Gastropathy	0 (0%)	9 (11.25%)	6 (7.5%)	0 (0%)	15 (18.75%)				
Gastritis	0 (0%)	14 (17.5%)	16 (20%)	0 (0%)	30 (37.5%)	162.347	0.000	0.036	0.754
Duodenitis	0 (0%)	6 (7.5%)	0 (0%)	0 (0%)	6 (7.5%)				
Prepyloric ulcer	0 (0%)	0 (0%)	2 (2.5%)	0 (0%)	2 (2.5%)				
Total	0 (0%)	44 (55%)	36 (45%)	0 (0%)	80 (100%)				
Presentation	Cellular Fas gene expressed in lamina propria								
Gastric ulcer	0 (0%)	0 (0%)	0 (0%)	15 (18.75%)	15 (18.75%)				
Du	0 (0%)	0 (0%)	0 (0%)	12 (15%)	12 (15%)				
Gastropathy	0 (0%)	0 (0%)	0 (0%)	15 (18.75%)	15 (18.75%)				
Gastritis	0 (0%)	0 (0%)	0 (0%)	30 (37.5%)	30 (37.5%)	162.347	0.000	-0.036	0.754
Duodenitis	0 (0%)	0 (0%)	0 (0%)	6 (7.5%)	6 (7.5%)				
Prepyloric ulcer	0 (0%)	0 (0%)	0 (0%)	2 (2.5%)	2 (2.5%)				
Total	0 (0%)	0 (0%)	0 (0%)	80 (100%)	80 (100%)				

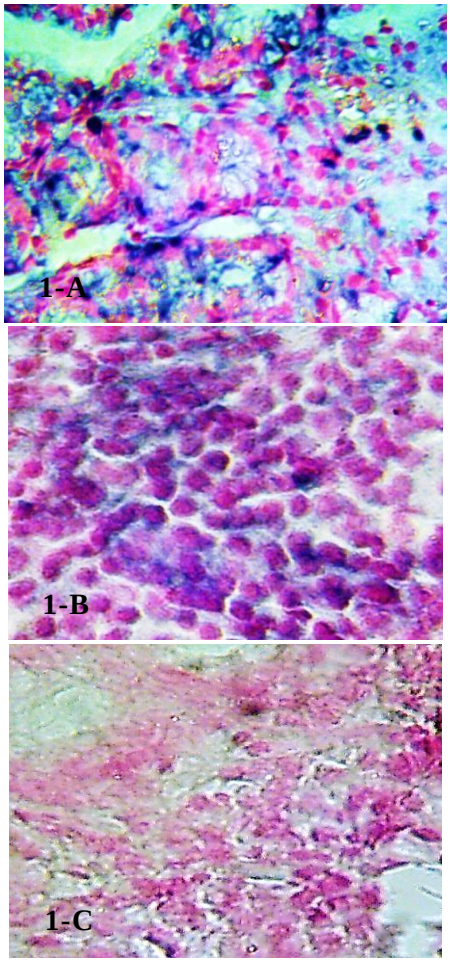

Figure 1: In situ hybridization for human Fas in gastric tissue section mainly in inflammatory cell infiltrates (A) lymphocytes; (B) PMNS, (C) negative expression. Bar size = 50 µm. staining by BCIP/NBT (bluish purple) counterstained with nuclear fast red

Mild (55%) to moderate (45%) Fas gene expression on inflammatory cells (lymphocytes and PMNs) infiltrated in gastric epithelial lining were reported. Mild Fas expression detected mainly in in gastritis (17.5%) and lastly in DU (5%). Moderate Fas expression detected in gastritis (20%), and finally in prepyloric ulcer (2.5%). There was significant difference ($p < 0.001$) in epithelial localization of lymphocytes & PMNs Fas among disorders without correlation ($p = 0.754$). Fas overexpression was detected in lymphocytes and PMNs infiltrated in lamina propria, mainly in gastritis (37.5%), gastric ulcer & gastropathy (18.75%), prepyloric ulcer (2.5%). There was significant difference ($p < 0.001$) in lamina propria localization of Fas expressing lymphocytes & PMNs among disorders without correlation ($p = 0.754$).

As shown in Table 2, overexpression of Fas on gastric cells reported in (61.25%), moderate expression (31.25%), mild expression (5%). A sum of (58.75%) of gastroduodenal disorders infected with *CagA* positive *H. pylori* (Figure 2), (43.75%) have gastric Fas overexpression. Significant difference in Fas expression among disorders correlated with between *CagA* expression (p value < 0.001, p value $= 0.001$).

Fas over expression reported in (7.5%) gastric disorders with hypopepsinogenemia, (31.25%) cases with normal PGI; (22.5%) with PGI hypersecretion. Moderate Fas expression reported in (27.5%) cases with normal PGI and (3.75%) with PGI hypersecretion. Significant difference among disorders in grade of Fas expression (p value < 0.001) without correlation with serum level of PGI (p value $= 0.643$).

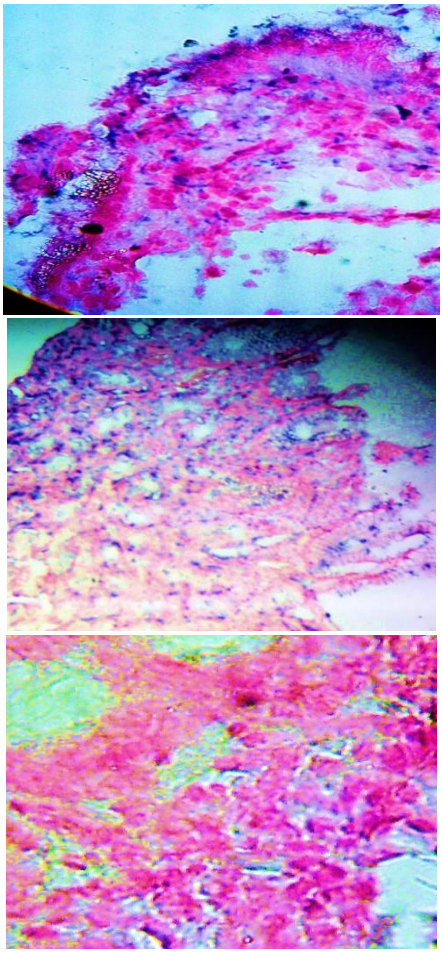

Figure 2: In situ hybridization for CagA Positive H. pylori in gastric tissue section .staining by BCIP/NBT (bluish purple) counterstained with nuclear fast red. Bar size=50 µm. A) Gastric epithelia, B) CagA expression extended to gastric pits, C) negative expression

Fas over expression reported in (15%) gastric disorders with normal PGII level; (46.25%) of cases with hypersecretion of PGII. Significant difference among disorders in grade of Fas expression (p value < 0.001) with correlation with serum level of PGII (p value $= 0.01$).

Gastric Fas over expression reported in (27.5%) of cases with PGI/PGII hyposecretion; (33.75%) of normal PGI/PGII. Significant difference among disorders in grade of Fas expression (p value

< 0.001) without correlation with serum level of PGI/PGII (p value = 0.156). Significant difference in grade of Fas gene expression (p value < 0.001) without correlation with gastrin17 level (p value = 0.858).

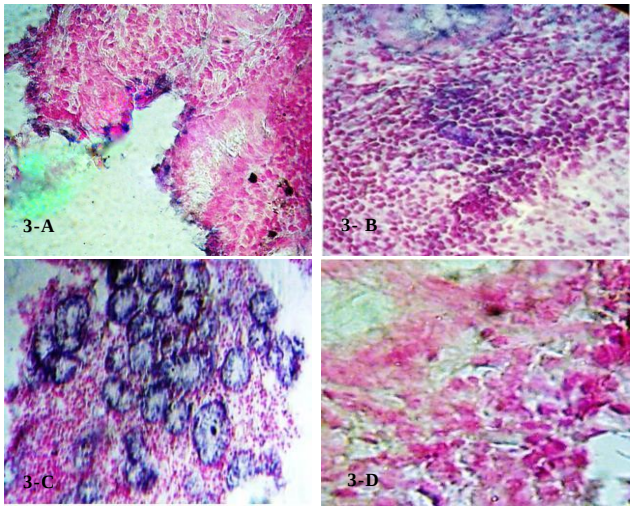

Figure 3: In situ hybridization for Fas gene in gastric tissue section. Staining by BCIP/NBT (bluish purple) counterstained with nuclear fast red. Bar size = 50 µm. A) positive Fas gene In situ hybridization expression in gastric epithelia; (B) positive Fas gene In situ hybridization expression in gastric lamina propria; (C) positive Fas gene In situ hybridization expression in gastric glands; (D) Negative In situ hybridization for Fas gene in gastric tissue section.

As in Table 3, gastric epithelia have mild Fas expression (61.25%) versus (38.75%) for moderate. *H. pylori CagA* infected gastric epithelia has moderate Fas expression in (26.25%) versus (32.5%) in mild with significant difference and correlation between *CagA* expression and grade of Fas expression (p value < 0.001, p value = 0.05) (Figure3). Significant difference in grade of Fas gene expression (p value < 0.001) with correlation between serum level of PGI and Fas grade (p value < 0.001).

Significant difference in gastric epithelia Fas grade (p value < 0.001) with correlation between PGII levels (p value = 0.0002). Among (58.75%) patients of normal PGI/PGII, (25%) have moderate grade of gastric Fas expression, (33.75%) mild. Significant difference in grade of gastric epithelial Fas expression (p value < 0.001) without correlation between level of PGI/PGII and grade Fas expression (p value = 0.487). Normal Gastrin17 detected in (87.5%), (37.5%) have moderate Fas expression and (50%) was mild. Hypergastrinemia was detected in (12.5%), (1.25%) have moderate Fas expression, (11.25%) mild. Significant difference in grade of Fas expression (p value < 0.001) without correlation between gastrin17 and grade of Fas expression (p value = 0.508).

As shown in Table 4, mild Fas expression in lamina propria (56.25%) versus (43.75%) moderate. Significant difference and correlation between *CagA* expression and Fas expression in lamina propria (p value = 0.001, p value = 0.056) (Figure3). Significant difference among disorders in grade of Fas expression (p value < 0.001) with significant correlation between serum PGI and grade of Fas expression in gastric lamina propria (p value = 0.05). Normal PGII level detected in (23.75%) patients, (15%) have moderate grade of Fas expression and (8.75%) was mild. Hypersecretion of PGII was determined in (76.25%) patients, (28.75%) have moderate grade of gastric Fas expression, (47.5%) was mild. Significant difference in grade of Fas expression (p value < 0.001) with correlation between PGII level and grade of Fas expression in gastric lamina propria (p value = 0.001). Significant difference in grade of Fas expression in gastric lamina propria (p value < 0.001) without correlation between serum PGI/PGII and grade of gastric lamina propria Fas expression (p value = 0.519). Significant difference in grade of Fas expression (p value < 0.001) without correlation between gastrin17 level and grade of gastric lamina propria Fas expression (p value = 0.575).

Table 2: Correlation of Gastric cells Fas gene expression and gastric secretion

Parameters		Gastric cells Fas gene					χ^2	P value	R	P value
		<5%	5-25%	26-50%	50% >	Total				
CagA genotype	Positive	0(0%)	4 (5%)	8 (10%)	35 (43.75%)	47 (58.75%)	60.363	0.000	0.350	0.001
	Negative	2 (2.5%)	0 (0%)	17 (21.25%)	14 (17.5%)	33 (41.25%)				
PGI	<30 µg/L	0 (0%)	0 (0%)	0 (0%)	6 (7.5%)	6 (7.5%)	1149.995	0.000	-0.053	0.643
	30-160 µg/L	2 (2.5%)	0 (0%)	22 (27.5%)	25 (31.25%)	49 (61.25%)				
	>160 µg/L	0 (0%)	4 (5%)	3 (3.75%)	18 (22.5%)	25 (31.25%)				
PGII	<3 µg/L	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1088.09	0.000	0.285	0.01
	3-15 µg/L	0 (0%)	0 (0%)	7 (8.75%)	12 (15%)	19 (23.75%)				
	>15 µg/L	2 (2.5%)	4 (5%)	18 (22.5%)	37 (46.25%)	61 (76.25%)				
PGI/PGII	<3 µg/L	2 (2.5%)	0 (0%)	9 (11.25%)	22 (27.5%)	33 (41.25%)	1833.79	0.000	-0.163	0.156
	3-20 µg/L	0 (0%)	4 (5%)	16 (20%)	27 (33.75%)	47 (58.75%)				
	>20 µg/L	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)				
Gastrin-17	< 1 pmol/ml	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	586.08	0.000	0.020	0.858
	1-7 pmol/ml	2 (2.5%)	4 (5%)	19 (23.75%)	45 (56.25%)	70 (87.5%)				
	>7 pmol/ml	0 (0%)	0 (0%)	6 (7.5%)	4 (5%)	10 (12.5%)				
Total		2 (2.5%)	4 (5%)	25 (31.25%)	49 (61.25%)	80 (100%)				

Table 3: Correlation of Gastric epithelial cells Fas gene expression and gastric secretion

Parameters		Gastric cells epithelial Fas gene					χ^2	P value	R	P value
		<5%	5-25%	26-50%	50% >	Total				
CagA Genotype	Positive	0 (0%)	26 (32.5%)	21 (26.25%)	0 (0%)	47 (58.75%)	40.177	0.000	0.213	0.058
	Negative	0 (0%)	23 (28.75%)	10 (12.5%)	0 (0%)	33 (41.25%)			0.218*	0.052*
PGI	<30 µg/l	0 (0%)	6 (7.5%)	0 (0%)	0 (0%)	6 (7.5%)	671.43	0.000	0.405	0.000
	30-160 µg/L	0 (0%)	34 (42.5%)	15 (18.75%)	0 (0%)	49 (61.25%)				
	>160 µg/L	0 (0%)	9 (11.25%)	16 (20%)	0 (0%)	25 (31.25%)				
PGII	<3 µg/l	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	650.29	0.000	0.341	0.002
	3-15 µg/L	0 (0%)	15 (18.75%)	4 (5%)	0 (0%)	19 (23.75%)				
	>15 µg/L	0 (0%)	34 (42.5%)	27 (33.75%)	0 (0%)	61 (76.25%)				
PGI/PGII	<3 µg/l	0 (0%)	22 (27.5%)	11 (13.75%)	0 (0%)	33 (41.25%)	1044.64	0.000	0.079	0.487
	3-20 µg/L	0 (0%)	27 (33.75%)	20 (25%)	0 (0%)	47 (58.75%)				
	>20 µg/L	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)				
Gastrin-17	< 1 pmol/ml	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	348.498	0.000	-0.075	0.508
	1-7 pmol/ml	0 (0%)	40 (50%)	30 (37.5%)	0 (0%)	70 (87.5%)				
	>7 pmol/ml	0 (0%)	9 (11.25%)	1 (1.25%)	0 (0%)	10 (12.5%)				

* Spearman Correlation

As shown in Table 4, Fas overexpression appear to be more frequent (53.75%) than moderate (41.25%) and mild (5%) grade of expression in gastric glands. Significant difference without correlation between *CagA* expression and grade of Fas in gastric glands (p value = 0.001, p value = 0.743).

Significant difference in grade of Fas expression (p value < 0.001) without significant correlation between serum PGI and grade of gastric glands Fas expression (p value = 0.296).Significant difference among disorders in grade of Fas (p value < 0.001) without correlation between serum PGII and grade of gastric glands Fas expression (p value = 0.501). Significant difference in grade of gastric gland Fas expression (p value < 0.001) without correlation between PGI/PGII serum level and grade of gastric cells Fas (p value = 0.947). Significant difference among disorders in grade of Fas gene expression (p value < 0.001) without correlation between gastrin17 and grade of gastric glands Fas expression (p value = 0.286).

As shown in Table 5, moderate lymphocytes Fas expression detected in (31.25%) versus (27.5%) mild among *CagA* positive cases. Significant difference without correlation between *CagA* expression and grade of Fas in lymphocytes (p value = 0.001, p value = 0.112). Significant difference in grade of Fas (p value < 0.001) with significant correlation between serum PGI and grade of lymphocytes Fas expression (p value = 0.009).Normal PGII level detected in (23.75%) of patients, (6.25%) have moderate and mild grades of lymphocytes Fas expression. Hypersecretion of PGII determined in (76.25%) of patients, (25%) have moderate grade of lymphocytes Fas, (55%) was mild. Significant difference in grade of lymphocytes Fas (p value < 0.001) with correlation between PGII levels and grade of gastric lymphocytes Fas (p value = 0.035). Significant difference in gastric lymphocytes Fas (p value < 0.001) with correlation between serum PGI/PGII and grade of gastric lymphocytes Fas expression (p value = 0.046).

Table 4: Correlation of Gastric lamina propria, gastric glands Fas gene expression and gastric secretion

Parameters		Gastric Fas gene expressed in lamina propria					χ^2	P value	r	P value
		<5%	5-25%	26-50%	50% >	Total				
Cag A genotype	Positive	0 (0%)	32 (40%)	15 (18.75%)	0 (0%)	47 (58.75%)	44.315	0.001	-0.215	0.056
	Negative	0 (0%)	13 (16.25%)	20 (25%)	0 (0%)	33 (41.25%)				
Pgi	<30 µg/l	0 (0%)	6 (7.5%)	0 (0%)	0 (0%)	6 (7.5%)	782.410	0.000	-.212	0.05
	30-160 µg/L	0 (0%)	19 (23.75%)	30 (37.5%)	0 (0%)	49 (61.25%)				
	>160 µg/L	0 (0%)	20 (25%)	5 (6.25%)	0 (0%)	25 (31.25%)				
Pgii	<3 µg/l	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	746.31	0.000	-0.361	0.001
	3-15 µg/L	0 (0%)	7 (8.75%)	12 (15%)	0 (0%)	19 (23.75%)				
	>15 µg/L	0 (0%)	38 (47.5%)	23 (28.75%)	0 (0%)	61 (76.25%)				
Pgi/pgii	<3 µg/l	0 (0%)	19 (23.75%)	14 (17.5%)	0 (0%)	33 (41.25%)	1300.262	0.000	-0.073	0.519
	3-20 µg/L	0 (0%)	26 (32.5%)	21 (26.25%)	0 (0%)	47 (58.75%)				
	>20 µg/L	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)				
Gastrin17	< 1 pmol/ml	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	444.840	0.000	-0.064	0.575
	1-7 pmol/ml	0 (0%)	40 (50%)	30 (37.5%)	0 (0%)	70 (87.5%)				
	>7 pmol/ml	0 (0%)	5 (6.25%)	5 (6.25%)	0 (0%)	10 (12.5%)				
Parameters		Fas gene expressed in Gastric glands					X2	P value	r	P value
Cag A genotype	Positive	0 (0%)	2 (2.5%)	21 (26.25%)	24 (30%)	47 (58.75%)	42.496	0.000	0.037	0.743
	Negative	0 (0%)	2 (2.5%)	12 (15%)	19 (23.75%)	33 (41.25%)				
Pgi	<30 µg/l	0 (0%)	0 (0%)	0 (0%)	6 (7.5%)	6(7.5%)	723.02	0.000	-0.118	0.296
	30-160 µg/L	0 (0%)	4 (5%)	22 (27.5%)	23 (28.75%)	49 (61.25%)				
	>160 µg/L	0 (0%)	0 (0%)	11 (13.75%)	14 (17.5%)	25 (31.25%)				
Pgii	<3 µg/l	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	673.75	0.000	0.08	0.501
	3-15 µg/L	0 (0%)	0 (0%)	7 (8.75%)	12 (15%)	19 (23.75%)				
	>15 µg/L	0 (0%)	4 (5%)	26 (32.5%)	31 (38.75%)	61 (76.25%)				
Pgi/pgii	<3 µg/l	0 (0%)	4 (5%)	5 (6.25%)	24 (30%)	33 (41.25%)	1067.56	0.000	0.008	0.947
	3-20 µg/L	0 (0%)	0 (0%)	28 (35%)	19 (23.75%)	47 (58.75%)				
	>20 µg/L	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)				
Gastrin17	< 1 pmol/ml	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	363.42	0.000	0.121	0.286
	1-7 pmol/ml	0 (0%)	4 (5%)	30 (37.5%)	36 (45%)	70 (87.5%)				
	>7 pmol/ml	0 (0%)	0 (0%)	3 (3.75%)	7 (8.75%)	10 (12.5%)				

Table 5: Correlation of Lymphocytes Fas gene and gastric secretion

Parameters		Lymphocyte Fas gene					χ^2	P value	r	P value
		<5%	5-25%	26-50%	50% >	Total				
Cag A genotype	Positive	0 (0%)	22 (27.5%)	25 (31.25%)	0 (0%)	47 (58.75%)	51.219	0.000	0.179	0.112
	Negative	2 (2.5%)	27 (33.75%)	4 (5%)	0 (0%)	33 (41.25%)				
PGI	<30 µg/L	0 (0%)	5 (6.25%)	1 (1.25%)	0 (0%)	6 (7.5%)	763.23	0.000	-0.291	0.009
	30-160 µg/L	2 (2.5%)	28 (35%)	17 (21.25%)	0 (0%)	47 (58.75%)				
	>160 µg/L	4 (5%)	16 (20%)	7 (8.75%)	0 (0%)	27 (33.75%)				
PGII	<3 µg/L	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	644.31	0.000	0.236	0.035
	3-15 µg/L	0 (0%)	5 (6.25%)	5 (6.25%)	0 (0%)	10 (12.5%)				
	>15 µg/L	6 (7.5%)	44 (55%)	20 (25%)	0 (0%)	70 (87.5%)				
PGI/PGII ratio	<3 µg/L	2 (2.5%)	18 (22.5%)	13 (16.25%)	0 (0%)	33 (41.25%)	1259.89	0.000	-0.224	0.046
	3-20 µg/L	4 (5%)	31 (38.75%)	12 (15%)	0 (0%)	47 (58.75%)				
	>20 µg/L	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)				
Gastrin17	< 1 pmol/ml	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	494.53	0.000	-0.050	0.663
	1-7 pmol/ml	6 (7.5%)	39 (48.75%)	25 (31.25%)	0 (0%)	70 (87.5%)				
	>7 pmol/ml	0 (0%)	10 (12.5%)	0 (0%)	0 (0%)	10 (12.5%)				
Parameters		PMNs Fas gene					χ^2	P value	r	P value
Cag A genotype	Positive	20 (25%)	24 (30%)	3 (3.75%)	0 (0%)	47 (58.75%)	29.177	0.015	0.262	0.019
	Negative	23 (28.75%)	10 (12.5%)	0 (0%)	0 (0%)	33 (41.25%)				
PGI	<30 µg/L	3 (3.75%)	3 (3.75%)	0 (0%)	0 (0%)	6 (7.5%)	654.03	0.000	-0.296	0.008
	30-160 µg/L	21 (26.25%)	25 (31.25%)	3 (3.75%)	0 (0%)	49 (61.25%)				
	>160 µg/L	16 (20%)	9 (11.25%)	0 (0%)	0 (0%)	25 (31.25%)				
PGII	<3 µg/L	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	534.59	0.000	-.018	0.874
	3-15 µg/L	9 (11.25%)	7 (8.75%)	3 (3.75%)	0 (0%)	19 (23.75%)				
	>15 µg/L	31 (38.75%)	30 (37.5%)	0 (0%)	0 (0%)	61 (76.25%)				
PGI/PGII	<3 µg/L	16 (20%)	17 (21.25%)	0 (0%)	0 (0%)	33 (41.25%)	1033.23	0.000	-0.014	0.905
	3-20 µg/L	24 (30%)	20 (25%)	3 (3.75%)	0 (0%)	47 (58.75%)				
	>20 µg/L	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)				
Gastrin17	< 1 pmol/ml	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	324.95	0.000	-0.007	0.952
	1-7 pmol/ml	34 (42.5%)	33 (41.25 %)	3 (3.75%)	0 (0%)	70 (87.5 %)				
	>7 pmol/ml	6 (7.5 %)	4 (5%)	0 (0%)	0 (0%)	10 (12.5 %)				

Significant difference in grade of Fas expression (p value < 0.001) without correlation between level of gastrin17 and grade of gastric lymphocyte Fas expression (p value = 0.663). Significant difference with correlation between *CagA* expression and grade of PMNs Fas (p value = 0.015, p value = 0.019), (Table 5). Significant difference in grade of PMNs Fas (p value < 0.001) with significant correlation between level of PGI and PMNs Fas grade (p value = 0.008). Significant difference in grade of PMNs Fas (p value < 0.001) without correlation with PGII level (p value = 0.874). Significant difference in grade of PMNs Fas expression (p value < 0.001) without correlation between level of PGI/PGII and grade of PMNs Fas (p value = 0.905). Normal gastrin17 detected in (87.5%), (3.75%) have moderate PMNs Fas grade, (41.25 %) have mild PMNs Fas.

Table 6: Correlation of cellular Fas gene and gastric secretion

Parameters		Cellular Fas gene expressed in epithelia					χ^2	P value	r	P value
		<5%	5-25%	26-50%	50% >	Total				
Cag A genotype	Positive	0 (0%)	25 (31.25%)	22 (27.5%)	0 (0%)	47 (58.75%)	43.225	0.000	-0.104	0.358
	Negative	0 (0%)	19 (23.75%)	14 (17.5%)	0 (0%)	33 (41.25%)				
PGI	<30 µg/L	0 (0%)	4 (5%)	2 (2.5%)	0 (0%)	6 (7.5%)	762.552	0.000	0.038	0.737
	30-160 µg/L	0 (0%)	27 (33.75%)	22 (27.5%)	0 (0%)	49 (61.25%)				
	>160 µg/L	0 (0%)	13 (16.25%)	12 (15%)	0 (0%)	25 (31.25%)				
PGII	<3 µg/L	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	689.201	0.000	0.148	0.191
	3-15 µg/L	0 (0%)	15 (18.75%)	4 (5%)	0 (0%)	19 (23.75%)				
	>15 µg/L	0 (0%)	29 (36.25%)	32 (40%)	0 (0%)	61 (76.25%)				
PGI/PGII	<3 µg/L	16 (20%)	17 (21.25%)	0 (0%)	0 (0%)	33 (41.25%)	1120.384	0.000	-0.068	0.547
	3-20 µg/L	24 (30%)	20 (25%)	3 (3.75%)	0 (0%)	47 (58.75%)				
	>20 µg/L	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)				
Gastrin17	< 1 pmol/ml	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	341.613	0.000	-0.098	0.388
	1-7 pmol/ml	34 (42.5%)	33 (41.25%)	3 (3.75%)	0 (0%)	70 (87.5%)				
	>7 pmol/ml	6 (7.5%)	4 (5%)	0 (0%)	0 (0%)	10 (12.5%)				
Parameters		Cellular Fas gene expressed in lamina propria								
		<5%	5-25%	26-50%	50% >	Total	χ^2	P value	r	P value
Cag A genotype	Positive	0 (0%)	32 (40%)	15 (20%)	0 (0%)	47 (58.75%)	44.395	0.001	-.215	0.056
	Negative	0 (0%)	13 (16.25%)	20 (25%)	0 (0%)	33 (41.25%)				
PGI	<30 µg/L	0 (0%)	3 (3.75%)	3 (3.75%)	0 (0%)	6 (7.5%)	762.552	0.000	-0.038	0.737
	30-160 µg/L	0 (0%)	22 (27.5%)	27 (33.75%)	0 (0%)	49 (61.25%)				
	>160 µg/L	0 (0%)	20 (25%)	5 (6.25%)	0 (0%)	25 (31.25%)				
PGII	<3 µg/L	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	689.201	0.000	0.148	0.191
	3-15 µg/L	0 (0%)	0 (0%)	0 (0%)	19 (23.75%)	19 (23.75%)				
	>15 µg/L	0 (0%)	0 (0%)	0 (0%)	61 (76.25%)	61 (76.25%)				
PGI/PGII	<3 µg/L	0 (0%)	19 (23.75%)	14 (17.5%)	0 (0%)	33 (41.25%)	1120.384	0.000	0.068	0.547
	3-20 µg/L	0 (0%)	26 (32.5%)	21 (26.25%)	0 (0%)	47 (58.75%)				
	>20 µg/L	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)				
Gastrin17	< 1 pmol/ml	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	586.079	0.000	0.020	0.858
	1-7 pmol/ml	0 (0%)	40 (50%)	30 (37.5%)	0 (0%)	70 (87.5%)				
	>7 pmol/ml	0 (0%)	5 (6.25%)	5 (6.25%)	0 (0%)	10 (12.5%)				

Hypergastrinemia detected in (12.5%), (5%) have mild grade of PMNs Fas. Significant difference in grade of PMNs Fas (p value < 0.001) without correlation between serum gastrin17 and grade of gastric glands Fas (p value = 0.952).

As shown in Table 6, moderate grade of PMNs & lymphocytes Fas in gastric epithelia reported in (27.5%) of cases, (31.25%) have mild expression. Significant difference without correlation between *CagA* and grade of PMNs & lymphocytes Fas expression (p value = 0.015, p value = 0.358). Significant difference among disorders in grade of PMNs &lymphocytes Fas expression (p value < 0.001) without significant correlation between PGI levels and grade of PMNs & lymphocytes Fas expression (p value = 0.737). Significant difference among disorders in grade of PMNs & lymphocytes Fas (p value < 0.001) without correlation with PGII levels (p value = 0.191). Hyposecretion of PGI/PGII detected in (41.25%), (21.25%) have mild grade of PMNs & lymphocytes Fas expression. Significant difference in grade of PMNs & lymphocytes Fas (p value < 0.001) without correlation (p value = 0.547). Significant difference among disorders in grade of PMNs Fas expression (p value < 0.001) without correlation between serum level of gastrin-17 and grade of PMNs & lymphocytes Fas expression (p value = 0.388).

As shown in Table 6, significant difference with marginal correlation between *CagA* gene expression and grade of PMNs & lymphocytes Fas (p value = 0.001, p value = 0.056). Significant difference in grade of PMNs & lymphocytes Fas (p value < 0.001) without significant correlation between serum level of PGI and grade of PMNs &lymphocytes Fas (p value = 0.737). Significant difference among disorders in grade of PMNs & lymphocytes Fas (p value < 0.001) without correlation with PGII levels (p value = 0.191).

As shown in Table 6, significant difference among disorders in grade of PMNs & lymphocytes Fas expression in lamina propria (p value < 0.001) without correlation (p value = 0.547). Significant difference among disorders in grade of PMNs Fas gene (p value < 0.001) without correlation between level of gastrin-17 and grade of PMNs & lymphocytes Fas expression (p value = 0.858).

Discussion

In this cross sectional study, (80) of *H. pylori* infected patients, age range 16-80 years, mean (47.24 ± 18.82) years were studied. The frequency of *H. pylori* associated disorders in current study starting from gastritis (37.5%), gastropathy & gastric ulcer (18.75%), DU(15%), duodenitis (7.5%), prepyloric ulcer (2.5%) which come in line with others[4] .

In current study high grade of Fas gene expression was not detected at all. Mild lymphocytes expression (5-25%) reported in (61.25%), followed by moderate grade (26-50%) of expression (31.25%) among different disorders. In PMNs, mild Fas expression was reported in (46.25%) versus (3.75%) moderate the frequency of Fas gene expression more frequently reported in gastritis and gastropathy. Even there was significant difference in lymphocytes Fas expression among disorders, there was no correlation between this expression and type of gastric disorder. This is a logical finding because the induction of Fas expression via infiltrating lymphocytes and PMNs is associated and attributed to the induction via *H. pylori* infection [10]. Low Fas expression in infiltrating PMNs compared with infiltrating lymphocytes may attributed to the chronic active inflammatory reaction among different disorders [11].

One of interesting points that the lymphocytes and PMNs Fas expression in epithelial lining range from mild (gastric ulcer; gastritis; gastropathy) to moderate (gastritis ; DU; gastropathy) with significant difference. While in lamina propria, infiltrating lymphocytes and PMNs among all disorders have high grade of Fas gene expression. This may attributed to the ability of *H. pylori* to invade deeply in gastric tissue gastric beyond mucosal epithelia, which indicates initiation of interaction between *H. pylori* and inflammatory cells in lamina propria leading to activation of NFkB gene in gastric as well as Lymphocytes and PMNs, finally IL8 production and increase oxidative stress in gastric tissue leading final to increase Fas gene production which is more obvious in lamina propria than in gastric epithelia, reflecting the underling pathology in near future [1, 10, 11].

In current study *CagA* genotype have a positive effect and correlation on the gastric cells Fas over expression in (43.75%). Mild expression detected in (10%). This results come in line with [1, 10]. This result come in concordance with opinion of [10, 18] stated that whenever *H. pylori* have potent Cag PAI, bacterial adhesion, good signals will be received by the gastric epithelial cells which reflect its response by increasing of expression of MHCII to play as antigen presenting cell (APC) and strong Th1 response will be occur with obvious IFNγ secretion which acts as good stimulator for up-regulation of FAS and even FASL in gastroduodenal tissue and tissue infiltrating lymphocytes (TILs).

One of exciting things in this study, the fluctuation of gastric Fas expression independently from a status of gastric hormones. All cases with hyposecretion of PGI; PGI/PGII characterized by gastric Fas overexpression, (7.5%) vs. (27.5%). On the other hand all cases with normal gastric hormone

level also associated with gastric Fas overexpress, PGI (31.25%), PGII (15%), PGI/PGII (33.75%), G17 (56.25%) without any significant correlation between a status of PGI, PGI/PGII, Gastrin 17 and gastric Fas overexpression. PGII have positive correlation with gastric Fas overexpression among different disorders.

These results indicating a *H. pylori* induced pangastric type of mucosal inflammation even to duodenal region that reflects the disruption in gastric hormones secretion mainly over production of PGI (31.25%), PGII (76.25%), G17 (5%) [19]. Gastric Fas expression and gastric hormones have indirect correlation. Mucosal colonization by the *H. pylori* ignites a cascade of events that result in large increases in inflammatory cytokines in the infected tissue which were originated from the gastric mucosa as well as from infiltrating inflammatory cells. Among these inflammatory cytokines, IL-1, IL-2, TNF-α, and IFNγ that have been shown to up-regulate the expression of Fas antigen in gastric as well as inflammatory cells. T-helper type 1 (Th1) cells are selectively increased during *H. pylori* infection [20]. Th1 cytokines, such as IFNγ and TNF-α, can increase the release of proinflammatory cytokines, such as IL-8 from the epithelium as well as Fas and Fas ligand (FasL) 34. Furthermore, these cytokines can also increase the expression of MHC class II molecules by gastric epithelial cells, thereby increasing the binding of *H. pylori* to the gastric epithelium [21].

One of frustrating things that there was no clinical study to evaluates the correlation between gastric Fas expression and the level of gastric hormones. One of remarkable finding in current study when making topographical analysis for the correlation between gastric hormones and gastric Fas expression in gastric epithelia, lamina propria and gastric glands. This study reported a positive correlation between gastric Fas expression and PGI;PGII in gastric epithelia, lamina propria but not in gastric gland even with presence of significant difference in gastric hormones according to Fas gene expression.

Attachment of *H. pylori* on specific molecules expressed and acts as a receptor in the gastric epithelia, MHCII and CD74 using multiple adhesins that have been identified on the outer membrane of *H. pylori* such as BabA, SabA. BabA and SabA bind to fucosylated and sialylated blood group antigens, respectively. While the attachment of *H. pylori* using BabA as an adhesin does not appear to induce signaling or immune responses from host cells, SabA appears to be required for activation of neutrophils and the resulting oxidative burst by binding to sialylated neutrophil receptors [22]. This inflammatory signal extended throughout gastric epithelia to lamina propria also via the effect of neutrophil activating protein and urease as well as *CagA* production by *H. pylori* which in turn leads to activation of lymphocytes and gastric NFkB causing production of proinflammatory cytokines, mainly IL8 and hence the gastric epithelia and lamina propria sinking with inflammatory reaction and cells leading to increase of Fas expression on gastric epithelia and lamina propria as well as inflammatory cells [1, 11, 23]. Because *CagA* interacts with important signaling mediators in the gastric cells, it is considered responsible for changes in cell morphology, adhesion and turnover [23]. *CagA* cause increase in Fas expression independently or in conjunction with other virulence factors such as urease [10, 11]. At the same time as a results of pan gastric inflammatory reaction this leads to direct effects on PGII producing cells causing increase in PGII production and as a results of neuroendocrine activity through vagus nerve and gastrin, PGI also affected. Fas expression on gastric glands has no correlation with gastric hormones even in the presence of significant differences in hormones according to grade of Fas expression may be associated with fact that gastric glands appear to be Immunological privileged site.

In current study, *H. pylori* influence on apoptosis of lymphocytes in gastric mucosa .lymphocytes Fas expression has positive correlation with PGI; PGII, PGI/PGII. Obvious fluctuation of lymphocytes Fas according to gastric hormones from mild to moderate expression as in Table 5. One of remarkable finding was the mild to moderate expression of Fas on PMNS which has positive correlation with PGI serum level only. This may attributed to chronic active pangastric inflammation affecting the PGI, PGII producing chief cells within gastric mucosa, reflecting by (3.75%) mild expression of PMNs, (6.25%) in lymphocytes and clinically patients suffered from atrophic corpus gastritis. The mild to moderate expression of Fas on gastric tissue infiltrating lymphocytes give an indication about the immunopathological role of these cells in underling clinical diseases .by relatively low expression of apoptotic marker (Fas) in lymphocytes as well as PMNs this will leads to delay spontaneous apoptosis and prolong their survival. It results in the prolonged activity of cytokines secreted by these cells and therefore augments their damaging action upon gastric mucosa [24, 25]. On the other hand the mild to moderate expression of Fas receptors on lymphocytes and PMNs act in delay of their proliferation and affects its ability to get rid of *H. pylori* from gastric tissue, and at sum this will cause persistence infection and a protective strategy for *H. pylori* [24, 26].

When making topographical analysis for the correlation between gastric hormones and inflammatory cells (lymphocytes & PMNs) Fas expression in gastric epithelia, lamina propria; No correlation was reported between inflammatory cells (lymphocytes & PMNs) Fas expression and gastric hormones secretion in gastric epithelia, lamina propria even with presence of significant difference in gastric hormones according to Fas gene expression as in Table 6. This finding reflect the role of; and give an

indication that *H. pylori* acts as inducer for Fas gene expression on gastric cells as well as lymphocytes & PMNs, directly through its virulence factors, mainly products of Cag pathogenicity island, *CagA* and others, and indirectly through cytokines produce by TH1 cells such as IFNγ as well as the oxidative stress produced by reactive oxygen species from inflammatory cells infiltrated into gastric tissue due to *H. pylori* infection [10, 11, 24].

In conclusion, Fas gene expression and localization on gastric and inflammatory cells affected directly by *H. pylori CagA* and indirectly by gastric hormones. This contributes to progression of various gastric disorders according to severity of *CagA* induced gastric pathology and gastric hormones disturbance throughout the course of infection and disease.

References

1. Al-Ezzy AIA. Molecular and Immunopathological role of Gastric versus lymphocytes Interleukin 8 Gene Expression in H. pylori induced Fas-Fasl apoptotic pathway in Gastroduodenal Ulcer in Iraqi patients. Journal of Biology, Agriculture and Healthcare. 2015;5:141-53.

2. AL-Ezzy AIA. Evaluation of Clinicopathological and Risk Factors for Nonmalignant H. Pylori Associated Gastroduodenal Disorders in Iraqi Patients. OA Maced J Med Sci. 2015;3:655-60. http://dx.doi.org/10.3889/oamjms.2015.113

3. Shafii M, Nikzad SE, Kasiri H, Naghipour M. Histopathological evaluation of chronic gastritis with and without Helicobacter pylori colonization: a study from Iran. Malays J Pathol. 2008;30:27-30. PMid:19108408

4. Xu X-Q, Wang Z-H, Liao J-X, Chen X-Y, Liu W-Z, Xiao S-D, et al. Predictive value of neutrophil infiltration as a marker of Helicobacter pylori infection. World journal of gastroenterology: WJG. 2012;18:5101. http://dx.doi.org/10.3748/wjg.v18.i36.5101 PMid:23049221 PMCid:PMC3460339

5. Chowers MY, Keller N, Tal R, Barshack I, Lang R, Bar-Meir S, et al. Human gastrin: a Helicobacter pylori specific growth factor. Gastroenterology. 1999;117:1113-8. http://dx.doi.org/10.1016/S0016-5085(99)70396-3

6. Rau T, Sonst A, Rogler A, Burnat G, Neumann H, Oeckl K, et al. Gastrin mediated down regulation of ghrelin and its pathophysiological role in atrophic gastritis. J Physiol Pharmacol. 2013;64:719-25. PMid:24388886

7. Sarker SA, Sultana S, Sattar S, Ahmed T, Beglinger C, Gyr N, et al. Influence of Helicobacter pylori Infection on Gastric Acid Secretion in PreSchool Bangladeshi Children. Helicobacter. 2012;17:333-9. http://dx.doi.org/10.1111/j.1523-5378.2012.00965.x PMid:22967116

8. Galmiche A, Rassow J, Doye A, Cagnol S, Chambard JC, Contamin S, de Thillot V, Just I, Ricci V, Solcia E, Van Obberghen E, Boquet P. The N-terminal 34 kDa fragment of Helicobacter pylori vacuolating cytotoxin targets mitochondria and induces cytochrome c release. EMBO J. 2000;19(23):6361-70. http://dx.doi.org/10.1093/emboj/19.23.6361 PMid:11101509 PMCid:PMC305856

9. Fan X, Gunasena H, Cheng Z, Espejo R, Crowe SE, Ernst PB, Reyes VE. Helicobacter pylori urease binds to class II MHC on gastric epithelial cells and induces their apoptosis. J Immunol. 2000;165(4):1918-24. http://dx.doi.org/10.4049/jimmunol.165.4.1918 PMid:10925273

10. AL-Ezzy AIA. Immunopathological Role of FAS-FASL Apoptotic Pathway in H. pylori CagA Positive Associated Chronic Atrophic Gastritis in Iraqi Patients. Journal of Biology, Agriculture and Healthcare. 2014;4:67-74.

11. Al-Ezzy AIA. Molecular and Immunopathological role of nuclear factor k b detected by insitu hybridization in pathogenesis of chronic atrophic gastritis in Iraqi patients. International Journal of Advanced Research. 2014;2:67-78.

12. Serim RC. PyloriTek® For H. pylori http://www.serim.com.

13. Subbukesavaraja V, Balan K. Comparative study of invasive methods for diagnosis of Helicobacter pylori in humans. Int J Curr Microbiol App Sci. 2013;2:63-8.

14. Tzeng J-E, Lin Y-L, Chung S-M, Chu Y-T. Comparison of four diagnostic methods for Helicobacter pylori. Tzu Chi Med J. 2005;17:339-43.

15. Maxim biotech. Insitu hybridization detection system.

16. Pity IS, Baizeed AM. Identification of helicobacter pylori in gastric biopsies of Patients with chronic gastritis: histopathological and Immunohistochemical study. Duhok Medical Journal. 2011;5.

17. Biohit H. Biohit gastro panel http://www.biohithealthcare.co.uk/.

18. Zhang Z-W, Dorrell N, Wren BW, Farthing MJ. Helicobacter pylori adherence to gastric epithelial cells: a role for non-adhesin virulence genes. Journal of medical microbiology. 2002;51:495-502. http://dx.doi.org/10.1099/0022-1317-51-6-495 PMid:12018657

19. Marcis L, Sanita L, Inese P, Dace R, Ilona V, Ilva D, et al. Pepsinogen testing for evaluation of the success of Helicobacterpylori eradication at 4 weeks after completion of therapy. Medicina. 2015;50:8-13.

20. Bamford KB, Fan X, Crowe SE, Leary JF, Gourley WK, Luthra GK, et al. Lymphocytes in the human gastric mucosa during Helicobacter pylori have a T helper cell 1 phenotype. Gastroenterology. 1998;114:482-92. http://dx.doi.org/10.1016/S0016-5085(98)70531-1

21. Fan X, Crowe SE, Behar S, Gunasena H, Ye G, Haeberle H, et al. The effect of class II major histocompatibility complex expression on adherence of Helicobacter pylori and induction of apoptosis in gastric epithelial cells: a mechanism for T helper cell type 1mediated damage. The Journal of experimental medicine. 1998;187:1659-69. http://dx.doi.org/10.1084/jem.187.10.1659 PMid:9584144 PMCid:PMC2212295

22. Unemo M, Aspholm-Hurtig M, Ilver D, Bergström J, Borén T, Danielsson D, et al. The sialic acid binding SabA adhesin of Helicobacter pylori is essential for nonopsonic activation of human neutrophils. J Biol Chem. 2005;280:15390-7. http://dx.doi.org/10.1074/jbc.M412725200 PMid:15689619

23. Beswick EJ, Suarez G, Reyes VE. H. pylori and host interactions that influence pathogenesis. World J Gastroenterol. 2006;12:5599-605. PMid:17007010 PMCid:PMC4088158

24. Kotłowska-Kmieć A, Bąkowska A, Szarszewski A, Kamińska B, Łuczak G, Radys W, et al. Helicobacter pylori increases expression of proapoptotic markers Fas and FasL on CD4 lymphocytes in children. Acta Biochemica Polonica. 2009;56:433-8. PMid:19572057

25. Bussiere F, Chaturvedi R, Asim M. Low multiplicity of infection of Helicobacter pylori suppresses apoptosis of B lymphocytes. Cancer Res. 2006;66:6834-42. http://dx.doi.org/10.1158/0008-5472.CAN-05-4197 PMid:16818661

26. Schmees C, Prinz C, Treptau T, Rad R, Hengst L, Voland P, et al. Inhibition of T-cell proliferation by Helicobacter pylori -glutamyl transpeptidase. Gastroenterology. 2007;132:1820-33. http://dx.doi.org/10.1053/j.gastro.2007.02.031 PMid:17484877

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):374-380.
http://dx.doi.org/10.3889/oamjms.2016.064
eISSN: 1857-9655
Clinical Science

Diagnostic Accuracy of Acoustic Radiation Force Impulse (ARFI) in Diagnosis of Liver Fibrosis among Egyptian Patients with Chronic HCV Infection

Yasser A. Elhosary[1], Saleh M. Saleh[2], Wafaa M. Ezzat[1*], Dirk-André Clevert[3]

[1]Department of Internal Medicine, National Research Center, Cairo, Egypt; [2]Department of Internal Medicine, National Liver Institute, Menoufia, Egypt; [3]Department of Clinical Radiology, Munich University Hospital, Munich, Germany

Citation: Elhosary YA, Saleh SM, Ezzat WM, Clevert D-A. Diagnostic Accuracy of Acoustic Radiation Force Impulse (ARFI) in Diagnosis of Liver Fibrosis among Egyptian Patients with Chronic HCV Infection. Open Access Maced J Med Sci. 2016 Sep 15; 4(3):374-380. http://dx.doi.org/10.3889/oamjms.2016.064

Keywords: HCV; ARFI; liver fibrosis; diagnostic accuracy.

***Correspondence:** Dr. Wafaa M Ezzat. National Research Center, Internal Medicine, Elbohoos st., Cairo 12311, Egypt. E-Mail: wafaa_3t@yahoo.com

Received: 21-Apr-2016; **Revised:** 14-May-2016; **Accepted:** 15-May-2016; **Online first:** 08-Jun-2016

Competing Interests: The authors have declared that no competing interests exist.

List of abbreviations: ARFI: Acoustic Radiation Force Impulse; HCV: hepatitis C virus infection; AST: aspartate transaminase; ALT: alanine transaminase; INR: international normalized ratio; TE: Transient elastography; RTE: real-time elastography; ROI: region of interest; AUROC: area under receiving operating curve

Abstract

BACKGROUND: Acoustic radiation force impulse (ARFI) evaluates liver stiffness non-invasively and was invented recently. This technique can easily and accurately assess the degree of liver fibrosis in clinical practice.

AIM: The aim of this study was to detect the diagnostic performance of ARFI elastography in the staging of fibrosis in some Egyptian patients with chronic HCV infection.

PATIENTS AND METHODS: One hundred ninety patients with chronic HCV infection; 142 men and 48 women were enrolled in the study. They underwent liver biopsy examination for liver fibrosis detection. All demographic; clinical and biochemical data were recoded. ARFI examination was done for all subjects to detect liver stiffness measurement in relation to liver fibrosis detected by pathological examination of liver biopsies.

RESULTS: Medians of liver stiffness measurement by shear wave velocity showed a significant increase as a grade of liver fibrosis increases ($p \leq 0.0001$, highly significant). Liver stiffness was directly correlated to age, AST; ALT; INR and liver steatosis (p values were: 0.009; 0.0001; 0.013; 0.006 and 0.04 respectively, significant). On the other hand, liver stiffness was inversely correlated to albumin; prothrombin concentration and platelets (p values were: 0.0001; 0.001, and 0.0001, respectively, significant). We found that shear wave velocity can predict F1; F2; F3 and F4 at cut-off values: 1.22; 1.32; 1.44 and 1.8 respectively.

CONCLUSION: ARFI is a diagnostic noninvasive promising technique for liver fibrosis diagnosis among Egyptian patients with chronic HCV infection.

Introduction

The incidence of chronic hepatitis C has been increasing gradually and is a potentially life-threatening problem especially among Egyptians. Sustained and repeated inflammatory stimuli can cause a stress repair response in the body, leading to the accumulation of a large number of extracellular matrixes in the liver tissue, leading to fibrosis [1]. Progression of fibrosis can cause cirrhosis or even liver cancer. Therefore, early detection is important for the prevention and control of liver fibrosis and cirrhosis. Liver biopsy is the main diagnostic method for liver fibrosis. However, it is an invasive method and associated complications limit its safety [2].

Recently, various non-invasive techniques for liver fibrosis assessment have been developed, including serum biomarkers and elastography techniques. Transient elastography (TE), real-time elastography (RTE), acoustic radiation force impulse imaging (ARFI) are the most frequent elastography techniques [3, 4].

ARFI evaluates liver stiffness non-invasively and was invented recently. This technique can easily and accurately assess the degree of liver fibrosis in clinical practice [5-7]. Fierbinteanu-Braticevici et al [8] reported that ARFI elastography showed very good accuracy in assessing all stages of liver fibrosis. In

addition, a meta-analysis by Nierhoff et al [9] also demonstrated good diagnostic accuracy.

Nowadays, early and continuous detection of liver fibrosis has become increasingly important in order to make therapeutic decisions, determine prognosis and to follow-up disease progression. Hepatic fibrogenesis is a dynamic process reflecting an imbalanced extracellular matrix turnover. Evaluating the evolution of fibrosis over time can be therefore more important than a “once only” diagnosis. Recent evidence suggesting that liver fibrosis can be reversible [10, 11], further emphasizes the importance to monitor fibrosis over time, other than simply diagnosing its presence and staging its severity. Accurate, reproducible and easily applied methods are therefore required for the assessment of hepatic fibrosis

The aim of this study was to detect the diagnostic performance of ARFI elastography in the staging of fibrosis in some Egyptian patients with chronic HCV infection.

Patients and Methods

Patients

The study was conducted on 190 patients with chronic HCV infection; 142 men and 48 women with documented chronic HCV infection, recruited from Agouza Liver Center and National Research Center, Cairo. Chronic HCV diagnosis was based on elevated serum transaminase levels for at least six months and positive HCV antibody by the second-generation enzyme-linked immunosorbent assay and confirmed by detection of circulating HCV RNA using polymerase chain reaction (PCR). A liver biopsy specimen was taken from every patient. The current study was approved by ethical committee in the national research center. An informed consent was signed by every patient before enrollment in the study. Demographic data; clinical data; biochemical investigations; results of abdominal ultrasonography; histopathological examination of liver biopsies and data of ARFI examination were recoded for every enrolled patient.

We excluded any patients with history renal disorder, the recent history of cardiovascular disease or patients with hepatitis B infection or human immunodeficiency virus infection, autoimmune or metabolic liver diseases; abnormal coagulation profiles that preclude liver biopsies; international normalized ratio (INR) > 1.5, prothrombin time > 50 seconds and platelet count < 50,000/mL. Relative and absolute contraindications to liver biopsy, e.g. biliary ductal dilatation, ascites, and the presence of hepatocellular carcinoma were also excluded.

Histopathological Examination of Liver Biopsies

Ultrasound-guided percutaneous liver biopsy specimens were taken from the patients and examined by two different pathologists , experienced in liver histology, who were unaware of the laboratory results or clinical diagnosis. Only specimens with the inter-observer agreement of stage of hepatic fibrosis were included in the study. METAVIR scoring system was used for staging hepatic fibrosis. Every biopsy specimen was staged on a scale of F0 to F4 (F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = few septa, F3 = numerous septa without cirrhosis, and F4 = cirrhosis) [12, 13].

Acoustic radiation force impulse elastography

Acoustic radiation force impulse elastography was performed for all subjects within two months of the date of liver biopsy with a Siemens Acuson S3000 Virtual Touch ultrasound system (Siemens AG, Erlangen, Germany) with a 6CI transducer. The principle underlying ARFI elastography is that sharing of the examined tissue induces a strain in the tissues. An acoustic ‘‘push’’ pulse is automatically produced by the ultrasound probe and directed to the side of a region of interest (ROI), which is where the speed of the shear wave is measured. This ROI has a predefined size, provided by the system (10 mm long and 5 mm wide).

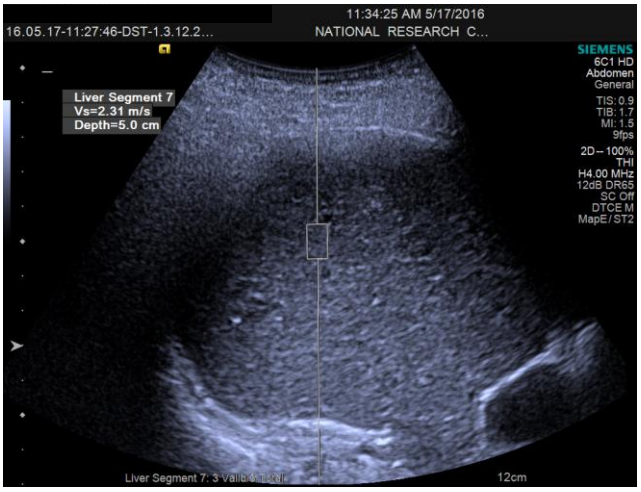

Figure 1: Ultrasonographic picture of ARFI examination for liver fibrosis

The acoustic ‘‘push’’ pulse generates shear waves that propagate into the tissue, perpendicular to the ‘‘push’’ axis. Detection waves are also generated by the transducer to measure the propagation speed of these shear waves, which increases with fibrosis severity [14]. The speed of the shear waves, measured in meters per second, as well as measurement depth, is displayed by the system. For each patient, 10 valid ARFI measurements were

performed under fasting conditions, with the patient in supine position with the right arm in maximum abduction, by the intercostal approach in the right liver lobe, 1–2 cm under the liver capsule. Minimal scanning pressure was applied by the operator; the patient was asked to stop normal breathing for a moment to minimize breathing motion. The mean of 8-10 valid measurements was calculated and considered indicative of the severity of fibrosis (Fig. 1, Fig. 2). The operators were blinded to any clinical or elastographic data.

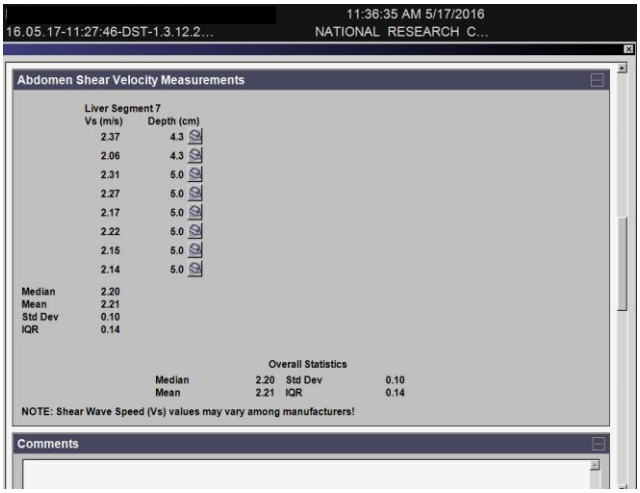

Figure 2: Output report of ARFI examination wit means, median and standard deviation

Statistical analysis

Data are expressed as median or mean ± SD or median and range where appropriate. Categorical data are described as the frequency of the subjects with a specific characteristic. Chi-square test or Fisher's exact test was used for comparing categorical data and Student's t-test, Mann-Whitney-U-test, or Kruskal-Wallis test, when appropriate, was used for comparing continuous variables. The sensitivity-to-specificity relationship of each noninvasive diagnostic test was assessed by using receiver operating characteristic curves. Area under the receiver operating characteristic curve and the 95% confidence interval were calculated for detection of histologic fibrosis stage 2 or higher disease (F ≥ 2), detection of histologic fibrosis stage 3 or higher disease (F ≥ 3), and detection of cirrhosis (F4) in the entire study population. P ≤ .05 was considered indicative of a significant difference. Statistical analysis was performed using SPSS software version 12.0 (SPSS Inc., Chicago, IL, USA).

Results

Base line features of the studied patients were present in Table 1. One hundred ninety patients with chronic HCV infection who underwent liver biopsies for pathological examination were enrolled in the current study from May 2014 to May 2015. They were 142 men and 48 women with mean age of 53.74 ± 12.05.

Table 1: Patients' characteristics

Variables		HCV patients N = 190
Age in years	Mean ± SD	53.74 ± 12.05
Sex		
	Male No (%)	142 (74.7)
	Female No (%)	48 (25.3)
PH of Schisto:		
	Negative No (%)	63 (33.2)
	Positive No (%)	127 (66.8)
Viral load IU/mL	Median (range)	114000 (0- 33400000)
AST IU/L	Median (range)	44.50 (1.97-283)
ALT IU/L	Median (range)	38.00 (8.00-270)
Albumin gm/dl	Median (range)	3.90 (1.40-4.01)
Total bilirubin mg/dl	Median (range)	1.20 (0.20-4.5)
Direct bilirubin mg/dl	Median (range)	0.50 (0.00-3.9)
P C %	Median (range)	79.00 (31-199)
INR	Median (range)	1.20 (0.8-3.9)
GGT IU/L	Median (range)	31.00 (1.3-271)
Platelets $\times 10^3$	Median (range)	130.50 (0.95-512.00)
AFP ng/ml	Median (range)	6.50 (0.20-7900)
CHOL mg/dl	Mean ± SD	167.69 ± 40.36
TRG mg/dl	Mean ± SD	114.85 ± 39.30
Histopathological examination		
Steatosis % median(range)		9 (4-17)
Fibrosis :		
F 1	No (%)	25 (13.2)
F 2	No (%)	28 (14.7)
F 3	No (%)	29 (15.3)
F 4	No (%)	108 (56.8)
Activity:		
A 0	No (%)	8 (4.2)
A 1	No (%)	64 (33.7)
A 2	No (%)	100 (52.7)
A 3	No (%)	18 (9.4)

PH of Schisto: past history of Schistosomiasis; AST: aspartate transaminase; ALT: alanine transaminase; PC%: prothrombin concentration; INR: international normalized ratio; GGT: Gamma-glutamyl transpeptidase; AFP: α-fetoprotein; CHOL: cholesterol; TRG: triglycerides.

The frequency of past history of Schistosomiasis was 66.8%. Histopathological examination of liver biopsies revealed that distribution of liver fibrosis grades: F1; F2; F3; F4 was 13.2%; 14.7%; 15.3% and 56.8% respectively. Medians of liver stiffness measurement by sheer wave velocity showed a significant gradual increase as a grade of liver fibrosis increases (p ≤ 0.0001, highly significant) as shown in Table 2 and Fig. 3.

Table 2: Distribution of liver stiffness measurements by ARFI among different grades of liver fibrosis

Grades of fibrosis	Shear wave velocity m/sec Median	Shear wave velocity m/sec Range	X^2	P value
F1	1.20	1.10 - 1.40	26.460	0.0001*
F2	1.43	1.26 - 1.62		
F3	1.90	1.43 - 2.80		
F4	2.70	1.52 - 3.90		

*: p is highly significant.

Analyzing correlation of liver stiffness measurement by shear wave velocity to the studied parameters revealed that liver stiffness was directly correlated to age, AST; ALT; INR and liver steatosis (p values were: 0.009; 0.0001; 0.013; 0.006 and 0.04 respectively, significant).

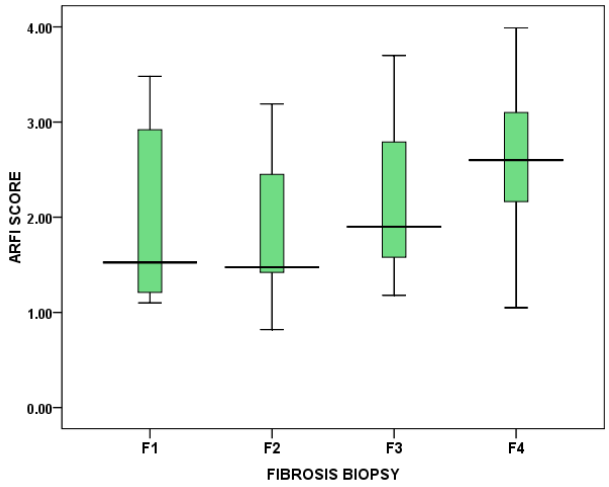

Figure 3: Box Plot diagram for distribution of liver stiffness measurement by ARFI among different grades of liver fibrosis

On the other hand, liver stiffness was inversely correlated to albumin; prothrombin concentration and platelets (p values were: 0.0001; 0.001 and 0.0001, respectively, significant) as shown in Table 3.

Table 3: Correlation of liver stiffness measurements by ARFI with different studied variables among the studied patients

Variables	Correlation Coefficient R	Coefficient of determination R2.	P value
Age	0.175**	0.031	0.009
AST	0.295**	0.087	0.000
ALT	0.165*	0.027	0.013
Albumin	-0.329**	0.108	0.000
Total bilirubin	0.127	0.016	0.057
Direct bilirubin	0.062	0.004	0.354
P C	-0.224**	0.050	0.001
INR	0.183**	0.033	0.006
GGT	0.036	0.001	0.593
Platelets	-0.300**	0.09	0.000
Viral load	-0.036-	0.001	0.596
AFP	0.110	0.012	0.099
CHOL	-0.086	0.007	0.200
TRG	-0.089	0.008	0.186
Liver steatosis	0.141*	0.020	0.042

AST: aspartate transaminase; ALT: alanine transaminase; PC%: prothrombin concentration; INR: international normalized ratio; GGT: Gamma-glutamyl transpeptidase; AFP: α-fetoprotein; CHOL: cholesterol; TRG: triglycerides; *: p is significant; **: p is highly significant.

Multiple regression (assessed by standardized B coefficients) analysis to find an independent predictor for an increase of liver stiffness revealed that platelet count is the independent factor for increasing liver stiffness measurement by ARFI (B = -0.006, P = 0.048, significant). There was no any significant impact of sex or past history of Schistosomiasis on liver stiffness measurements (p values were: 0.812 and 0.262 respectively, insignificant).

As regards diagnostic accuracy of ARFI technique for liver fibrosis detection, we found that shear wave velocity can predict F1 at a cut-off value 1.22 with 67.6% sensitivity and 75% specificity. A cut off value1.32 can predict F2 with 75.0 % sensitivity and 90.9 % specificity. A cut-off value 1.44 can predict F3 with 96.6% sensitivity and 75% specificity. A cut-off value 1.8 can predict F4 with 95.7 % sensitivity and 100% specificity Table 4 and Fig. 4.

Table 4: Diagnostic performance of ARFI for liver fibrosis diagnosis

	Shear wave velocity m/sec Cut off value	AUR	Specificity	Sensitivity	PPV	NPV
F1	1.22	0.639	75%	67.6%	87.50%	60%
F2	1.32	0.727	90.9 %	75.0 %	90.9 %	75.0 %
F3	1.44	0.905	75%	96.6%	96.6%	100%
F4	1.80	0.989	100%	95.7 %	100%	40%

AUR: area under curve; PPV: positive predictive value; NPV: negative predictive value.

Discussion

Early detection of liver fibrosis is very important for proper management of chronic liver disease patients, especially in our country. To best of our knowledge, this study is the first to detect accuracy of ARFI as a non-invasive diagnostic technique for liver fibrosis among Egyptian patients. All enrolled patients were recruited from Agouza Liver Center and outpatient's clinics of National Research Center., these patients come from all governorates of Egypt seeking medical advice, thus, we think our patient's sample represents patients all over the country.

a) b)

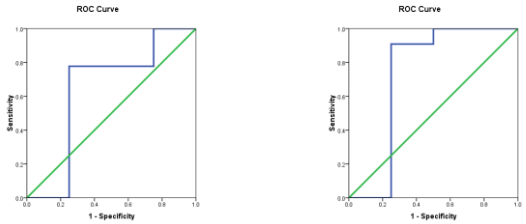

Figure 4: a) ROC curve for diagnosis of liver fibrosis grade 1 by ARFI; b) ROC curve for diagnosis of liver fibrosis grade 2 by ARFI

In the current study, we found that ARFI elastography can predict different grades of liver fibrosis with reasonable sensitivity and specificity. The area under the curve was highest for F ≥ 3 and F = 4. Thus, the ability to diagnose late fibrosis (F ≥ 3) and cirrhosis (F = 4) is higher than that for early fibrosis (F ≥ 1 and F ≥ 2). Our findings agree with previous studies, a meta-analysis of nine studies [15-23] showed that ARFI imaging had excellent diagnostic accuracy for the staging of liver fibrosis in various chronic liver diseases; compared with liver biopsy, ARFI was highly accurate in the diagnosis of fibrosis stage F ≥ 3 (AUROC = 0.91) and for the diagnosis of liver cirrhosis (AUROC = 0.93) [24]. Moreover, an international multicenter study of 911 HCV mono-infected patients found that ARFI was highly accurate in the diagnosis of fibrosis stage F ≥ 3

(AUROC = 0.83) [25].

Spore and his colleagues reported that in daily practice, seldom more than one noninvasive electrographic method for liver fibrosis assessment is available at any center. Thus, the hepatologist must use the most cost-effective one. According to their results, ARFI elastography could be this method as it has a significantly higher rate of reliable liver stiffness (LS) measurements, compared with transient elastography (TE). Also, as previously noted, most of the published studies [26-31] have reported similar accuracy for ARFI and TE in the evaluation of liver fibrosis. ARFI has another advantage compared with TE: It can be integrated into a standard ultrasound system (which can also be used for standard ultrasound evaluation, contrast-enhanced ultrasound, and/or Doppler examinations).

According to Rizzo and his colleagues [32], their study included 139 patients with chronic HCV infection, ARFI was more accurate than TE for the noninvasive staging of both significant (≥ F2) (AUROC: 0.86 vs. 0.78, P = 0.024) and severe (F3-F4) fibrosis (AUROC: 0.94 vs. 0.83, P = 0.02). Importantly, no cases of invalid measurements were recorded vs. 6.5% of unreliable results in patients undergoing TE (P = 0.029). Moreover, in contrast to TE, liver steatosis does not seem to influence ARFI [33]. Another advantage is that it can be easily incorporated into a modified U/S machine. Further validation including inter- and intra-operator reproducibility is required before ARFI can be used in routine clinical practice.

Lupsor, and his colleagues [34] reported that the cut-off values (m/s) for fibrosis stages were: 1.19 (F ≥ 1), 1.34 (F ≥ 2), 1.61 (F ≥ 3) and 2.00 (F4). The study was performed in 112 patients. When they compared ARFI and TE, the areas under the receiver operating characteristic curves (AUROC) were 0.709 vs. 0.902, P = 0.006 (F ≥ 1), 0.851 vs. 0.941, P = 0.022 (F ≥ 2), 0.869 vs. 0.926, P = 0.153 (F ≥ 3) and 0.911 vs. 0.945, P = 0.331 (F4). Sporea, and his colleagues [10] concluded that the cut-off values for predicting the stages of fibrosis were 1.19 m/s for F ≥ 1 (based on Metavir staging system), 1.21 m/s, (F ≥ 2), 1.58 m/s (F ≥ 3) and 1.82 m/s (F = 4). This was conducted in 247 patients with hepatitis C.

Our findings with that of previous studies contribute to confirming the accuracy of ARFI as a noninvasive promising device for liver fibrosis and cirrhosis. From a practical point of view, management of early fibrosis (F1 and F2) is different from that of late fibrosis (F3) or cirrhosis (F4).Most of these studies reported higher sensitivities and specificities for F3 and F4.So that the ability of ARFI can discriminate late fibrosis (F3) and cirrhosis (F4) from early fibrosis (F1 and F2) increase its feasibility as a noninvasive diagnostic tool for liver fibrosis.

ARFI elastography was properly correlated with biochemical markers for hepatitis as it was directly correlated to AST; ALT; INR while it was inversely correlated to albumin; prothrombin concentration and platelets. Moreover, ARFI elastography increased gradually with the advance of live fibrosis. This is a more added evidence for ARFI as a diagnostic technique for liver fibrosis.

Hepatic fibro genesis is the corner stone of progression of any chronic liver disease. So that, we think it's time to pay more efforts in the field of detection of liver fibrosis. We search for an accurate; easy; cheap and can be used the frequent method to help us not only in diagnosing liver fibrosis but we can follow up our patients to detect progression or even regression of fibrosis especially among patients who receive antiviral treatment for chronic viral infection. Although Egypt has the highest prevalence of HCV infection, we think that with continuous health promotion and new antiviral treatments, the incidence of chronic HCV infection among Egyptian will be decreased in the near future. But there is another big health problem that threatens Egyptian livers which are a nonalcoholic fatty liver disease (NAFLD). We previously searched for NAFLD among some Egyptian healthy subjects; we found that NAFLD was detected in 47 (65.3%) children and in 52 (62.7%) adults [35]. Fatty liver disease patients in our country usually consider that NAFLD is a normally associated condition with obesity and overweight cases. They do not know the progression of NAFLD into nonalcoholic steatohepatitis (NASH); liver cirrhosis or even hepatocellular carcinoma. These patients cannot agree for their liver to be biopsied for pathological examination. Thus, use of noninvasive accurately, a diagnostic device for liver fibrosis is mandatory for patients and doctors.

For the past 50 years, liver biopsy has been considered to be the gold standard for staging of liver fibrosis. However, many recent studies clearly highlight several crucial drawbacks of liver biopsy, including variable accessibility, high cost, sampling errors and inaccuracy due to inter-and intra-observer variability of pathologic interpretations [36]. In addition, there is a small but important risk of liver biopsy-associated morbidity and mortality, with pain and hypotension as the most frequent complications and intra peritoneal bleeding and injury to the biliary system as the most serious complications. Studies reveal that the risk for hospitalization after liver biopsy is 1-5%, the risk for severe complications is 0.57%, and mortality rates vary from 0.009% to 0.12% [37]. Because of these reasons, some patients may opt to forgo liver biopsy and may not know the stage of their liver disease with important prognostic implications. The liver biopsy is not a golden standard method for liver fibrosis any more, but it could be considered the best available standard method. The activating validity of noninvasive techniques for liver fibrosis detection will help us to observe the liver continuously. Moreover, paving the way for therapeutic intervention

aiming to reverse liver fibrosis or at least stop its progression.

In conclusion, acoustic radiation force impulse elastography is a feasible method for assessment of liver fibrosis among Egyptian patients with chronic HCV infection.

References

1. Sporea I, Sirli R, Bota S, Fierbinteanu-Braticevici C, Petrisor A, Badea R, Lupsor M, Popescu A, Danila M. Is ARFI elastography reliable for predicting fibrosis severity in chronic HCV hepatitis? World J Radiol. 2011;3:188–193. http://dx.doi.org/10.4329/wjr.v3.i7.188 PMid:21860715 PMCid:PMC3158897

2. Ratziu V, Charlotte F, Heurtier A, Gombert S, Giral P, Bruckert E, Grimaldi A, Capron F, Poynard T. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. Gastroenterology. 2005;128:1898-1906. http://dx.doi.org/10.1053/j.gastro.2005.03.084 PMid:15940625

3. Sporea I. Is there a real future for liver elastography? J Gastrointestin Liver Dis. 2012;21:129–131. PMid:22720299

4. Colombo S, Buonocore M, Del Poggio A, Jamoletti C, Elia S, Mattiello M, Zabbialini D, Del Poggio P. Head-to-head comparison of transient elastography (TE), real-time tissue elastography (RTE), and acoustic radiation force impulse (ARFI) imaging in the diagnosis of liver fibrosis. J Gastroenterol. 2012;47:461–469. http://dx.doi.org/10.1007/s00535-011-0509-4 PMid:22223175

5. Friedrich-Rust M, Nierhoff J, Lupsor M, Sporea I, Fierbinteanu-Braticevici C, Strobel D, Takahashi H, Yoneda M, Suda T, Zeuzem S. Performance of Acoustic Radiation Force Impulse imaging for the staging of liver fibrosis: a pooled meta-analysis. J Viral Hepat. 2012;19:e212-e219. http://dx.doi.org/10.1111/j.1365-2893.2011.01537.x PMid:22239521

6. Kircheis G, Sagir A, Vogt C, Vom Dahl S, Kubitz R, Häussinger D. Evaluation of acoustic radiation force impulse imaging for determination of liver stiffness using transient elastography as a reference. World J Gastroenterol. 2012;18:1077-1084. http://dx.doi.org/10.3748/wjg.v18.i10.1077 PMid:22416182 PMCid:PMC3296981

7. Nightingale K. Acoustic Radiation Force Impulse (ARFI) Imaging: a Review. Curr Med Imaging Rev. 2011;7:328-339. http://dx.doi.org/10.2174/157340511798038657 PMid:22545033 PMCid:PMC3337770

8. Fierbinteanu-Braticevici C, Andronescu D, Usvat R, Cretoiu D, Baicus C, Marinoschi G. Acoustic radiation force imaging sonoelastography for noninvasive staging of liver fibrosis. World J Gastroenterol. 2009;15:5525-5532. http://dx.doi.org/10.3748/wjg.15.5525 PMid:19938190 PMCid:PMC2785054

9. Nierhoff J, Chávez Ortiz AA, Herrmann E, Zeuzem S, Friedrich-Rust M. The efficiency of acoustic radiation force impulse imaging for the staging of liver fibrosis: a meta-analysis. Eur Radiol. 2013;23:3040-3053. http://dx.doi.org/10.1007/s00330-013-2927-6 PMid:23801420

10. Chang TT, Liaw YF, Wu SS, et al. Long-term entecavir therapy results in the reversal of fibrosis/cirrhosis and continued histological improvement in patients with chronic hepatitis B. Hepatology. 2010;52:886–893. http://dx.doi.org/10.1002/hep.23785 PMid:20683932

11. George SL, Bacon BR, Brunt EM, et al. Clinical, virologic, histologic, and biochemical outcomes after successful HCV therapy: a 5-year follow-up of 150 patients. Hepatology. 2009;49:729–738. http://dx.doi.org/10.1002/hep.22694 PMid:19072828 PMCid:PMC2731713

12. Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, et al. Histological grading and staging of chronic hepatitis. J Hepatol. 1995;22(6):696–9. http://dx.doi.org/10.1016/0168-8278(95)80226-6

13. Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. Lancet. 1997;349(9055):825–32. http://dx.doi.org/10.1016/S0140-6736(96)07642-8

14. Palmeri ML, Wang MH, Dahl JJ, Frinkley KD, Nightingale KR. Quantifying hepatic shear modulus in vivo using acoustic radiation force. Ultrasound Med Biol. 2008;34:546–558. http://dx.doi.org/10.1016/j.ultrasmedbio.2007.10.009 PMid:18222031 PMCid:PMC2362504

15. Friedrich-Rust M, Wunder K, Kriener S et al. Liver fibrosis in viral hepatitis: noninvasive assessment with acoustic radiation force impulse imaging versus transient elastography. Radiology. 2009;252: 595–604. http://dx.doi.org/10.1148/radiol.2523081928 PMid:19703889

16. Lupsor M, Badea R, Stefanescu H et al. Performance of a new elastographic method (ARFI technology) compared to unidimensional transient elastography in the noninvasive assessment of chronic hepatitis C. Preliminary results. J Gastrointestin Liver Dis. 2009;18: 303–310. PMid:19795024

17. Takahashi H, Ono N, Eguchi Y et al. Evaluation of acoustic radiation force impulse elastography for fibrosis staging of chronic liver disease: a pilot study. Liver Int. 2010;30: 538–545. http://dx.doi.org/10.1111/j.1478-3231.2009.02130.x PMid:19874490

18. Fierbinteanu-Braticevici C, Andronescu D, Usvat R, Cretoiu D, Baicus C, Marinoschi G. Acoustic radiation force imaging sonoelastography for noninvasive staging of liver fibrosis. World J Gastroenterol. 2009;15: 5525–5532. http://dx.doi.org/10.3748/wjg.15.5525 PMid:19938190 PMCid:PMC2785054

19. Goertz RS, Zopf Y, Jugl V et al. Measurement of liver elasticity with acoustic radiation force impulse (ARFI) technology: an alternative noninvasive method for staging liver fibrosis in viral hepatitis. Ultraschall Med. 2010;31:151–155. http://dx.doi.org/10.1055/s-0029-1245244 PMid:20306380

20. Yoneda M, Suzuki K, Kato S et al. Nonalcoholic fatty liver disease: US-based acoustic radiation force impulse elastography. Radiology. 2010; 256: 640–647. http://dx.doi.org/10.1148/radiol.10091662 PMid:20529989

21. Sporea I, Sirli RL, Deleanu A, et al. Acoustic radiation force impulse elastography as compared to transient elastography and liver biopsy in patients with chronic hepatopathies. Ultraschall Med. 2011;32(Suppl. 1):46–52. http://dx.doi.org/10.1055/s-0029-1245360 PMid:20603783

22. Osaki A, Kubota T, Suda T, et al. Shear wave velocity is a useful marker for managing nonalcoholic steatohepatitis. World J Gastroenterol. 2010;16:2918–2925. http://dx.doi.org/10.3748/wjg.v16.i23.2918 PMid:20556839 PMCid:PMC2887589

23. Haque M, Robinson C, Owen D, Yoshida EM, Harris A. Comparison of acoustic radiation force impulse imaging (ARFI) to liver biopsy histologic scores in the evaluation of chronic liver disease: a pilot study. Ann Hepatol. 2010;9:289–293. PMid:20720270

24. Friedrich-Rust M, Nierhoff J, Lupsor M, Sporea I, Fierbinteanu-Braticevici C, Strobel D, Takahashi H, Yoneda M, Suda T, Zeuzem S, Herrmann E: Performance of Acoustic Radiation Force Impulse imaging for the staging of liver fibrosis: a pooled meta-analysis. J Viral Hepat. 2012;19:e212-e219. http://dx.doi.org/10.1111/j.1365-2893.2011.01537.x PMid:22239521

25. Sporea I, Bota S, Peck-Radosavljevic M, Sirli R, Tanaka H, Iijima H, Badea R, Lupsor M, Fierbinteanu-Braticevici C, Petrisor A, Saito H, Ebinuma H, Friedrich-Rust M, Sarrazin C, Takahashi H, Ono N, Piscaglia F, Borghi A, D'Onofrio M, Gallotti A, Ferlitsch A, Popescu A, Danila M: Acoustic Radiation Force Impulse

elastography for fibrosis evaluation in patients with chronic hepatitis C: an international multicenter study. Eur J Radiol. 2012;81:4112-4118. http://dx.doi.org/10.1016/j.ejrad.2012.08.018 PMid:23000186

26. Bota S, Herkner H, Sporea I, Salzl P, Sirli R, Neghina A, Popescu A, Danila A, Peck-Radosavljevic M. Acoustic radiation force impulse elastography versus transient elastography for the evaluation of liver fibrosis: Systematic review. In: Abstract Book. Madrid: EUROSON. 2012;24:22–24.

27. Ebinuma H, Saito H, Komuta M, Ojiro K, Wakabayashi K, Usui S, Chu PS, Umeda R, Ishibashi Y, Takayama T, Kikuchi M, Nakamoto N, Yamagishi Y, Kanai T, Ohkuma K, Sakamoto M, Hibi T. Evaluation of liver fibrosis by transient elastography using acoustic radiation force impulse: Comparison with Fibroscan. J Gastroenterol. 2011;46:1238–1248. http://dx.doi.org/10.1007/s00535-011-0437-3 PMid:21779759

28. Friedrich-Rust M, Schwarz A, Ong M, Dries V, Schirmacher P, Herrmann E, Samaras P, Bojunga J, Bohle RM, Zeuzem S, Sarrazin C. Real-time tissue elastography versus FibroScan for noninvasive assessment of liver fibrosis in chronic liver disease. Ultraschall Med. 2009;30:478–484. http://dx.doi.org/10.1055/s-0028-1109488 PMid:19813157

29. Friedrich-Rust M, Wunder K, Kriener S, Sotoudeh F, Richter S, Bojunga J, Herrmann E, Poynard T, Dietrich CF, Vermehren J, Zeuzem S, Sarrazin C. Liver fibrosis in viral hepatitis: Noninvasive assessment with acoustic radiation force impulse imaging versus transient elastography. Radiology. 2009;252:595–604. http://dx.doi.org/10.1148/radiol.2523081928 PMid:19703889

30. Kircheis G, Sagir A, Vogt C, Vom Dahl S, Kubitz R, H€aussinger D. Evaluation of acoustic radiation force impulse imaging for determination of liver stiffness using transient elastography as a reference. World J Gastroenterol. 2012;18:1077–1084. http://dx.doi.org/10.3748/wjg.v18.i10.1077 PMid:22416182 PMCid:PMC3296981

31. Sporea I, Sirli R, Popescu A, Danila M. Acoustic Radiation Force Impulse (ARFI): A new modality for the evaluation of liver fibrosis. Med Ultrason. 2010;12:26–31. PMid:21165451

32. Rizzo L, Calvaruso V, Cacopardo B, et al. Comparison of transient elastography and acoustic radiation force impulse for non-invasive staging of liver fibrosis in patients with chronic hepatitis C. Am J Gastroenterol. 2011;106:2112–2120. http://dx.doi.org/10.1038/ajg.2011.341 PMid:21971536

33. Rifai K, Cornberg J, Mederacke I, et al. Clinical feasibility of liver elastography by acoustic radiation force impulse imaging (ARFI) Dig Liver Dis. 2011;43:491–497. http://dx.doi.org/10.1016/j.dld.2011.02.011 PMid:21439919

34. Lupsor M, Badea R, Stefanescu H, Sparchez Z, Branda H, Serban A, Maniu A. Performance of a new elastographic method (ARFI technology) compared to unidimensional transient elastography in the noninvasive assessment of chronic hepatitis C. Preliminary results. J Gastrointestin Liver Dis. 2009;18: 303-10. PMid:19795024

35. Ezzat WM, Ragab S, Ismail NA, Elhosary YA, Nour AM, ElBaky EA, Farouk H, Rasheed IA. Frequency of non-alcoholic fatty liver disease in overweight/obese children and adults: Clinical, sonographic picture and biochemical assessment. Journal of Genetic Engineering and Biotechnology. 2012;10:221–227. http://dx.doi.org/10.1016/j.jgeb.2012.05.006

36. Sebastiani G, Alberti A. Non invasive fibrosis biomarkers reduce but not substitute the need for liver biopsy. World J Gastroenterol. 2006;12(23):3682-3694. http://dx.doi.org/10.3748/wjg.v12.i23.3682 PMid:16773685 PMCid:PMC4087461

37. West J, Card TR. Reduced mortality rates following elective percutaneous liver biopsies. Gastroenterology. 2010;139(4):1230-7. http://dx.doi.org/10.1053/j.gastro.2010.06.015 PMid:20547160

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):381-387.
http://dx.doi.org/10.3889/oamjms.2016.068
eISSN: 1857-9655
Clinical Science

Risk Factors of End Stage Renal Disease in Peshawar, Pakistan: Odds Ratio Analysis

Salahuddin Khan[1*], Tariq Hussain[2], Najma Salahuddin[3], Salahuddin Mehreen[4]

[1]Department of Family and Community Medicine, College of Medicine, University of Dammam, Kingdom of Saudi Arabia; [2]Higher Education Department, Peshawar, Pakistan; [3]Department of Statistics, Shaheed Benazir Bhutto Women University, Peshawar, Pakistan; [4]Medical Officer, North West Hospital, Phase 5 Hayatabad, Peshawar, Pakistan

Citation: Khan S, Hussain T, Salahuddin N, Mehreen S. Risk Factors of End Stage Renal Disease in Peshawar, Pakistan: Odds Ratio Analysis. Open Access Maced J Med Sci. 2016 Sep 15; 4(3):381-387. http://dx.doi.org/10.3889/oamjms.2016.068

Keywords: Odds ratio; confidence interval; ESRD; risk factors; association.

***Correspondence:** Salahuddin Khan. Department of Family and Community Medicine, College of Medicine, University of Dammam, Kingdom of Saudi Arabia. Second address: Institute of Management and Information Sciences, CECOS University of IT and Emerging Sciences, Sector H/3, Phase II, Hayatabad, Peshawar, Pakistan. E-mail: salahuddin_90@yahoo.com

Received: 06-Apr-2016; **Revised:** 31-may-2016; **Accepted:** 01-Jun-2016; **Online first:** 10-Jul-2016

Competing Interests: The authors have declared that no competing interests exist.

Abstract

AIM: The basic aim of this study was to discover the association of End Stage Renal Disease (ESRD) with various risk factors. End Stage Renal Failure is the last stage of the chronic renal failure in which kidneys become completely fail to function.

MATERIALS AND METHODS: The data were collected from the patients of renal diseases from three major hospitals in Peshawar, Pakistan. Odds ratio analysis was performed to examine the relationship of ESRD (a binary response variable) with various risk factors: Gender, Diabetic, Hypertension, Glomerulonephritis, Obstructive Nephropathy, Polycystic kidney disease, Myeloma, SLE Nephritis, Heredity, Hepatitis, Excess use of Drugs, heart problem and Anemia.

RESULTS: Using odds ratio analysis, the authors found that the ESRD in diabetic patients was 11.04 times more than non-diabetic patients and the ESRD were 7.29 times less in non-hypertensive patients as compared to hypertensive patients. Similarly, glomerulonephritis patients had 3.115 times more risk of having ESRD than non-glomerulonephritis. Other risk factors may also, to some extent, were causes of ESRD but turned out insignificant due to stochastic sample.

CONCLUSION: The authors concluded that there is a strong association between ESRD and three risk factors, namely diabetes, hypertension and glomerulonephritis.

Introduction

Statistical methods are applied frequently in medical research, which deals with issues that are of great concern for the general public. It is now a well-known fact that no research could be carried out without having sufficient knowledge of Statistics. Particularly, medical research requires a good understanding of statistical methods.

End Stage Renal Disease (ESRD) is the last stage of the chronic renal failure in which kidneys fail to function completely. At this stage, the kidney stops its functions to remove the impurities and control electrolytes. The symptoms of ESRD comprise less urine output, swelling of legs, face, nausea and vomiting [1].

ESRD is one of the major health problems throughout the world. Several investigations have been carried out to study various risk factors of the ESRD. The United States Renal Data System (USRDS) established in 1989. This system is the largest and most comprehensive national Chronic Kidney Disease (CKD) and ESRD surveillance system [2]. The death rate due to ESRD in western countries especially in the USA is higher, but in Asian countries like Pakistan, India, Bangladesh; there are also a significant number of deaths due to ESRD [3].

It has been established that both low estimated Glomerular Filtration Rate (eGFR) and high albuminuria were independently associated with

mortality and ESRD regardless of age across a wide range of populations [4]. A retrospective cross-sectional study was conducted to investigate the prevalence and associated comorbidities of Stage 3 (GFR 30-59 ml/min/1.73 m^2) and Stages 4 and 5 (GFR <30 mL/min/1.73 m^2) CKD among Chinese nursing home older adults. The researchers concluded that stages 3 to 5 CKD are widespread in Chinese nursing home older adults [5]. Regardless of higher risks of mortality and ESRD in diabetes, the relative risks of these outcomes by eGFR and Albumin-to-Creatinine Ratio (ACR) are much the same irrespective of the presence or absence of diabetes, highlighting the significance of kidney disease as a predictor of clinical outcomes [6].

It has been recognized that males and females face increased risk of all-cause mortality, cardiovascular mortality, and ESRD with lower estimated GFR and higher albuminuria [7]. It has been shown that declines in estimated GFR smaller than a doubling of serum creatinine concentration occurred more commonly and were strongly and consistently associated with the risk of ESRD and mortality, supporting consideration of lesser declines in estimated GFR (such as a 30% reduction over 2 years) as an alternative end point for CKD development [8]. A kidney failure definition, including treated and untreated disease identifies more cases than linkage to the United States Renal Data System registry alone, particularly among older adults [9]. It has been found that CKD is increasingly common in older adults. Competing risks of death influence the risk of development to ESRD [10].

By using survival analysis through the Cox proportional hazard model, the researchers found that the elevated C - reactive protein (CRP) was a robust predictor of mortality in ESRD patients. In a study of 663 ESRD patients (374 males and 289 females), the researchers also found that CRP was a strong predictor. CRP had positive correlation (= 0.369; p-value equal to 0.001) in addition, the coefficient of correlation for females (= 0.519; p-value < 0.0001) and male correlation (= 0.372; p-value < 0.0001) [11].

A univariate Cox regression analysis was carried out and the researchers found that the chlamydia pneumonia infection was related to the cardiovascular risk of ESRD patients. In a cohort of 227 ESRD patients, the Hazard Ratio of mortality was 1.08 with 95% confidence interval (0.678 to 1.722); p-value = 0.737. The researchers concluded that the chlamydia pneumonia infection is a major risk factor in patients with ESRD [12].

In a prospective cohort study of 143802 patients in China, having an age of 40 years or above, the researchers found that the Body Mass Index (BMI) is strongly associated with ESRD. The multivariate-adjusted risks for ESRD are 1.389 with 95% confidence interval (1.021 to 1.909) for BMI < 18.52 kg/m, 1.213 with 95% confidence interval (0.919 to 1.598) for BMI (25.01 to 29.93 kg/m) and 2.142 with confidence interval 95% (1.392 to 3.289) for BMI ≥ 30.01 kg/m with J-shaped association [13]. A cohort study was carried out in survival analysis and the researchers concluded that the independent risk factors of ESRD are sex, race, anemia and heredity [14].

In this study, odds ratio analysis was used to examine the relationship of ESRD with various risk factors.

Materials and Methods

To determine the effects of various risk factors on ESRD, this study was carried out based on the data obtained from three major hospitals in Peshawar: (i) Hayatabad Medical Complex, (ii) Lady Reading Hospital and (iii) Khyber Teaching Hospital. The association of various risk factors with the occurrence of ESRD was determined through the statistical technique of odds ratio analysis. A total of 407 patients was examined for the presence or absence of ESRD. The statistical analyses were performed with SPSS software package.

Odds and the odds ratio: The probability of interested events divided by the probability of non-interested events are called the Odds, i.e. Odd = P/1-P, where P is the probability of interested events. If the observed dichotomous data contain 'X' number of interested events in 'n' outcomes, then the odds ratio of interest can be calculated as:

$$\text{Odds} = \frac{x}{n - x}.$$

'X' denotes the number of occurrences of interested events and 'n-X' indicates the number of non-interested events.

In order to compare two binary data sets, the ratio of odds of interest in one set to the odds of the other data set, is a relative measure of odds of interest. The odds ratio is denoted by Ψ, and mathematically, it is defined as:

$$\Psi = \frac{P_1/1 - P_1}{P_2/1 - P_2}$$

If the probability of interest in two data sets is equal, then the odds ratio (Ψ) = 1 and if odd ratio (Ψ) < 1, then the odds of interest will be less in the first data set than in the second one. On the other hand, if the odds ratio (Ψ) > 1, then the odds of an interest will be greater in the first data set [15].

Statistical inference based on odds ratio: To estimate the odds ratio, the binary data are needed to arrange in (2x2) contingency table given as:

Contingency Table

	No. of Success	No. of Failure	Total
Data Set 1	A	B	**a+b**
Data Set 2	C	D	**c+d**
Total	**a+c**	**b+d**	**N**

The probabilities of interest obtained from two data sets are $\hat{P}_1 = \frac{a}{a+b}$ and $\hat{P}_2 = \frac{c}{c+d}$. The estimated odds ratio (Ψ) is given by:

$$\hat{\psi} = \frac{\hat{P}_1 / 1-\hat{P}_1)}{\hat{P}_2 / (1-\hat{P}_2)} = \frac{ad}{bc}$$

This estimated odds ratio ($\hat{\psi}$) is usually termed as "cross-product ratio", as it is obtained by multiplying the two pairs of diagonal values in the (2 x 2) contingency table [16].

To test such an association, the hypothesis is considered as:

$H_0 : \psi = 1$ or equivalently, $H_0 : \ln(\psi) = 0$, means that the two variables (ESRD and Risk factors) are independent, that is, risk factors do not affect ESRD.

The test-statistic is: $Z = \frac{\ln(\hat{\psi})}{S.E\{\ln(\hat{\psi})\}}$, which has an approximate standard normal distribution. An approximate 100 (1- α) % confidence interval for $\ln(\psi)$ is constructed as:

$$\ln(\hat{\psi}) \pm Z_{\alpha/2} S.E\{\ln(\hat{\psi})\} \text{ . (I)}$$

For example, a 95% confidence interval for $\ln(\psi)$ is given by

$$\ln(\hat{\psi}) \pm 1.96 S.E\{\ln(\hat{\psi})\} \quad .$$

The confidence interval given by equation (I) on inversion will give us the confidence interval for ψ as: $\hat{\psi} e^{-z\alpha/2S.E} < \psi < \hat{\psi} e^{z\alpha/2S.E}$.

If the interval contains unity, it indicates independence; otherwise an association between risk factor and ESRD is significant.

Results

Several researchers have investigated the association of ESRD and its various risk factors. A meta-analysis study was conducted. The interpretation of this study was that CKD should be regarded as at least an equally relevant risk factor for mortality. These researchers further interpreted that ESRD in individuals without hypertension should be regarded as it is in those with hypertension [17]. It has been revealed that diabetes, higher systolic blood pressure, lower estimated glomerular filtration rate and black race were risk factors for developing treated chronic kidney failure irrespective of albuminuria status, although the absolute risk of kidney failure in participants without albuminuria was very low. These researcher also showed that their findings support testing for kidney disease in high-risk populations, which often have otherwise unrecognized kidney disease [18].

To investigate the relationship of ESRD with various risk factors, we used odds ratio analysis.

ESRD versus gender

Contingency table of ESRD versus gender is given in Table (1). The calculated values are: Odd Ratio = 1.40, Chi-square = 2.628, p-value = 0.105 and the confidence interval is (0.931, 2.104).

Table 1: Contingency table of ESRD versus gender

Gender	ESRD		Total (%)
	No	Yes	
Female	104	59	163 (40)
Male	136	108	244 (60)
Total (%)	240 (59)	167 (41)	407

The odds of ESRD show that the males are 1.4 times more exposed to ESRD than the females and the Log of the odds ratio is 0.336 (with a standard error = 0.208). The confidence interval for the odds ratio is (0.931, 2.104) at the 5% level of significance. The interval contains unity; it indicates independence (no association between Gender and ESRD). Also, p-value is greater than 0.05, the result is insignificant. It is concluded that there is no association between Gender and ESRD.

ESRD versus diabetic

Contingency table of ESRD versus diabetic is given in Table 2. The calculated values are: Odd Ratio = 11.04, Chi-square = 141.883, p-value < 0.001 and the confidence interval is (6.913, 17.63).

Table 2: Contingency table of ESRD versus diabetic

Diabetic	ESRD		Total (%)
	No	Yes	
No	185	39	224 (55)
Yes	55	128	183 (45)
Total (%)	240 (59)	167 (41)	407

The odds of ESRD show that the diabetic patients are 11.04 times more exposed to ESRD than the non-diabetic patients and the Log of odds ratio is 2.402 (with a standard error = 0.239). The confidence interval for the odds ratio is (6.913, 17.63) at the 5% level of significance. The interval does not contain

unity; it indicates that there is an association between Diabetic and ESRD. Also, observed p-value is less than 0.05, the result is significant. It is concluded that there is a strong association between diabetes and ESRD.

ESRD versus hypertension

Contingency table of ESRD versus hypertension is given in Table 3. The calculated values are: Odd Ratio = 7.287, Chi-square = 77.56, p-value < 0.001 and the confidence interval is (4.571, 11.616).

Table 3: Contingency table of ESRD versus hypertension

Hypertension	ESRD		Total (%)
	No	Yes	
No	152	32	184 (45)
Yes	88	135	233 (55)
Total (%)	240 (59)	167 (41)	407

The odds of ESRD show that the hypertensive patients are 7.287 times more exposed to ESRD than the non-hypertensive patients and the Log of odds ratio is 1.986 (with a standard error = 0.238). The confidence interval for the odds ratio is (4.571, 11.616) at the 5% level of significance. The interval does not contain unity; it indicates there is an association between hypertension and ESRD. Also, observed p-value is less than 0.05, the result is significant. It is concluded that there is a strong association between hypertension and ESRD.

ESRD versus glomerulonephritis

Contingency table of ESRD versus glomerulonephritis is given in Table 4. The calculated values are: Odd Ratio = 3.115, Chi-square = 29.826, p-value < 0.001 and the confidence interval is (2.059, 4.712).

Table 4: Contingency table of ESRD versus glomerulonephritis

Glomerulonephritis	ESRD		Total (%)
	No	Yes	
No	171	74	245 (60)
Yes	69	93	162 (40)
Total (%)	240 (59)	167 (41)	407

The odds of ESRD show that the glomerulonephritis patients are 3.115 times more exposed to ESRD than the non-glomerulonephritis patients and the Log of odds ratio is 1.136 (with a standard error = 0.211). The confidence interval for the odds ratio is (2.059, 4.712) at the 5% level of significance. The interval does not contain unity; it indicates there is association between glomerulonephritis and ESRD. Also, observed p-value is less than 0.05, the result is significant. It is concluded that there is a strong association between glomerulonephritis and ESRD.

ESRD versus obstructive nephropathy

Contingency table of ESRD versus obstructive nephropathy is given in Table 5. The calculated values are: Odd Ratio = 1.2, Chi-square = 0.542, p-value = 0.462 and the confidence interval is (0.738, 1.952).

Table 5: Contingency table of ESRD versus obstructive nephropathy

Obstructive Nephropathy	ESRD		Total (%)
	No	Yes	
No	194	130	324 (80)
Yes	46	37	83 (20)
Total (%)	240 (59)	167 (41)	407

The odds of ESRD show that the obstructive nephropathy patients are 1.2 times more exposed to ESRD than the non-obstructive nephropathy patients and the Log of odds ratio is 0.182 (with standard error = 0.248). The confidence interval for the odds ratio is (0.738, 1.952) at the 5% level of significance. The interval contains unity; it indicates independence. Also, observed p-value is greater than 0.05, the result is insignificant. It is concluded that there is no association between obstructive nephropathy and ESRD.

ESRD versus polycystic kidney disease

Contingency table of ESRD versus Polycystic kidney is given in Table 6. The calculated values are: Odd Ratio = 1.67, Chi-square = 0.186, p-value = 0.403 and Confidence Interval is (0.553, 2.527).

Table 6: Contingency table of ESRD versus polycystic kidney disease

APKD	ESRD		Total (%)
	No	Yes	
No	224	154	378 (93)
Yes	16	13	29 (7)
Total (%)	240 (59)	167 (41)	407

The odds of ESRD show that the patients, who had Polycystic kidney disease, are 1.67 times more exposed to ESRD than the patients who do not have Polycystic kidney disease and the Log of odds ratio is 0.167 (with standard error = 0.288). The confidence interval for the odds ratio is (0.553, 2.527) at the 5% level of significance. The interval contains unity; it indicates independence. Also, observed p-value is greater than 0.05, the result is insignificant. It is concluded that there is no association between polycystic kidneydisease and ESRD.

ESRD versus myeloma

Contingency table of ESRD versus myeloma is given in Table 7. The calculated values are: Odd Ratio = 1.081, Chi-square = 0.20, p-value = 1.000 and Confidence Interval is (0.368, 3.174).

Table 7: Contingency table of ESRD verses myeloma

Myeloma	ESRD		Total (%)
	No	Yes	
No	232	161	393 (97)
Yes	8	6	14 (3)
Total (%)	240 (59)	167 (41)	407

The odds of ESRD show that the myeloma patients are 1.081 times more exposed to ESRD than the non-myeloma patients and the Log of odds ratio is 0.078 (with a standard error = 0.302). The confidence interval for the odds ratio is (0.368, 3.174) at the 5% level of significance. The interval contains unity; it indicates independence. Also, observed p-value is greater than 0.05, the result is insignificant. It is concluded that there is no association between myeloma and ESRD.

ESRD versus SLE nephritis

Contingency table of ESRD versus SLE nephritis is given in Table 8. The calculated values are: Odd Ratio = 1.132, Chi-square = 0.051, p-value = 0.802 and Confidence Interval is (0.41, 3.077).

Table 8: Contingency table of ESRD versus SLE nephritis

SLE Nephritis	ESRD		Total (%)
	No	Yes	
No	231	160	391 (96)
Yes	9	7	16 (4)
Total (%)	240 (59)	167 (41)	407

The odds of ESRD show that the SLE nephritis patients are 1.123 times more exposed to ESRD than the non-SLE nephritis patients and the Log of odds ratio is 0.116 (with standard error = 0.551). The confidence interval for the odds ratio is (0.41, 3.077) at the 5% level of significance. The interval contains unity; it indicates independence. Also, observed p-value is greater than 0.05, the result is insignificant. It is concluded that there is no association between SLE nephritis and ESRD.

ESRD versus heredity

Contingency table of ESRD versus heredity is given in Table 9. The calculated values are: Odd Ratio = 1.757, Chi-square = 1.818, p-value = 0.202 and Confidence Interval is (0.767, 4.024).

Table 9: Contingency table of ESRD versus heredity

Heredity	ESRD		Total (%)
	No	Yes	
No	229	154	383 (94)
Yes	11	13	24 (6)
Total (%)	240 (59)	167 (41)	407

The odds of ESRD show that the patients, who have a family history of ESRD, are 1.757 times more exposed to ESRD than the patients who do not have a family history of ESRD and the Log of odds ratio is 0.564 (with standard error = 0.423). The confidence interval for the odds ratio is (0.767, 4.024) at the 5% level of significance. The interval contains unity; it indicates independence. Also, p-value is greater than 0.05, the result is insignificant. It is concluded that there is no association between heredity and ESRD.

ESRD versus hepatitis

Contingency table of ESRD versus hepatitis is given in Table 10. The calculated values are: Odd Ratio = 1.792, Chi-square = 4.495, p-value = 0.063 and Confidence Interval is (0.747, 2.277).

Table 10: Contingency table of ESRD versus hepatitis

Hepatitis	ESRD		Total (%)
	No	Yes	
No	211	134	345 (85)
Yes	29	33	62 (15)
Total (%)	240 (59)	167 (41)	407

The odds of ESRD show that the hepatitis patients are 1.792 times more exposed to ESRD than the non-hepatitis patients and the Log of odds ratio is 0.253 (with a standard error = 0.277). The confidence interval for the odds ratio is (0.747, 2.277) at the 5 % level of significance. The interval contains unity; it indicates independence. Also, observed p-value is greater than 0.05, the result is insignificant. It is concluded that there is no association between hepatitis and ESRD.

ESRD versus drug usage

Contingency table of ESRD versus drug usage is given in Table 11. The calculated values are: Odd Ratio = 1.157, Chi-square = 0.091, p-value = 0.809 and Confidence Interval is (0.100, 2.994).

Table 11: Contingency table of ESRD versus drug usage

Hepatitis	ESRD		Total (%)
	No	Yes	
No	230	159	389 (96)
Yes	10	8	18 (4)
Total (%)	240 (59)	167 (41)	407

The odds of ESRD show that the patients, who used a lot of drugs, are 1.157 times more exposed to ESRD than the patients who do not use a lot of drugs and the Log of odds ratio is 0.146 (with standard error = 0.485). The confidence interval for the odds ratio is (0.100, 2.994) at the 5% level of significance. The interval contains unity; it indicates independence. Also, observed p-value is greater than 0.05, the result is insignificant. It is concluded that there is no association between drug usage and ESRD.

ESRD versus heart problem

Contingency table of ESRD versus heart problem is given in Table 12. The calculated values

are: Odd Ratio = 1.15, Chi-square = 0.0243, p-value = 0.672 and Confidence Interval is (0.100, 2.994).

Table 12: Contingency table of ESRD verses heart problem

Heart Problem	ESRD		Total (%)
	No	Yes	
No	227	156	383 (94)
Yes	13	11	24 (6)
Total (%)	240 (59)	167 (41)	407

The odds of ESRD show that the patients who have heart problem are 1.231 times more exposed to ESRD than the patients who do not have heart problems and the Log of the odds ratio is 0.208 (with standard error = 0.422). The confidence interval for the odds ratio is (0.543, 2.285) at the 5% level of significance. The interval contains unity; it indicates independence. Also, observed p-value is greater than 0.05, the result is insignificant. It is concluded that there is no association between heart problem and ESRD.

ESRD versus anemia

Contingency table of ESRD versus anemia is given in Table 13. The calculated values are: Odd Ratio = 1.083, Chi-square = 0.088, p-value = 0.788 and Confidence Interval is (0.676, 4.024).

The odds of ESRD show that the anemia, patients are 1.083 times more exposed to ESRD than the non-anemia patients and the Log of odds ratio is 0.080 (with a standard error = 0.269). The confidence interval for the odds ratio is (0.767, 4.024) at the 5 % level of significance.

Table 13: Contingency table of ESRD verses anemia

Anemia	ESRD		Total (%)
	No	Yes	
No	201	138	339 (83)
Yes	39	29	68 (17)
Total (%)	240 (59)	167 (41)	407

The interval contains unity; it indicates independence. Also, observed p-value is greater than 0.05, the result is insignificant. It is concluded that there is no association between anemia and ESRD.

Discussion

The major aim of this study was to determine the most important risk factors of ESRD in Peshawar. A total of 407 patients was examined in the three major hospitals of Peshawar and the phenomena of ESRD was studied in relation to different risk factors like diabetic, hypertension, glomerulonephritis, obstructive nephropathy, polycystic kidney diseases, myeloma, SLE nephritis, heredity, hepatitis, excess use of drugs, heart problem and anemia.

Out of 407 patients, 244 (60%) were males and 163 (40%) were females. The average age of male patients was 43.38 years and the average age of female patients was 42.4 years. Out of 244 male patients, 108 patients were in an uncontrolled group (ESRD cases) and out of 163 female patients, 59 were in an uncontrolled group (ESRD cases).

The total number of diabetic patients was 183 in which 128 patients had ESRD. On the other hand, out of 185 non-diabetic patients, 39 patients had ESRD. The total number of 233 (55%) patients had hypertension in which 135 (60.5%) had ESRD and out of 184 non-hypertension patients, 32 patients had ESRD. A total of 162 patients had glomerulonephritis in which 93 (57.4%), patients had ESRD and 74 patients out of 240 non-glomerulonephritis patients, had ESRD. The total number of 62 patients had hepatitis, in which 33 patients had ESRD and 134 patients out of 345 non-hepatitis patients, had ESRD.

The total number of 83 patients had obstructive nephropathy problem in which 37 patients had ESRD. On the other hand, 120 patients out of 324 non-obstructive nephropathy patients had ESRD. The total number of 29 patients had polycystic kidney disease problem in which 13 have ESRD. On the other hand, 154 patients out of 324 non-polycystic kidney disease patients had ESRD. There were 14 myeloma patients out of 407, in which 6 patients had ESRD. On the other hand, 161 patients out of 378 non-myeloma patients had ESRD. There were 16 SLE nephropathy patients out of 407, in which 7 had ESRD. On the other hand, 160 patients out of 393 non-SLE nephropathy patients had ESRD. A total of 13 patients out of 24 patients had a family history of ESRD. On the other hand, 154 patients out of 389 non-heredity patients had ESRD. There were 18 patients who used a lot of drugs in which 8 patients had ESRD. On the other hand, 159 patients out of 384 non-drug user had ESRD. A total of 24 patients had heart problems in which 11 had ESRD. On the other hand, out of 383 non-heart problem patients, 156 patients had ESRD. Out of 68 anemic patients, 29 patients had ESRD. On the other hand, 138 patients out of 339 non-anemia patients had ESRD.

Using odds ratio analysis, it was found that the ESRD in diabetic patients were 11.04 times more than non-diabetic patients and the ESRD in hypertensive patients were 7.29 times more than non-hypertensive patients. Similarly, glomerulonephritis patients have 3.115 times more chances to have ESRD than non-glomerulonephritis. This analysis shows that there was a strong association between ESRD and the three risk factors diabetes, hypertension and glomerulonephritis. The odds of ESRD for heredity were 1.76 times more than non-heredity patients. The odds of ESRD for non-hepatitis patients were 1.792 times less than hepatitis patients.

Based on odds ratio analysis, using data from 407 patients from three major hospitals of Peshawar,

the researchers concluded that the main causes of ESRD were the three risk factors i.e. diabetes, hypertension and glomerulonephritis. Other risk factors, i.e. obstructive nephropathy, heredity and hepatitis may also, to some extent, causes of ESRD but in this study, it turned out to be insignificant due to stochastic sample.

The researchers concluded that the main finding of this study is that there is a strong association between ESRD and the three risk factors namely diabetic, hypertension & glomerulonephritis.

References

1. Schrier RW. Diseases of the kidney and urinary tract, 8th Edition volume III. Lippincott Williams and Wilkins, 2007.

2. Allan JC, Robert NF, David TG, and Shu-Cheng C. United States Renal Data System public health surveillance of chronic kidney disease and terminal-stage renal disease. Kidney Int Suppl. 2015; 5 (1): 2–7. http://dx.doi.org/10.1038/kisup.2015.2 PMid:26097778 PMCid:PMC4455192

3. World Health Statistics. WHO Press, 2012.

4. Hallan SI, Matsushita K, Sang Y, Mahmoodi BK, Black C, Ishani A, Kleefstra N, Naimark D, Roderick P, Tonelli M, Wetzels JF, Astor BC, Gansevoort RT, Levin A, Wen CP, Coresh J. Age and association of kidney measures with mortality and end-stage renal disease. JAMA. 2012; 308 (22):2349-60. http://dx.doi.org/10.1001/jama.2012.16817 PMid:23111824 PMCid:PMC3936348

5. Chan TC, Yap DY, Shea YF, Luk KH, Chan HW, Chu LW. Prevalence and associated comorbidities of moderate to severe chronic renal impairment in Chinese nursing home older adults. J Am Med Dir Assoc. 2012; 13 (7): 630-3. http://dx.doi.org/10.1016/j.jamda.2012.05.007 PMid:22698953

6. Fox CS, Matsushita K, Woodward M, Bilo HJ, Chalmers J, Heerspink HJ, Lee BJ, Perkins RM, Rossing P, Sairenchi T, Tonelli M, Vassalotti JA, Yamagishi K, Coresh J, de Jong PE, Wen CP, Nelson RG. Associations of kidney disease measures with mortality and end-stage renal disease in individuals with and without diabetes: a meta-analysis. Lancet. 2012;380 (9854):1662-73. http://dx.doi.org/10.1016/S0140-6736(12)61350-6

7. Nitsch D, Grams M, Sang Y, Black C, Cirillo M, Djurdjev O, Iseki K, Jassal SK, Kimm H, Kronenberg F, Oien CM, Levey AS, Levin A, Woodward M, Hemmelgarn BR. Associations of estimated glomerular filtration rate and albuminuria with mortality and renal failure by sex: a meta-analysis. BMJ. 2013; 346: f324. http://dx.doi.org/10.1136/bmj.f324 PMid:23360717 PMCid:PMC3558410

8. Coresh J, Turin TC, Matsushita K, Sang Y, Ballew SH, Appel LJ, Arima H, Chadban SJ, Cirillo M, Djurdjev O, Green JA, Heine GH, Inker LA, Irie F, Ishani A, Ix JH, Kovesdy CP, Marks A, Ohkubo T, Shalev V, Shankar A, Wen CP, de Jong PE, Iseki K, Stengel B, Gansevoort RT, Levey AS. Decline in estimated glomerular filtration rate and subsequent risk of end-stage renal disease and mortality. JAMA. 2014;311(24) :2518-31. http://dx.doi.org/10.1001/jama.2014.6634 PMid:24892770 PMCid:PMC4172342

9. Rebholz CM, Coresh J, Ballew SH, McMahon B, Whelton SP, Selvin E, Grams ME. Kidney Failure and ESRD in the Atherosclerosis Risk in Communities (ARIC) Study: Comparing Ascertainment of Treated and Untreated Kidney Failure in a Cohort Study. Am J Kidney Dis. 2015;66(2):231-9. http://dx.doi.org/10.1053/j.ajkd.2015.01.016 PMid:25773483

10. Fung E, Kurella Tamura M. Epidemiology and Public Health Concerns of CKD in Older Adults. Adv Chronic Kidney Dis. 2016;23(1):8-11. http://dx.doi.org/10.1053/j.ackd.2015.10.001 PMid:26709057

11. Stenyinkel P, Wanner C, Metzger T, Heimbürger O, Mallamaci F, Tripepi G, et al. Inflammation and outcomes in ESRD: Does female gender constitute a survival advantage? Journal of Kidney International. 2002; 62(5): 1791-8. http://dx.doi.org/10.1046/j.1523-1755.2002.00637.x PMid:12371981

12. Zoccali C, Mallamaci F, Tripepi G, Parlongo S, Cutrupi S, Benedetto FA, et al. Chlamydia Pneumonia, overall and cardiovascular mortality in ESRD. Journal of Kidney International. 2003;64(2): 579-84. http://dx.doi.org/10.1046/j.1523-1755.2003.00095.x PMid:12846753

13. Reynolds K, Gu D, Muntner P, Chen J, Wu X, Yau CL, et al. Body Mass Index and risk of ESRD in China. Am J Kidney Diseases. 2007; 50 (5): 754 - 64. http://dx.doi.org/10.1053/j.ajkd.2007.08.011 PMid:17954288

14. Hsu CY, Iibarren C, Mc Culloch CE, Darbinian J and Alan SG. Risk factors of end stage renal disease. Arch Intern Med. 2009;169 (4): 342-350. http://dx.doi.org/10.1001/archinternmed.2008.605 PMid:19237717 PMCid:PMC2727643

15. Draper N and Smith H. Applied regression analysis. 2nd Edition. John Wiley and Sons; New York, 1981.

16. Rudes and Tarnas. Odds ratio in the analysis of contingency table. Thousand Okas, CA: Sage, 1998.

17. Mahmoodi BK, Matsushita K, Woodward M, Blankestijn PJ, Cirillo M, Ohkubo T, Rossing P, Sarnak MJ, Stengel B, Yamagishi K, Yamashita K, Zhang L, Coresh J, de Jong PE, Astor BC. Associations of kidney disease measures with mortality and end-stage renal disease in individuals with and without hypertension: a meta-analysis. Lancet. 2012; 380(9854): 1649-61. http://dx.doi.org/10.1016/S0140-6736(12)61272-0

18. Chang T, Li S, Chen SC, Peralta CA, Shlipak MG, Fried LF, Whaley-Connell AT, McCullough PA, Tamura MK. Risk factors for ESRD in individuals with preserved estimated GFR with and without albuminuria: results from the Kidney Early Evaluation Program (KEEP). Am J Kidney Dis. 2013, 61(4 Suppl 2):S4-11. http://dx.doi.org/10.1053/j.ajkd.2012.12.016 PMid:23507268 PMCid:PMC4117734

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):388-391.
http://dx.doi.org/10.3889/oamjms.2016.084
eISSN: 1857-9655
Clinical Science

Patients with Schizophrenia and Social Contacts

Slavica Arsova[1*], Gabriela Kopacheva Barsova[2]

[1]University Psychiatry Clinic, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia; [2]University Clinic for Ear, Nose and Throat, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Vodnjanska 17, Skopje 1109, Republic of Macedonia

Citation: Arsova S, Kopacheva Barsova G. Patients with Schizophrenia and Social Contacts. Open Access Maced J Med Sci. 2016 Sep 15; 4(3):388-391. http://dx.doi.org/10.3889/oamjms.2016.084

Keywords: schizophrenia; treatment; personal and social relations; psychosocial activity; Republic of Macedonia.

***Correspondence:** Slavica Arsova, MD. University Psychiatry Clinic, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia. E-mail: arsova_s@yahoo.com

Received: 21-Jun-2016; **Revised:** 09-Jul-2016; **Accepted:** 10-Jul-2016; **Online first:** 21-Jul-2016

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: Patients with schizophrenia have severe problems with personal and social relations which affect their quality of life.

AIM: The aim of the paper was to monitor personal and social relations in patients with schizophrenia and to find out the differences regarding socio-demographic characteristics and ambulatory and day hospital treatment.

MATERIAL AND METHODS: The investigation included 120 subjects each with diagnosis F20 according to ICD 10 criteria; divided into two groups of 60 patients regarding their actual treatment (the first group received ambulatory care whereas those from the second group had a day hospital treatment). Patients were of different age and gender, receiving regular antipsychotic therapy. They were included in individual and group psychosocial therapeutic procedures during the day hospital treatment. The investigation utilised the following diagnostic instruments: standardised clinical interview and Personal and social performance scale (PSP scale), a non-standardized questionnaire of socio-demographic data, family support and existence of mental disorder in other family members.

RESULTS: The results have shown better personal and social functioning in patients who had family support, in those who are employed, in those with no mental disorder in other family members and in patients on day hospital treatment against patients receiving ambulatory care.

CONCLUSION: Day hospital treatment, family support and social support improve the ability for personal and social contacts of patients with schizophrenia.

Introduction

Over the last two decades, psychosocial activities have been directed towards to improve the personal and social functioning of patients with schizophrenia which means not only treatment of the schizophrenic symptomatology. Schizophrenia is a chronic mental disorder that affects emotions, cognition behaviour. The consequences are poor psychosocial functioning and a low quality of life in those people.

The quality of life means the ability to play socially defined roles such as homemaker, worker, student, spouse and friend, and additionally, this gives the individual a feeling of satisfaction and the ability to take care of him/her and to enjoy the life [1].

Psychosocial interventions inducted on a day hospital treatment would enable better therapeutic collaboration, effective pharmacological treatment, better control of patient disorder and their life in general and taking self-care of themselves with greater personal satisfaction [2-4].

The aim of this study was to monitor self-care in patients with schizophrenia and to find out the differences regarding socio-demographic characteristics and ambulatory and day hospital treatment.

Materials and Method

The investigation included 120 subjects each with diagnosis F20 according to ICD 10 criteria.

Subjects were divided into two groups of 60 patients regarding their actual treatment. The first group received ambulatory care whereas those from the second group had a day hospital treatment). Patients were of different age and gender and were, receiving regular antipsychotic therapy. They were included in individual and group psychosocial therapeutic procedures during the day hospital treatment. The subjects of both groups were evaluated at the beginning of treatment and after 6 months, after ambulatory or day hospital treatment.

The investigation utilised the following diagnostic instruments: standardised clinical interview; personal and social performance scale (PSP scale) [5]; non-standardized questionnaire of socio-demographic data including, family support and existence of mental disorder in other family members.

Results

Distribution in Table 1 shows the absence of problems in personal and social contacts in only 4 (3.33%) single subjects, but severe problems were found in 18 (15%) of subjects and very severe in 32 (26.67%) of single subjects. The final results of the research have shown that the majority of the subjects who are with schizophrenia are singles not married with statistical signification p = 0.017.

Table 1: Personal and social contacts – marital status

Personal and social contacts	Marital status				Total
	Single/man/woman	Married	Divorced	Widow	
1 absent	4 (3.33%)	2 (1.67%)	1 (0.83%)	0	7 (5.83%)
2 mild	4 (3.33%)	1 (0.83%)	2 (1.67%)	0	7 (5.83%)
3 manifested	4 (3.33%)	6 (5.0%)	0	0	10 (8.33%)
4 marked	12 (10.0%)	14 (11.67%)	1 (0.83%)	1 (0.83%)	28 (23.33%)
5 severe	18 (15.0%)	5 (4.17%)	2 (1.67%)	0	25 (20.83%)
6 very severe	32 (26.67%)	6 (5.0%)	4 (3.33%)	1 (0.83%)	43 (35.83%)
Total	74 (61.67%)	34 (28.33%)	10 (8.33%)	2 (1.67%)	120 (100%)

Kruskal-Wallis H = 8.13, p = 0.017.

The subjects who have the lower education or they are not employed have significantly harder tasks in establishing of personal and social contacts versus the ones who are employed and possessing a higher level of education, p = 0.0025.

Table 2: Personal and social contacts – educational level

Personal and social contacts	*Education*			*Total*
	Low	High	Academic level	
1 absent	0	5 (4.17%)	2 (1.67%)	7 (5.83%)
2 mild	0	1 (0.83%)	6 (5.0%)	7 (5.83%)
3 manifested	1 (0.83%)	5 (4.17%)	4 (3.33%)	10 (8.33%)
4 marked	2 (1.67%)	19 (15.83%)	7 (5.83%)	28 (23.33%)
5 severe	3 (2.50%)	17 (14.17%)	5 (4.17%)	25 (20.83%)
6 very severe	11 (9.17%)	25 (20.83%)	7 (5.83%)	43 (35.83%)
Total	17 (14.17%)	72 (60.0%)	31 (25.83%)	120 (100%)

Kruskal-Wallis H = 11.99, p = 0.0025.

Distribution in Table 3 shows the absence of problems in personal and social contacts in 3 (2.50) unemployed, 1 (0.83%) employed subjects and in 2 (1.67%) retired persons. Manifested problems were found in 6 (5.0%) unemployed, 4 (3.33) employed subjects, 1 student and 2 retired subjects, whereas marked problems were found in 15 (12.50%) unemployed subjects. Very severe problems in social relations were experienced by 34 (28.33%) unemployed subjects.

Table 3: Personal and social contacts – employment status of the subjects

Personal and social contacts	*Employment*				*Total*
	Unemployed	Employed	Student	Retired persons	
1 absent	3 (2.50%)	1 (0.83%)	1 (0.83%)	2 (1.67%)	7 (5.83%)
2 mild	3 (2.50%)	1 (0.83%)	0	3 (2.50%)	7 (5.83%)
3 manifested	6 (5.0%)	4 (3.33%)	0	0	10 (8.33%)
4 marked	15 (12.50%)	13 (10.83%)	0	0	28 (23.33%)
5 severe	20 (16.67%)	1 (0.83%)	0	4 (3.33%)	25 (20.83%)
6 very severe	34 (28.33%)	4 (3.33%)	1 (0.83%)	4 (3.33%)	43 (35.83%)
Total	81 (67.50%)	24 (20.0%)	2 (1.67%)	13 (10.83%)	120 (100%)

Kruskal-Wallis H=7.13, p=0.028.

Subjects with different employment status (p<0.05) showed differences in the modalities of the personal and social contacts Scale. Unemployed subjects significantly more often had marked or severe problems regarding the personal and social relations, while employed subjects significantly less often had very severe problems.

Table 4: Personal and social contacts – family support

Self-care	*I think the family is supportive*						*Total*	
	No		Little		Very much			
1 absent	1	(0.83%)	4	(3.33%)	2	(1.67%)	7	(5.83%)
2 mild	1	(0.83%)	1	(0.83%)	5	(4.17%)	7	(5.83%)
3 manifested	1	(0.83%)	3	(2.50%)	6	(5.0%)	10	(8.33%)
4 marked	1	(0.83%)	9	(7.50%)	18	(15.0%)	28	(23.33%)
5 severe	2	(1.67%)	17	(14.17%)	6	(5.0%)	25	(20.83%)
6 very severe	11	(9.17%)	22	(18.33%)	10	(8.33%)	43	(35.83%)
Total	17	(14.17%)	56	(46.67%)	47	(39.17%)	120	(100%)

Chi-square = 45.65, df = 10, p = 0.000000.

Six months after the grouping of subjects, in the day hospital treatment group had - mild difficulties of self-care [34 (56.7%)], with the manifestation of problems in 16 (26.7%). In ambulatory treated patients 20 (33.3%) demonstrated manifest problems; 17 (28.3%) had marked problems and-14 (23.3%), severe difficulties in taking care of themselves. There was a high statistically significant difference between subjects who were treated daily in a hospital and those who were ambulatory-cared. Patients who were treated on a daily basis in the hospital did not have any hard times regarding their daily self-care (p<0.0001).

Table 5: Personal and social contacts – DC/Ambulatory Care

Personaland social contacts / 6 м	DC		Ambulatory Care	
	N	%	N	%
1 absent	6	10.0	2	3.33
2 mild	34	56.67	5	8.33
3 manifest	16	26.67	20	33.33
4 marked	3	5.0	17	28.33
5 severe	1	1.67	14	23.33
6 very severe	0	0	2	3.33
Total	60	100	60	100

U = 588.5, Z = 6.36, P = 0.00000.

Discussion

The results obtained in this study demonstrated unsatisfactory psychosocial functioning in both groups of patients. Thus, quality of life of patients with the schizophrenic disorder was observed. Most of the unemployed patients, about 30% lost their jobs after because of the psychosocial dysfunction and stigmatisation of the society.

However, the six–month continuous treatment brought improvement in functioning, which was statistically significant in those receiving day hospital treatment. Our results are in therefore in agreement with those presented by other authors, who suggested that integrated psychopharmacological and psychosocial treatment was indispensable for the inclusion of these patients again in the social functioning, establishing social contacts, employment, as part of, inclusion in societal life [1, 2, 4, 6, 8, 9].

NICE rec. for the treatment and recovery of the patients with schizophrenia is a community-based treatment which means individual treatment tailored for each patient, treatment in the community, ambulatory care, service level interventions, and acute day hospital treatment and in the day hospital centres. NICE rec. are CBT treatment, family interventions and art therapy in the recovery period and after for faster and better reintegration and socialisation [21].

Data presented in literature point out to the poor psychosocial functioning of patients with schizophrenic disorders and poor quality of life in general [9]. Koivumaa-Honkanen et al. in their investigation used different scales for assessment of the quality of life (QOL) in patients with schizophrenia and found out poorer functioning in these patients compared to the remaining psychiatric patients [10]. Sullivan et al. conducted a study among a population of schizophrenic patients divided into three groups (patients in psychiatric institutions, patients who live alone and those who live in centres for psychosocial support) and compared them with the healthy population. Using the interview for the assessment of QOL, they obtained results that revealed the poorer quality of life in all three groups of schizophrenic subjects against the healthy ones. The biggest differences were observed in satisfaction from social life, finances and employment.

Malm et al. using the semi-structured questionnaire (QOLC) for assessment of the quality of life of 40 schizophrenic subjects 2 years after their last hospitalisation, found out dissatisfaction in almost all aspects of living and especially in social relations, education, finances, etc. [11].

The majority of studies identify the relationship and diversity of quality of life in schizophrenic patients and some sociodemographic characteristics [12]. Shtasel et al. in their study of schizophrenic patients detected better functioning of female subjects than male [13, 14].

On the other hand, Lehman in his study revealed that individuals who were married had a better quality of life that those who were not married [15, 16]. With regard to education, many studies have revealed the poorer quality of life in those schizophrenic patients who had higher levels of education [17]. Other researchers have presented the correlation between the presence of neuroleptic symptomatology, negative schizophrenic symptomatology and distinct depression with low quality of life satisfaction [17, 18].

Relationship between the treatment of these patients and their quality of life is underlined in many studies the results obtained confirmed better psychosocial functioning with usage of the second-generation antipsychotics and, better quality of life in those subjects who had integrated psychopharmacological and psychosocial treatment (family interventions, supportive interventions, cognitive behavioural, training for social skills, and especially in day hospital settings or other similar psychosocial facilities) [1, 3, 19, 20].

In conclusion, daily hospital psychosocial therapeutic treatment in combination with regular antipsychotic therapy, family and social support helps in more rapid reintegration and re-socialization and better quality of life in patients with schizophrenia.

References

1. Essock S, Kontos N. Implementing assertive community treatment teams. Psychiatr Serv. 2005; 46:679-683.

2. Aberg-Wistedt A, Cressell T, Lidberg Y, Liljenberg B, Osby U. Two-year outcome of team-based intensive case management for patients with schizophrenia. Psychiatr Serv. 1995; 46:1263-6. http://dx.doi.org/10.1176/ps.46.12.1263 PMid:8590112

3. Arango C, Bombín I, González-Salvador T, García-Cabeza I, Bobes J. Randomised clinical trial comparing oral versus depot formulations of zuclopenthixol in patients with schizophrenia and previous violence. Eur Psychiatry. 2006; 21:34–40. http://dx.doi.org/10.1016/j.eurpsy.2005.07.006 PMid:16360311

4. Bachrach LL. Psychosocial rehabilitation and psychiatry in the care of long-term patients. American Journal of Psychiatry. 2002; 149:1455-1463.

5. Morosini PL, Magliano L, Brambilla L, Ugolini S, Pioli R. Development, reliability and acceptability of a new version of the DSM-IV Social and Occupational Functioning Assessment Scale (SOFAS) to assess routine social functioning. Acta Psychiatr Scand. 2000;101(4):323-9. http://dx.doi.org/10.1034/j.1600-0447.2000.101004323.x

6. Schwartz RC. Self-awareness in schizophrenia: its relationship to depressive symptomatology and broad psychiatric impairments. J Nerv Ment Dis. 2001; 189: 401–403. http://dx.doi.org/10.1097/00005053-200106000-00010 PMid:11434643

7. Sim K, Mahendran R, Siris SG, Heckers S, Chong SA. Subjective quality of life in first episode schizophrenia spectrum

disorders with comorbid depression. Psychiatry Res. 2004; 129:141–147. http://dx.doi.org/10.1016/j.psychres.2004.07.007 PMid:15590041

8. Slaughter J, Lehman A, Myers P. Quality of life of severely mentally ill adults in residential care facilities. Adult Residential Care Journal. 2001; 5:97-111.

9. Lieberman JA, Drake RE, Sederer LI, Belger A, Keefe R, Perkins D, Stroup S. Science and recovery in schizophrenia. Psychiatr Serv. 2008;59(5):487-96. http://dx.doi.org/10.1176/ps.2008.59.5.487 PMid:18451003

10. Koivumaa-Honkanen HT, Viinamäki H, Honkanen R, Tanskanen A, Antikainen R, Niskanen L, Jääskeläinen J, Lehtonen J. Correlates of life satisfaction among psychiatric patients. Acta Psychiatr Scand. 1996;94(5):372-8. http://dx.doi.org/10.1111/j.1600-0447.1996.tb09875.x PMid:9124086

11. Malm U, May P, Dencker S: Evaluation of the quality of life in schizophrenic outpatients: A checklist. Schizophr Bull. 2001; 7: 477-486. http://dx.doi.org/10.1093/schbul/7.3.477

12. Borge L, Martinsen EW, Ruud T, Watne O, Friis S. Quality of life, loneliness, and social contact among long-term psychiatric patients. Psychiatr Serv. 1999;50(1):81-4. http://dx.doi.org/10.1176/ps.50.1.81 PMid:9890584

13. Slaughter J, Lehman A, Myers P. Quality of life of severely mentally ill adults in residential care facilities. Adult Residential Care Journal. 2001; 5:97-111.

14. Skantze K, Malm U, Dencker S, et al. Comparison of quality of life with standard of living in schizophrenic outpatients. Br J Psychiatry. 2002; 161: 797-801. http://dx.doi.org/10.1192/bjp.161.6.797

15. Lehman A. Measures of quality of life among persons with severe and persistent mental disorders. Soc Psychiatry Psychiatr Epidemiol. 2006; 31:78-88. http://dx.doi.org/10.1007/BF00801903

16. Lehman A, Kerman E, DeForge B, et al. Effects of homelessness on the quality of life of persons with severe mental illness. Psychiatr Serv. 2005; 46:922-925.

17. Test MA, Greenberg JS, Long JD, Brekke JS, Burke SS. Construct validity of a measure of subjective satisfaction with life of adults with serious mental illness. Psychiatr Serv. 2005;56(3):292-300. http://dx.doi.org/10.1176/appi.ps.56.3.292 PMid:15746503

18. Salokangas RK, Honkonen T, Stengård E, Koivisto AM. Subjective life satisfaction and living situations of persons in Finland with long-term schizophrenia. Psychiatr Serv. 2006;57(3):373-81. http://dx.doi.org/10.1176/appi.ps.57.3.373 PMid:16524996

19. Corrigan PW, McCracken SG. Psychiatric rehabilitation and staff development: educational and organizational models. Clinical Psychology Review. 2005; 15:699-719. http://dx.doi.org/10.1016/0272-7358(95)00044-5

20. Fulton B, Goa K. Olanzapine, a review of its pharmacologic properties and therapeutic efficacy in the management of schizophrenia and related psychoses. Drugs. 2007; 53:281-298. http://dx.doi.org/10.2165/00003495-199753020-00007

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):392-396.
http://dx.doi.org/10.3889/oamjms.2016.085
eISSN: 1857-9655
Clinical Science

Ketamine Sedation in Gastrointestinal Endoscopy in Children

Ayman E. Eskander[1], Nevine R. El Baroudy[1], Amira S. El Refay[2*]

[1]Department of Pediatrics, Faculty of Medicine, Cairo University, Cairo, Egypt; [2]Departments of Child Health, National Research Centre, Cairo, Egypt (Affiliation ID: 60014618)

Citation: Eskander AE, El Baroudy NR, El Refay AS. Ketamine Sedation in Gastrointestinal Endoscopy in Children. Open Access Maced J Med Sci. 2016 Sep 15; 4(3):392-396. http://dx.doi.org/10.3889/oamjms.2016.085

Keywords: Pediatric endoscopy; sedation; Ketamine; safety; complications.

***Correspondence:** Amira S. El Refay, PhD, Department of Child Health, National Research Centre, El-Bohouth Street, Dokki, Cairo, Egypt P.O:12622, Affiliation ID: 60014618. Tel: +20 012 298 3076; fax: +37601877. E-mail: amirasayed.ak@gmail.com

Received: 06-Jul-2016; **Revised:** 16-Jul-2016; **Accepted:** 17-Jul-2016; **Online first:** 26-Jul-2016

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: Moderate sedation for gastrointestinal endoscopy has traditionally been provided by the endoscopist. Controversy has ensued over safe and efficient sedation practice as endoscopy has increased in numbers and complexity.

AIM: To evaluate the safety of ketamine sedation given by non-anesthesiologist during gastrointestinal endoscopy in children.

METHODS: A prospective study of 100 paediatric patients with gastrointestinal symptoms who were a candidate for upper or lower gastrointestinal endoscopy in paediatric endoscopy unit at Abo El-Reesh Paediatric Hospital, Cairo University. All children were > 2 years old and weighed > 6 kg. The analysis was performed in terms of sedation-related complications.

RESULTS: A total 100 paediatric patients including 53 males and 47 females with mean age of 5.04 years were involved in the study. All children were medicated with ketamine with a mean dose of 3.77mg/kg. No complications occurred in 87% of cases. Desaturation occurred in 13% of the cases and was reversible by supplemental nasal oxygen. Desaturation was more frequent during Upper GI Endoscopy and with the intramuscular route (p value=0.049). No apnea, bradycardia, arrest or emergence reactions were recorded.

CONCLUSION: Ketamine sedation found to be safe for paediatric gastrointestinal endoscopy in Egyptian children without co-morbidities. Transient Hypoxia (13%) may occur but easily reversed by nasal oxygen therapy.

Introduction

Although gastrointestinal endoscopy is widely accepted as fundamental to the diagnosis and treatment of digestive disorders in children, considerable controversy and practice differences persist with respect to the methods and agents used to achieve optimal endoscopic sedation [1]**.** Sedation must have a rapid onset, short duration of action, and should be safely administered by a non-anesthesiologist without significantly increased the risk of potential complications [2].

Ketamine is a general anaesthetic agent widely used for paediatric procedural sedation outside the operating theatre by non-anesthesiologists [3]. It is considered a dissociative anaesthetic. This means that the drug distorts the user's perception of sight and sound and produces feelings of detachment from the environment and one's self [4]. Ketamine has found many applications in paediatric anaesthetic practice. Insights into the mechanism of action and the pharmacokinetics and pharmacodynamics of its isomers have led to a re-evaluation of this drug, expanding the range of applications in children. Ketamine is a remarkably versatile drug that can be administered through almost any route. It can also be used for different purposes [5].

Prior studies of ketamine sedation for paediatric gastrointestinal endoscopy have been retrospective in nature and have used chart review to identify any clinical concerns for inadequate sedation [6, 7].

The aim of the present study was to

evaluate the safety of ketamine sedation given by non-anesthesiologist during upper and lower gastrointestinal endoscopy in children. Also, this study aims to detect possible complications from sedation in the upper and lower gastrointestinal endoscopy in children and relation between the disease history and clinical condition of the patient with possible complications of sedation by ketamine.

Patients and Methods

This prospective study was conducted upon 100 paediatric patients with gastrointestinal symptoms who were a candidate for upper or lower gastrointestinal endoscopy presented to Pediatric Endoscopy Unit, Abo El-Reesh Pediatric Hospital, Cairo University.

All patients had the appropriate instructions for gastrointestinal endoscopy according to its type.

The study followed the regulations of the medical ethical committee of Abo El-Reesh Pediatric Hospital, Cairo University and written Informed consent was obtained from the appropriately designated parent or guardian.

A full and detailed history was taken from one of the parents or guardians regarding the disease, nutritional and drug history including drug allergies.

A full clinical examination of the patient was done including general, cardiac, chest and abdominal examination.

Pre-sedation risk assessment of cardiopulmonary status including heart rate, respiratory rate, oxygen saturation and blood pressure measurement was done before ketamine administration.

Ketamine Administration

- Intravenous (IV) Route: A loading dose of 1.5 to 2 mg/kg was administrated. The procedure started 30 seconds to 1 minute after administration. Additional incremental doses of ketamine may be administered (0.5 to 1 mg/kg) if initial sedation was inadequate or to accomplish a longer procedure.
- Intramuscular (IM) Route: Ketamine was administrated with a dose of 3 to 5 mg/kg.

Clinical onset began within 5 minutes. Duration of effective dissociation was 20 to 30 minutes.

Sedation was actively administered and supervised by a paediatrician. Procedures were performed by four different endoscopists and also attended by two nurses educated in sedation pharmacology and gastrointestinal endoscopy.

Constant patient monitoring during and after the administration of ketamine was provided including continuous cardiac monitoring, respiratory rate, pulse oximetry and blood pressure monitoring.

Sedation-related complications include a drop in oxygen saturation to equal or less than 94%, respiratory distress (stridor or wheezes), apnea, bradycardia (< 60 beats per minute), cardiac arrest, emergency reactions and need for reversal medication were observed. All patients were put under observation for 2 hours after the procedure to observe any complications.

Statistical analysis

Statistical analysis was performed using the SPSS statistical package software for windows version 21 (SSPS Inc, Pennsylvania, USA). Parametric variables are expressed as the mean ± SD. P value <0.05 was considered significant.

Results

The patients included 53 males (53%) and 47 females (47%).Their age ranged from 2 to 12 years with a mean of 5.04 years, while their weight ranged from 6 to 36 kg with a mean of 16.92 kg and they received ketamine with a dose ranged from 2 to 5 mg/kg with a mean of 3.77 mg/kg (Table 1).

Table 1: Ketamine doses in studied cases

	Mean ± SD	Median	Minimum	Maximum
Age (years)	5.04 ± 3.120	4.50	2	12
Weight (kg)	16.92 ±7.388	15.00	6	36
Ketamine dose (mg/kg)	3.77 ± 0.851	4.00	2	5

Most of the studied cases (42%) were indicated for GI endoscopy for diagnostic purposes, while 24 cases (24%) were done for follow-up of oesophagal varies, 1 case (1%) was done routinely before renal transplantation (Table 2).

Table 2: Indications of endoscope in studied cases

	Frequency(No)	Percent (%)
Dilatation	23	23
Diagnostic	42	42
Foreign body extraction	10	10
Follow-up of oesophagal varices	24	24
Routine before renal transplantation	1	1
Total	100	100

In pre-sedation assessment and examination, we found 3 cases with tachycardia and 4 cases with hypertension for age, 2 cases with cardiac murmur, 4 cases with wheezy chest and respiratory distress, 5

cases with ascites and there was no significant relation between these findings and occurrence of complications except for those who had respiratory distress, all of them developed hypoxia during the procedure (p value=0.001)

The only complication that occurred in our study was desaturation; there were 13 patients who developed hypoxia during the procedure. These patients developed a decrease in oxygen saturation to less than 94% by pulse oximetry. These attacks of hypoxia were easily reversed by supplemental nasal oxygen.

In our study, Cases are classified into four groups according to the type of the procedure performed (Upper GI endoscopy, Colonoscopy) and the route of ketamine administration (IM, IV)

Group (1) had upper GI endoscopy and received ketamine intravenously. Group (2) had upper GI endoscopy and received ketamine intramuscularly. Group (3) had a colonoscopy and received ketamine intravenously. Group (4) had a colonoscopy and received ketamine intramuscularly.

There was a significant relation between the route of ketamine administration and type of performed procedure with occurrence of complications, hypoxia occurred during Upper GI Endoscopy (14.28%) more than that occurred during lower GI Endoscopy (8.69%) and with the intramuscular administration of ketamine (15.38%) more than the intravenous (8.57%) (p value=0.49), (Table 3).

Table 3: Classification of studied cases according to the type of the procedure performed (Upper GI endoscopy, Colonoscopy) and the route of ketamine administration (IM, IV)

	Group 1		Group 2		Group 3		Group 4		P value
	NO	%	NO	%	NO	%	NO	%	
Hypoxia	3	11.1	8	16	0	0	2	13.3	0.049
Normal	24	88.9	42	84	8	100	13	86.7	

There was no relation between ketamine dose and occurrence of complications. After the procedure, one of the studied cases had an attack of vomiting that didn't recur.

Discussion

This prospective study was designed to detect complications of ketamine sedation given by non-anesthesiologist in gastrointestinal endoscopy in children. A total of 100 cases presented to the Gastrointestinal Endoscopy Unit of Abo El-Reesh Pediatric hospital.

In our study, there were 13 patients who developed hypoxia during the procedure and it was reversed by supplemental oxygen. These patients developed a decrease in oxygen saturation to less than 94% by a pulse oximeter. Most of these patients developed desaturation within few minutes from the introduction of the endoscopy. This desaturation is thought to be due to laryngospasm. Although ketamine has been deemed safe based on it's cardiovascular and respiratory protective effect [6, 7] it is also associated with increased risk of laryngospasm [9, 10].

Most of the episodes of desaturation occurred during Upper GI Endoscopy (14.28%), where stimulation of the posterior pharynx may increase the risk of airway complications. Desaturation also occurred more frequent with the Intramuscular route (15.38%), so Desaturation was more frequent in the group (2) (16%) than group (1) (11.1%).

All patients who had respiratory distress by examination before injection of ketamine had hypoxia during the procedure (p value=0.001).

The study was done by Green and Johnson, 1990 [11] of 11,589 patients, the incidence of laryngospasm was 0.4%, and that when it did occur, it was easily treated, only two cases of ketamine-associated laryngospasm led to intubation.

Unlike to our study, the incidence of desaturation less than 94% was 13%, but destruction may be due to laryngospasm or other causes related (Increased bronchial secretions) or not related to ketamine sedation such as associated chest infection as all patients who had respiratory distress and wheezy chest by examination before injection of ketamine developed desaturation during procedure.

In our study, none of the patients developed apnea, bradycardia or arrest. In our study, none of the patients developed emergence reactions, this may be due to young age of the sample size that ranged from 2 to 12 years with mid age of 5 years and these reactions, unpleasant dreams or hallucinations are mainly found to be transient and mild in children [12], however recovery agitation occurred in (2.4%) in study done by Green and his colleagues, 2011 [13].

Ketamine was found to increase heart rate nearly for all children more than that measured before injection in pre-sedation assessment, but it was accepted for their age.

Also, ketamine was found to increase blood pressure nearly for all children more than that measured before in the pre-sedation assessment, but most of them were accepted for their age. Hypertension occurred above-accepted level for age in 4 cases (4%), 2 of them was in group (4) who had high blood pressure in pre-sedation assessment before ketamine injection, one in group (1), this patient was previously diagnosed with ITP and received steroids, and one in group (2), this patient

was Steroid Resistant Nephrotic Syndrome (SRNS), receiving steroids and the endoscopy was requested as a routine preparation of renal transplantation.

In all groups, there is no relation between ketamine dose and complications except in group (3), this could not be assessed as there is no complications occurred in this group.

One patient (1%) in the group (2) developed post-procedural vomiting, this female patient is SRNS, was receiving steroids, received ketamine at a dose of 2.5 mg/kg, had high blood pressure for her age during the procedure and her upper GI endoscopy revealed gastritis.

A randomised clinical trial suggested that the use of 0.01 mg/kg of atropine reduced hypersalivation and vomiting associated with IM ketamine; there are no data on this strategy when used with IV ketamine [14].

A study was done by Green et al., 2011 [13] which is different from our study as it was retrospective in 636 cases primarily by the intravenous (IV) route (98%), but our study is prospective study on 100 cases primarily on the intramuscular (IM) route (65%).

This study is similar to our study as it was done primarily for EGD (86%) and our study was done also primarily for EGD (76%).

Adverse effects of this study included transient laryngospasm (8.2%), vomiting (4.1%), recovery agitation (2.4%), partial airway obstruction (1.3%), apnea and respiratory depression (0.5%), and excessive salivation (0.3%) and this differs completely from our study as we had desaturation (13%),vomiting(1%) with no other adverse effects but it may be due to this study had nearly half (46%) the subjects had severe underlying illness (American Society of Anesthesiologists (ASA] class > or =3) and our study had exclusion criterion of any severe illness.

In this study, all instances of laryngospasm occurred during EGD (9.5% incidence), and the only independent predictor of laryngospasm in this sample was decreasing age. The incidence of laryngospasm was 13.9% in preschool-aged (< or = 6 years) children and was 3.6% in school-aged (> 6 years) children (difference 10.3%, 95% confidence intervals 5.5-14.9%). No dose relationship was noted with laryngospasm. This is similar to our study as desaturation occurred mainly in EGD (14%) and there is no relation between ketamine dose and desaturation but differs that no relations between age and desaturation in our study.

In Regional Hospital of Iquique, Iquique, Chile, over the past 24 years, with the authorization of the anesthesiology department, they have used ketamine in more than 900 paediatric endoscopic procedures. This experience has included upper endoscopy, intestinal biopsy, colonoscopy, percutaneous gastrostomy, ERCP, foreign-body removal, oesophagal dilation, and oesophagal sclerotherapy [15].

These indications are similar to our indications which included upper endoscopy for diagnosis and follow-up, colonoscopy (23%), foreign body removal (10%), oesophagal dilation (23%), and routine before Renal Transplantation.

Our data may be useful for planning larger studies required to determine whether laryngospasm or other adverse events occurs more or less frequently with ketamine than has been reported previously.

References

1. Schwarz SM, Lightdale JR, Liacouras CA. Sedation and anesthesia in pediatric endoscopy: one size does not fit all. J Pediatr Gastroenterol Nutr. 2007;44:295-7. http://dx.doi.org/10.1097/MPG.0b013e31802f6435 PMid:17325547

2. Lauven PM. Pharmacology of drugs for conscious sedation. Scand J Gastroenterol. 1990;Suppl.179:1-6. http://dx.doi.org/10.3109/00365529009093164

3. Deasy C, Babl FE. Intravenous vs intramuscular ketamine for pediatric procedural sedation by emergency medicine specialists: a review. Paediatr Anaesth. 2010;20:787-796. http://dx.doi.org/10.1111/j.1460-9592.2010.03338.x PMid:20716070

4. National Institute on Drug Abuse. NIDA Research Report Series, Hallucinogens and Dissociative Drugs. U.S. Department of Health and Human Services, 2001.

5. Roelofse JA. The evolution of ketamine applications in children. Paediatr Anaesth. 2010;20:240-245. http://dx.doi.org/10.1111/j.1460-9592.2009.03145.x PMid:19793346

6. Gilger MA, Spearman RS, Dietrich CL, Spearman G, Wilsey M J, Zayat MN. Safety and effectiveness of ketamine as a sedative agent for pediatric GI endoscopy. Gastrointestinal Endoscopy. 2004;59:659–663. http://dx.doi.org/10.1016/S0016-5107(04)00180-4

7. Green SM, Klooster M, Harris T, Lynch EL, Rothrock SG. Ketamine sedation for pediatric gastroenterology procedures.Journal of Pediatric Gastroenterology and Nutrition. 2001;32:26–33. http://dx.doi.org/10.1097/00005176-200101000-00010 PMid:11176320

8. Reich DL, Silvay G. Ketamine: an update on the first twenty-five years of clinical experience. Canadian Journal of Anaesthesia. 1989;36:186–197. http://dx.doi.org/10.1007/BF03011442 PMid:2650898

9. Aggarwal A, Ganguly S, Anand VK, Patwari AK. Efficacy and safety of intravenous ketamine for sedation and analgesia during pediatric endoscopic procedures. Indian Pediatrics. 1998;35:1211–1214. PMid:10216697

10. Spearman RS, Spearman G, Wilsey MJ, Gilger M. Is Ketamine a "safe" sedative agent for pediatric endoscopy? Journal of Pediatric Gastroenterology and Nutrition. 2000;31:29.

11. Green SM and Johnson NE. Ketamine sedation for pediatric procedures: Part 2, review and implications. Ann Emerg Med. 1990;19:1033–1046. http://dx.doi.org/10.1016/S0196-0644(05)82569-7

12. Howes MC. Ketamine for paediatric sedation/analgesia in the emergency department. Emerg Med J. 2004;21:275–280.

http://dx.doi.org/10.1136/emj.2003.005769 PMid:15107362 PMCid:PMC1726352

13. Green SM, Roback MG, Kennedy RM, Krauss B. Clinical Practice Guideline for Emergency Department Ketamine Dissociative Sedation: 2011 Update. Annals of Emergency Medicine. 2011;57:5: 449–461. http://dx.doi.org/10.1016/j.annemergmed.2010.11.030 PMid:21256625

14. Heinz P, Geelhoed BC, Wee C, Pascoe EM. Is atropine needed with ketamine sedation? A prospective, randomised double blind trial. Emerg Med J. 2006;23:206–209. http://dx.doi.org/10.1136/emj.2005.028969 PMid:16498158 PMCid:PMC2464444

15. Kirberg A, Sagredo R, Montalva G, Flores E. Ketamine for pediatric endoscopic procedures and as a sedation complement for adult patients. Gastrointest Endosc. 2005;61:501–502. http://dx.doi.org/10.1016/S0016-5107(04)02724-5

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):397-403.
http://dx.doi.org/10.3889/oamjms.2016.080
eISSN: 1857-9655
Clinical Science

Justification for Rhinoseptoplasty in Children – Our 10 Years Overview

Gabriela Kopacheva-Barsova*, Nikola Nikolovski

University Clinic for Ear, Nose and Throat, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Vodnjanska 17, Skopje, MK 1109, Republic of Macedonia

Citation: Kopacheva-Barsova G, Nikolovski N. Justification for Rhinoseptoplasty in Children – Our 10 Years Overview. Open Access Maced J Med Sci. 2016 Sep 15; 4(3):397-403. http://dx.doi.org/10.3889/oamjms.2016.080

Keywords: septo/rhinoplasty in children; indications; surgical procedures; nasal growth; midfacial development.

***Correspondence:** Gabriela Kopacheva-Barsova, PhD. University Clinic for Ear, Nose and Throat, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Vodnjanska 17, Skopje, MK 1109, Republic of Macedonia. E-mail: gabrielak70@yahoo.com

Received: 01-Jul-2016; **Revised:** 17-Jul-2016; **Accepted:** 19-Jul-2016; **Online first:** 01-Aug-2016

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: Background: Nasal septal surgery and rhinoplasty are controversial in children. Traditionally, an attitude of restraint has been employed by most surgeons till an empirical age of 16 to 18 years. This is to avoid the possible adverse effects that the growth spurts may have on the nose and midface region.

AIM: The aim of this paper was to present the results of rhinoplasty in children in order to restore the anatomy and function or to promote normal development and outgrowth of the nose.

MATERIAL AND METHODS: Ninety seven children aged 6-14, with severe nose deformities and breathing problems through the nose, were admitted for septo/rhinoplasty at the University Clinic for Ear, Nose and Throat, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Republic of Macedonia. At our Clinic, they have been observed and photographed (with parent permission) in the period of 10 years (2006-2016). The most frequent cause of these deformities was the nasal trauma in early childhood which was ignored or untreated. All of them rhino/septoplasty were indicated in accordance with the above-mentioned recommendations for rhino/septoplasty in early childhood and in adolescents.

RESULTS: In 51 children and adolescents septoplasty were prepared. Mostly there was a group of younger children age from 6-10 (68%) and adolescents (32%). In the other 31 children and adolescents, septorhinoplasty was prepared. Mostly there were children older than 12 years old and adolescents (70%). Only 30% were younger than 12 years, of course with severe nasal breathing problems, nasal septal deformities and deformities of the nasal pyramid.

CONCLUSION: The growth centres of the nose have to be avoided if possible; long-term nasal issues will theoretically be minimised. If the surgeon replaces it, the cartilage of the nose becomes straighter but still intact.

Introduction

There has been a lot of debates and controversy about the rhino/septoplasty in children and adolescents because for a long time it has been generally accepted that surgery of the bony and cartilaginous nasal pyramid and the especially nasal septum is justified until a young patient has reached the minimum age of sixteen years.

Frequently the deformities of the nasal septum and nasal pyramid occur due to the fractures of the facial and nasal bones especially in children younger than five years of age. The incidence increases with increasing age and peaks between 16 and 20 years. Numerous observations on the retarded growth of the nose after submucous resection caused the above-mentioned restriction in surgery at a young age. If the septum is determined to be the reason of nasal obstruction in a childhood, a clinical dilemma arises. Recent basic researches, assessment the results of the thesis of the negative influence of surgical trauma in growth and development of nasal and skull bone.

The behaviour of hyaline cartilage of the human nose appeared to be comparable to that of other mammals. Cartilage, although resilient, can be easily fractured whereas its tendency to integrated

healing is very low, even when the perichondrium has been saved. Also surgical procedures, like in septoplasty – may result in growth disturbances of the nasal skeleton like recurrent deviations or duplicature. Loss of cartilage, as might occur after a severe septal trauma, is never completely restored despite some cartilage regeneration. Still, there remains a lack of consensus in the literature concerning the developmental effects of rhino surgery in children [1-3].

Clinical reports have not produced solid evidence for the statement that septal surgery has no negative effect on nasal growth or can serve for correcting abnormal growth. The functional and esthetic problems of the patient, however, mean a continuous stimulus for further clinical and experimental investigations [4, 5].

Developmental aspects of the children nose

Children's nose is different than in adults. The children nose is growing and anatomical structures are developing, therefore, there are differences in size, form and structure of supporting cartilaginous and bony framework. The anatomy of the nasal skeleton is specific and different than in adults, and the wound healing capacity of nasal cartilage is poor. The restricted wound healing capacity of septum cartilage is the essential factor in limiting the effectiveness of surgical interventions and should be recognised during the planning and surgical intervention. Accidental or surgical injury have immediate and late consequences for the further growth of the midface [6- 8].

Figure 1: Facial profile of a child (7 years old) and her mother (44 years old). The differences in the proportions and size of the nose, facial and brain scull are obvious. The infant's face shows smaller vertical dimensions, less frontal projection of the nose and a larger nasolabial angle (with the permission of parents)

Children nose is smaller than adults, it has shorter nasal dorsum, less projection of the nasal tip and columella, rounder nostrils and a larger nasolabial angle. It has a flat tip with a shorter columella. The tissue of the nose is very soft and has a thicker subcutaneous layer. The septum of the children nose is the main is the main supporting mechanism of the nasal skeleton and it's cartilaginous. The septum forms a T-bar-shaped structure with the upper laterals. Upper lateral cartilages extended to reach the anterior scull base. In adults, upper lateral cartilages extend cephalically underneath the nasal bones.

Nasal growth is continued after puberty. Later, in the adolescents, the process ends, for male 18-20 years old, and 16-18 years old for female [9, 10] (Fig. 1).

The septo-dorsal cartilage is essential in the development of the midfacial bony and cartilaginous skeleton because growing cartilage and growing bone are in interaction. Interventions, surgical procedures, incisions on the septum cartilage, fractures, defects, interfere with normal development of the premaxilla and can seriously affect the development of all nasal supporting structures. This, special zones have special functions in the postnatal growth of the midface. The growing dorsolateral cartilage is interacting with the sutural growth of the midfacial skeleton.

There is two significant nasal growth spurts The first postnatal year which starts an endochondral ossification process (in the region of the anterior skull base) and the period of puberty when the nose grows faster compared to other periods in life.

Later, the perpendicular plate extends due to progressive ossification of nasal septal cartilage. Due to this ossification process, most of the cartilaginous part of the nasal septum loses contact with the sphenoid. The Vomer as a formation is the result of extra cartilaginous ossification.

There are two very important Growth zones responsible for the growth and development of the nose and nose elements. These two thicker areas with different mitotic activity and histological maturation are in the cartilaginous part of the nasal septum. These "growth zones", are extended from the sphenoid [11, 12].

First, the "sphenodorsal" zone is located between the sphenoid and the nasal dorsum. This zone is primarily responsible for the normal increase in length and height of the nasal dorsum. The second, "sphenospinal" zone is located between the sphenoid and the anterior nasal spine and is the driving force in the forward outgrowth of the (pre)maxilla region.

Common causes of the destruction of zones are septal hematomas and nasal septal abscess formation, surgery or trauma. That affects the midfacial growth related to the children age and thus it can cause deformities in young children, like saddle nose deformity or/with columellar retraction, or an over-rotation of the nasal tip and a retroposition of the midface [13, 14].

Septum surgery in children- when is justified

Rhinologic, orthodontic and cephalmometric data should be essential elements in the follow-up of children after injury and surgery of the nose. The effects of surgery (or injury) should be specified for subgroups of children with different ages, like 3–6, 7–10 years etc.

Patient (child) and parents should be informed of the potential benefits of the surgery, and of continuing a follow-up of the facial growth till after the adolescent growth spurt [15, 16] (Fig. 2).

Figure 2: Making photographic documentation is important to define qualifications as normal or abnormal, which are in essence subjective (with the permission of parents)

Indications for rhinosepoplasty in children

The aim to perform rhinoplasty in children is to restore the anatomy and function or to promote normal development and outgrowth of the nose. For every indication, the expected benefits of intervention should be weighed against the possible adverse outcomes on nasal and midfacial growth. In the ideal situation, surgery should be postponed till after the pubertal growth spurt. However, there are distinct indications for immediate intervention. Apart from malignancies, these indications include the destruction of the nasal skeleton jet, it seems neither possible not even advisable to act strictly to this rule. Rhinoseptoplasty in children can be required for certain reasons.

Actual indications are severe congenital malformations of the nose, recent traumatic deformities, external distortion of the nose due to septal abscess, breathing problems due to septum pathology, cleft lip nose, dermoid cysts.

Figure 3: Saddle nose (rhinolordosis) due to untreated septal hematoma after the nasal trauma in early childhood) (with the permission of parents)

Children with less evident pathology have to follow-up the progression of the pathology for a certain period before making a definite decision for surgery indication. The tendency towards rhino/septoplasty in children has increased considerably. This changes in attitude bear some certain risks, so is appropriate to review once again the methods of assessment and management of pathology of the nasal skeleton in children [17, 18] (Fig. 3).

Recommendations for Rhinosurgery in children

Before any surgery on the child's nose is performed, it is very important first to Identify defects (older or recent) and fractures of the septum, and their relation to the specific growth and support zones.

Indications for immediate intervention are malignancies, septal abscess (septal haematoma), severe nasal trauma, cleft lip or progressive distortion of the nose. In all other cases, the surgeon has to mobilise the deviated or overlapping cartilaginous fragments, to adapt form and size of the fragments, to reconstruct a straight septum in the midline, always avoiding incisions through the growing and supporting zones (spheno-ethmoido dorsal zone).

Mobilisation of nasal bones in combination with the reconstruction of a malformed septum is the risk of postoperative instability of the (corrected) supporting structures. Intraseptal blood collection should be avoided in order to minimise the chance of infection and subsequent cartilage necrosis. In exceptional cases, it is necessary to use a 2mm osteotome to produce a satisfactory alignment of the nasal bones. Nasal packing for a few days is only tolerated by older children. Alloplastic or biomaterials are not capable of growth and implanted in a growing septum may disturb septal growth [19, 20].

The aim of this paper was to present the results of rhinoplasty in children in order to restore the anatomy and function or to promote normal development and outgrowth of the nose.

Materials and Methods

Children aged 6-14, with severe nose deformities and breathing problems through the nose, were admitted for septo/rhinoplasty at the University Clinic for Ear, Nose and Throat, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Republic of Macedonia. At our Clinic, 97 children have been observed and photographed (with parental consent) in the period of 10 years (2006-2016). The most frequent cause of these deformities was the nasal trauma in early childhood which was ignored or untreated. All of them rhino/septoplasty were indicated in accordance

with the above-mentioned recommendations for rhino/septoplasty in early childhood and in adolescents.

Fifty-eight percentages of the children were observed for the period of at least 5-7 years after the operation. The remaining patients could not be observed because they were unavailable, changed the place of living or telephone number.

Results

At the University Clinic for Ear, Nose and Throat, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Republic of Macedonia, 97 children have been observed in the period of 10 years (2006-2016).

Children were on age 6-14 years old and were divided into 2 groups: the First group were from age 6-10 years old (36%), and second from 11-14 years old (64%). 52 of them were women and 45 were men. According to the nationality, 49 % were Albanian, 42% were Macedonian and 6% were from other nationality (Table 1).

Table 1: Distribution of the children patients by demographic characteristic

Demographic characteristics		
Gender	Women	52 (53.61%)
	Men	45 (46.39%)
Nationality	Macedonian	42 (43.3%)
	Albanian	49 (50.51%)
	Other nationality	6 (6.18%)

Comparing with the severity of nose deformity, nasal septal deviation and radiological investigation (CT scans or plane Radiographs founding's) children and adolescents were divided into 5 groups. In our study, we have been made the systematic classification of the deviations of the nasal septum taking into consideration not only the classifications in the region of the cartilaginous septum but the position of the nasal septum regarding the external configuration of the nose. The objectives of this classification were to encompass all the pathological alterations of the nasal septum and to document them, in order to implement an adequate surgical technique.

We classified the septal deviation in 5 groups: Group 1: 21 patients were with deviation in pars cartilaginea in area of spina septi nasi (anterior parts of nasal septum); Group 2: 15 patients were with nasal septal deviation in pars ossea (posterior parts of nasal septum including vomer); Group 3: 19 patients were with nasal septal deviation close to dorsum septi nasi; Group 4. 17 patients were with subluxation of nasal septum; and Group 5: 25 patients were with mixed deviation: spina septi nasi with deviation in pars cartilaginea and nasal septal deviation in pars ossea (Fig. 4).

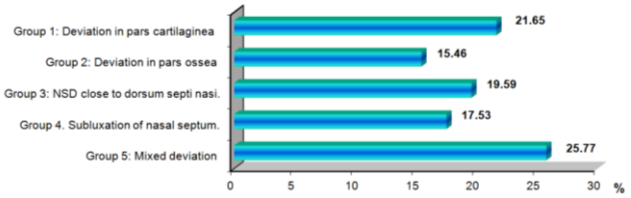

Figure 4: Classification of the deviations of the nasal septum in children by groups

According to the severity of the nose deformities and breathing problems septo/rhinoplasty were indicated in all of them. But in only, 82% septo/rhinoplasty were prepared of course with parental consent and in accordance with the above mentioned recommendations for rhino/septoplasty in early childhood and in adolescents. In 51 children and adolescents septoplasty were prepared. Mostly there was a group of younger children age from 6-10 (68%) and adolescents (32%). In the other 31 children and adolescents septorhinoplasty were prepared. Mostly there were children older than 12 years old and adolescents (70%). Only 30% were younger than 12 years, of course with severe nasal breathing problems, nasal septal deformities and deformities of the nasal pyramid (Table 2).

Table 2: Distribution of the patients by age and type of operative technique

Distribution of the patients by age and type of operative technique		
Septoplasty N=51	6-10 years	35 (68.6%)
	11 > years	16 (31.4%)
Septorhinoplasty N=31	< 12	9 (29.03%)
	12>	22 (70.97%)

Case Reports

Case 1**:** (8 years old boy) DSN. Rhinokyphosis (nasal trauma in early childhood). Nose under the tension of the long septum. Closed approach. Septal medioposition. Medial and lateral osteotomy. Cranial rotation and refinement of the nasal tip. Nasal hump reduction (Fig. 5).

Case 2: (12 years old girl). Tip asymmetry. Subluxation of nasal septum. Closed approach. Septal medioposition. Cranial rotation and refinement of the nasal tip. Preservation with the septal graft in the position of the nasal dorsum at a high level (Fig. 6).

Case 3: (11 years old girl) DSN. Rhinokyphosis (11 years girl/after nasal trauma in early childhood). Nose under the tension of the long septum. Closed approach. Septalmedioposition. Medial and lateral osteotomy. Cranial rotation and

refinement of the nasal tip. Nasal hump reduction (Fig. 7).

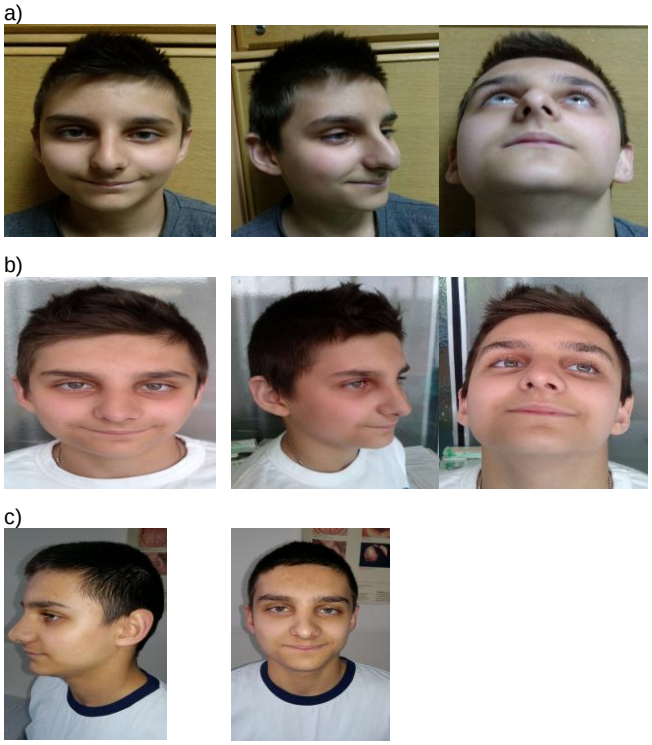

Figure 5: Eight years old boy with DSN. a) Pre operative results; b) 6 months after surgery; c) Two years after surgery (with the permission of parents)

Case 4: Fourteen years girl. Rhinoscoliosis. Rhinolordosis (after nasal trauma). Closed approach. Medial and lateral osteotomy. Septal media position.Graft (from auricula) augmentation on the nasal dorsum (Fig. 8).

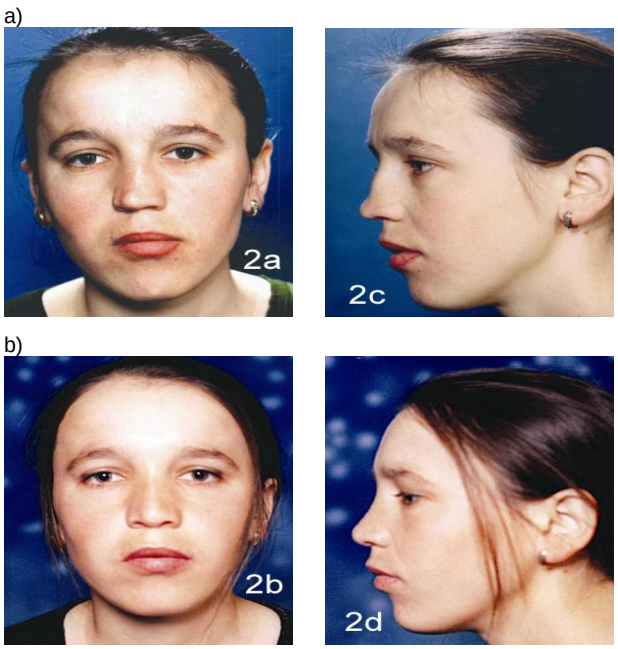

Figure 6: Twelve years old girl. a) Pre operative results; b) 1 year after surgery (with the permission of parents)

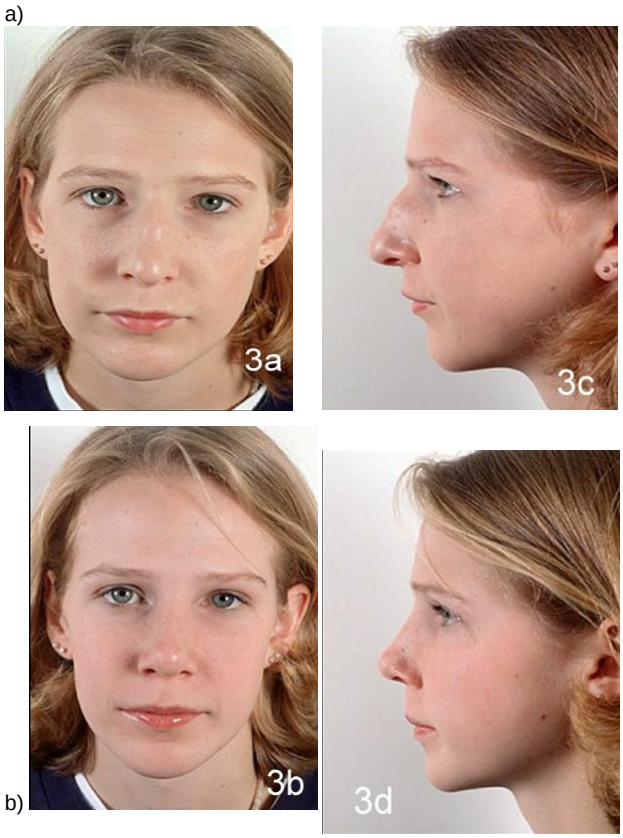

Figure 7: Eleven years old girl with DSN. Rhinokyphosis after nasal trauma in early childhood. a) Pre operative results; b) after surgery (with the permission of parents)

Discussion

The aim to perform rhinoplasty in children is to restore the anatomy and function or to promote normal development and outgrowth of the nose. The ideal situation for every surgeon is to wait for the surgery to be performed till after the pubertal growth spurt.

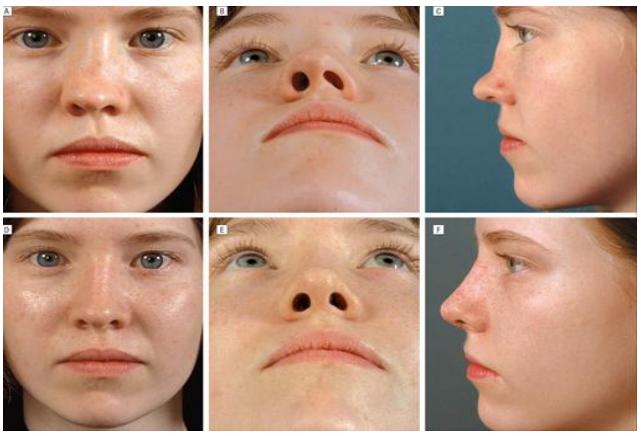

Figure 8: Fourteen years girl. Rhinoscoliosis and rhinolordosis (after nasal trauma). Graft (from auricula) augmentation on the nasal dorsum (with the permission of parents)

A correct diagnosis of fracture or dislocation of the bony pyramid is more difficult than in adults. The examination should be repeated after 3-5 days, when the soft tissue swelling has diminished. General anesthesia is necessary. Repositioning of fractured or deviated nasal bones is nearly always possible without an open reduction. Septal hematoma and abscesses should be treated as in adults. In case when nasal cartilage is destructed - should be made a direct reconstruction using autologous cartilage grafts.

The behaviour of hyaline cartilage of the human nose appeared to be comparable to that of other mammals. Cartilage, although resilient, can be easily fractured whereas its tendency to integrated healing is very low, even when the perichondrium has been saved. Also surgical procedures, like in septoplasty – may result in growth disturbances of the nasal skeleton like recurrent deviations or duplicature. Loss of cartilage, as might occur after a severe septal trauma, is never completely restored despite some cartilage regeneration.

Often, difficult septal deformations in children are followed with deformation of nasal pyramid (rhinoscoliosis, rhinolordosis). In those cases, we cannot solve septal pathology without nasal pyramid intervention in the same time and opposite. Only severe deformities of the septum or the nasal pyramid, mostly in older children are treated by conservative septoplasty or septorhinoplasty [21-24].

Whatever type of nasal surgery should be performed in the children, the parents and the young patient should be informed, because the late results cannot be predicted. Because of the growing nose in this sensitive age, the recurrent septum pathology may occur after several years. So, information should be given about long follow-up and the possibility of a second operation after the adolescent growth spurt [25, 26].

In summary, the growth centres of the nose have to be avoided if possible; long-term nasal issues will theoretically be minimised. If the surgeon replaces it, the cartilage of the nose becomes straighter but still intact.

References

1. Poublon RML, Verwoerd CDA, Verwoerd-Verhoef HL. Anatomy of the upper lateral cartilages in the human newborn. Rhinology. 1990;28:41-46. PMid:2336524

2. Verwoerd CDA, van Loosen J, Schutte HE, Verwoerd-Verhoef HL, van Velzen D. Surgical aspects of the anatomy of the vomer in children and adults. Rhinology. 1989;9:87-96.

3. Potsic WP, Cotton RT, Handler SD, Zur KB, eds. Surgical Pediatric Otolaryngology. 2nd ed. Thieme, 2016:pp 900.

4. NolstTrenite GJ, Verwoerd CDA, Verwoerd-Verhoef HL. Reimplantation of autologous septal cartilage in the growing nasal septum I. The influence of resection and reimplantation of septal cartilage upon nasal growth: an experimental study in growing rabbits. Rhinology 1987;25:225-236New York: Thieme medical publishers, 1997:168-180.

5. Graber TM. Postnatal development of cranial, facial and oral structures: the dynamics of facial growth. In: Orthodontics: Principles and Practice. Philadelphia: WB Saunders, 1966:69-78.

6. Pirsig W. Morphological aspects of the injured septum in children. Rhinology. 1979;17:65-76. PMid:493821

7. Verwoerd CDA, Verwoerd-Verhoef HL. Rhinosurgery in children: Basic Concepts. Facial Plastic Surgery. 2007;23:219-230. http://dx.doi.org/10.1055/s-2007-995814 PMid:18085496

8. Pirsig W. The influence of trauma on the growing nose. In: Mladina R, passali D, eds. Pediatric Rhinology. Siena Tipografia Sense, 2000:145-159.

9. Menger DJ, Tabink I, NolstTrenite GJ. Treatment of septal hematomas and abscesses in children. Facial Plastic Surgery. 2007;23:239-243. http://dx.doi.org/10.1055/s-2007-995816 PMid:18085498

10. Killian G. Beitragezur sub submukosenfensterresektion der nasenscheidewand. Passow U. Schaefer Teits. 1908:183-192.

11. Menger DJ, Tabink I, NolstTrenite GJ. Nasal septal abscess in children, reconstruction with autologous cartilage grafts on Polydioxanone Plate. Arch Otolaryngol Head Neck Surg 2008;134(8):1-6. http://dx.doi.org/10.1001/archotol.134.8.842 PMid:18711058

12. Menger DJ, Tabink IC, TrenitéGJ.Nasalseptal abscess in children: reconstruction with autologous cartilage grafts on polydioxanoneplate. Arch Otolaryngol Head Neck Surg. 2008; 134(8):842-7. http://dx.doi.org/10.1001/archotol.134.8.842 PMid:18711058

13. Fattahi T, Steinberg B, Fernandes R, Mohan M, Reitter E.J.Repair of nasal complex fractures and the need for secondary septo-rhinoplasty. Oral Maxillofac Surg. 2006; 64(12):1785-9. http://dx.doi.org/10.1016/j.joms.2006.03.053 PMid:17113446

14. Bae JS, Kim ES, Jang YJ.Treatment outcomes of pediatric rhinoplasty: the Asan Medical Center experience. Int J Pediatr Otorhinolaryngol. 2013; 77(10):1701-10. http://dx.doi.org/10.1016/j.ijporl.2013.07.030 PMid:23958392

15. Verwoerd CD, Verwoerd-Verhoef HL. Rhinosurgery in children: developmental and surgical aspects of the growing nose]. Laryngorhinootologie. 2010; 89(Suppl 1):S46-71. http://dx.doi.org/10.1055/s-0029-1246162 PMid:20352570

16. Pediatric rhinoplasty in an academic setting. Facial Plast Surg. 2007;23(4):245-57. http://dx.doi.org/10.1055/s-2007-995817 PMid:18085499

17. Riechelmann H, Rettinger G. Three-step reconstruction of complex saddle nose deformities. Arch Otolaryngol Head Neck Surg. 2004;130(3):334-8. http://dx.doi.org/10.1001/archotol.130.3.334 PMid:15023843

18. Dennis SC, den Herder C, Shandilya M, NolstTrenité GJ. Open rhinoplasty in children. Facial Plast Surg. 2007;23(4):259-66. http://dx.doi.org/10.1055/s-2007-995818 PMid:18085500

19. Septoplasty pearls. Dobratz EJ, Park SS. Otolaryngol Clin North Am. 2009; 42(3):527-37. http://dx.doi.org/10.1016/j.otc.2009.03.003 PMid:19486747

20. Kühnel TS, Reichert TE. Trauma of the midface. GMS Curr Top Otorhinolaryngol Head Neck Surg. 2015; 14:Doc06. PMid:26770280 PMCid:PMC4702055

21. El-Hakim H, Crysdale WS, Abdollel M, Farkas LG. A study of anthropometric measures before and after external septoplasty in children: a preliminary study.Arch Otolaryngol Head Neck Surg. 2001;127(11):1362-6. http://dx.doi.org/10.1001/archotol.127.11.1362 PMid:11701075

22. Dennis SCR, den Herder C, Shandilya M, NolstTrenite GJ. Open rhinoplasty in children. Facial Plastic Surgery. 2007;23(4):259-266. http://dx.doi.org/10.1055/s-2007-995818 PMid:18085500

23. Bradbury E. Meeting the psychological needs of patients with

facial disfigurement. Br J Oral Maxillofac Surg. 2012;50(3):193-6. http://dx.doi.org/10.1016/j.bjoms.2010.11.022 PMid:21440966

24. Boenisch M, Hajas T, NolstTrenite GJ. Influence of Polydioxanone foil on growing septal cartilage after surgery in an animal model. Arch Facial Plast Surg. 2003;5:316-319. http://dx.doi.org/10.1001/archfaci.5.4.316 PMid:12873869

25. Verwoerd CDA, Verwoerd-Verhoef HL. Anatomy and development of the nasal septum in relation to septal surgery in children. In: Sih T, Clement PAR, eds. Pediatric Nasal and sinus disorders. Boca Raton, FL: Taylor & Francis Group, 2005:127-145. http://dx.doi.org/10.1201/b14160-9

26. NolstTrenite GJ. Postoperative care and complications. In; NolstTrenite GJ, ed. Rhinoplasty, 3rd ed. Chapter 5. The Hague: Kugler Publications, 2005:31-37.

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):404-409.
http://dx.doi.org/10.3889/oamjms.2016.076
eISSN: 1857-9655
Clinical Science

Automated Versus Manual Blood Pressure Measurement: A Randomized Crossover Trial in the Emergency Department of a Tertiary Care Hospital in Karachi, Pakistan: Are Third World Countries Ready for the Change?

Kanaan Mansoor[1*], Saba Shahnawaz[2], Mariam Rasool[3], Huwad Chaudhry[4], Gul Ahuja[1], Sara Shahnawaz[1]

[1]Dr. Ziauddin University Hospital, Karachi, Pakistan; [2]Dialysis Unit, The Kidney Centre, Karachi, Pakistan; [3]Sindh Government Hospital, Karachi, Pakistan; [4]New Mehran Medical Centre, Karachi, Pakistan

Citation: Mansoor K, Shahnawaz S, Rasool M, Chaudhry H, Ahuja G, Shahnawaz S. Automated Versus Manual Blood Pressure Measurement: A Randomized Crossover Trial in the Emergency Department of a Tertiary Care Hospital in Karachi, Pakistan: Are Third World Countries Ready for the Change? Open Access Maced J Med Sci. 2016 Sep 15; 4(3):404-409. http://dx.doi.org/10.3889/oamjms.2016.076

Keywords: Sphygmomanometers; Automated oscillometer; blood pressure.

***Correspondence:** Dr. Ziauddin Hospital. 4/B , Shahrahe Ghalib, Block-6, Clifton, Karachi, Pakistan. Phone: + 92 3002334895. Facsimile: +92 2134553205. E-mail: kanaanm@gmail.com

Received: 08-Jun-2016; **Revised:** 30-Jun-2016; **Accepted:** 02-Jul-2016; **Online first:** 27-Jul-2016

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: Hypertension has proven to be a strong liability with 13.5% of all mortality worldwide being attributed to elevated blood pressures in 2001. An accurate blood pressure measurement lies at the crux of an appropriate diagnosis. Despite the mercury sphygmomanometer being the gold standard, the ongoing deliberation as to whether mercury sphygmomanometers should be replaced with the automated oscillometric devices stems from the risk mercury poses to the environment.

AIM: This study was performed to check the validity of automated oscillometric blood pressure measurements as compared to the manual blood pressure measurements in Karachi, Pakistan.

MATERIAL AND METHODS: Blood pressure was recorded in 200 individuals aged 15 and above using both, an automated oscillometric blood pressure device (Dinamap Procare 100) and a manual mercury sphygmomanometer concomitantly. Two nurses were assigned to each patient and the device, arm for taking the reading and nurses were randomly determined. SPSS version 20 was used for analysis. Mean and standard deviation of the systolic and diastolic measurements from each modality were compared to each other and P values of 0.05 or less were considered to be significant. Validation criteria of British Hypertension Society (BHS) and the US Association for the Advancement of Medical Instrumentation (AAMI) were used.

RESULTS: Two hundred patients were included. The mean of the difference of systolic was 8.54 ± 9.38 while the mean of the difference of diastolic was 4.21 ± 7.88. Patients were further divided into three groups of different systolic blood pressure <= 120, > 120 to = 150 and > 150, their means were 6.27 ± 8.39 (p-value 0.175), 8.91 ± 8.96 (p-value 0.004) and 10.98 ± 10.49 (p-value 0.001) respectively. In our study 89 patients were previously diagnosed with hypertension; their difference of mean systolic was 9.43 ± 9.89 (p-value 0.000) and difference of mean diastolic was 4.26 ± 7.35 (p-value 0.000).

CONCLUSIONS: Systolic readings from a previously validated device are not reliable when used in the ER and they show a higher degree of incongruency and inaccuracy when they are used outside validation settings. Also, readings from the right arm tend to be more precise.

Introduction

One of the most common medical tests done on thousands of patients every day is a blood pressure measurement. An accurate measurement is vital in providing appropriate treatment. It aids in the diagnosis of various conditions; ranging from dehydration in diarrhoea patients with low readings to vascular disease patients with elevated readings. A BP reading can be the defining point of treatment in patients with chest pain or altered mental status.

Hypertension is one of the leading causes of developing atherosclerosis, cerebrovascular disease, stroke, ischemic cardiac disease, congestive heart failure and myocardial infarction [1]. Elevated blood pressure is also associated with the development of

renal failure and dementia [2, 3]. The prevalence of Hypertension in Pakistan was 10% in 1997 [4] while in Canada it is estimated to be 20% prevalent (that is every 1 in 5 persons has HTN) [5]. HTN related diseases are on the rise and are a global burden. According to a report published in 2008, 54% of strokes, 47% of ischemic heart diseases and 13.5% of all mortality worldwide was attributable to elevated blood pressures in 2001 [6].

Physicians are thus advised to routinely check for BP elevation in all of their patients. Overestimation of BP can expose the patient to the potential adverse effects of drug treatments as well as unnecessary medical costs and dietary restrictions. While with an underestimated reading, the patient is at a risk of HTN related diseases, which can significantly reduce life expectancy. Therefore an accurate reading is essential [7].

There are three non-invasive modalities commonly used to check blood pressure throughout the world, namely the manual mercury sphygmomanometer, aneroid meter and the automated oscillometric device. The manual mercury sphygmomanometer is considered to be the gold standard [8] that is, if used by a trained nurse or doctor. Recently, however, there is an ongoing debate about whether mercury sphygmomanometers should be replaced with the automated oscillometric devices because of health concerns. Mercury is a toxic substance and is considered an environmental hazard. It has been banned in various European countries such as Sweden and The Netherlands as well as in numerous hospitals in the United States [8, 9].

A myriad of factors can affect manual blood pressure measurements such as the site of placement of the cuff, the size of the cuff, type of stethoscope, following the proper protocol, patient's age group, pregnancy, exercise, arrhythmias and the white coat response [10]. Readings can also vary depending on whether the nurse or the doctor is conversing while taking the measurement and whether there is background noise or silence [7, 11]. All these factors contribute towards possibly inaccurate BP readings, with a potential for misdiagnosis.

Apart from the above-mentioned causes that are mostly associated with the manual mercury sphygmomanometer, there are causes that might influence the readings of both AO BP devices and the manual BP like respiration, emotions, tobacco, alcohol, temperature, bladder distension, pain and exercise. Most of these are controllable, while some are non-modifiable like age, race and diurnal variation [12-15]. Automated Oscillometric devices are seen to be less influenced by most of these factors and recent studies indicate that they virtually eliminate the white coat response [16].

Multiple studies suggest that the AO devices should replace the conventional manual mercury sphygmomanometer, as the latter is destined to become obsolete [17, 18]. However, with limited resources and the high costs involved in attaining the latest medical equipment, a significant question arises: Are third world countries ready for the change?

This study was conducted at a tertiary care hospital in Karachi, Pakistan, using the resources at hand, with the available AO BP instruments and the mercury sphygmomanometer. The study was performed to check the validity of AOBP measurements as compared to the manual BP measurements in Karachi, Pakistan.

Subjects and Methods

The study was conducted at a tertiary care hospital in Karachi, Pakistan. This hospital is equipped with AOBP monitors, Dinamap Procare 100 and manual mercury sphygmomanometers with a Littman classic II stethoscope. This was a double-blind randomised clinical crossover trial. Every patient was assigned two staff nurses who used an automated oscillometric blood pressure device and a manual mercury sphygmomanometer on the patient concurrently. The device, arm for taking the reading and nurses were randomly determined. Both the nurses remained blind of the readings they recorded.

All patients were in a supine position when their BP was checked. Patients aged 15 and above were included, whereas patients with complaints of chest pain, altered mental status, GCS < 12/15 and patients who had smoked within the last 30 minutes were all excluded.

Randomization was done by giving the staff nurses printed forms that they filled and then sealed in the same envelope for a given patient. The crossover was achieved by taking manual than auto or auto and then manual measurements. The form consisted of fields that required the name of the device, arm from which the reading was taken, presenting complaint, history of HTN, DM, IHD, renal diseases, neurological disorders, and chronic respiratory tract diseases.

The analysis was done using SPSS version 20. Test of significance was T test and *p-values* of 0.05 or lower were considered to be significant. Bland-Altman plots were employed to graphically represent the data. This study was done from February 2015 to May 2015. The sample size was 200.

Results

The Emergency Department is one of the most vital departments in the hospital. Decisions

made in the ED shape the rest of the therapy for the patient. EDs all over the world receive a diverse array of patients from all age groups with a variety of clinical conditions. Accurate blood pressure measurements are pivotal for treatment, especially for patients with deteriorating conditions. In 2011, a study suggested that substituting automated oscillometric devices for auscultatory devices could cause grave repercussions for patients in specific circumstances; and in cases of trauma or deteriorating patient condition, manual BP should be given preference [19]. Despite this, oscillometric devices are gaining popularity and are steadily replacing auscultatory devices [8].

Even at very low levels, environmental mercury can act as a potent neurotoxin and cause serious harm. Health care facilities are one of the main sources of mercury pollution via emissions from incineration of medical waste. Mercury sphygmomanometers, collectively, are the largest reservoir of mercury in the health care setting, often containing 80-100 g/unit. The WHO considers them a major occupational hazard, as inadequate care may result in dangerous exposures to patients and health care staff [20]. WHO and other organisations around the world are working towards the removal of Mercury from hospitals and other health care settings due to the potential threat it poses [20]**.** In 1998, after an agreement between the American Hospitals Association and the U.S Environmental Protection Agency, Hospitals for Healthy Environment (H2E) was launched to virtually eradicate mercury from the healthcare setup in the U.S. [21]. Many countries have already switched from mercury sphygmomanometers to alternative devices [22].

Automated oscillometric devices, on the other hand, are not only considered environmentally safe but they also have a significant advantage over their manual counterparts (mercury or aneroid): they don't require a trained professional to take a reading. This makes them perfect for ambulatory readings for patients and to monitor BP at home. Primarily for this reason, oscillometric devices are gaining fame. A bias commonly faced by physicians is white coat hypertension, and it has been proven that automated oscillometric devices substantially remove this effect leading to more accurate readings [23, 24].

Many oscillometric devices are available in the market. Most of these devices are not put through any validation and yet they are still being sold and used by the population. Thus there is a growing concern regarding many of these devices [25, 26]. The British Hypertension Society (BHS) and the US Association for the Advancement of Medical Instrumentation have devised protocols that are widely used for validating BP monitors [27]. They have compiled a list of devices that are approved for home as well as clinical use [28]. If a device fulfils the criteria set by these two organisations it can be recommended. Unfortunately, most devices are not evaluated for accuracy independently by using these two protocols [26].

Oscillometric devices can have unreliable readings when used on diabetic patients, pregnant women, elderly patients and patients with arrhythmias [27, 29]. They have to be independently validated and also need to be calibrated at regular intervals to ensure that their readings are accurate. Oscillometric devices have been reported to overstate blood pressure and at times understate blood pressure. In both of these instances, it can put patient management at risk. It has been reported that on some devices, there is an inherent flaw in the algorithms used which leads to skipping of certain values; this can influence results [30].

In our study, the mean of difference in manual systolic and automated systolic blood pressure was 8.54 with a standard deviation of ± 9.38, while the mean of difference in manual diastolic and automated diastolic was noted to be 4.21 with a standard deviation of ± 7.88. The range of difference between manual systolic and automated systolic blood pressure was calculated to be -20 to +38, while the difference between the manual diastolic and automated diastolic blood pressure was -17 to +29. The 95% CI for systolic blood pressure lies in the range of -9.84 to +26.92.

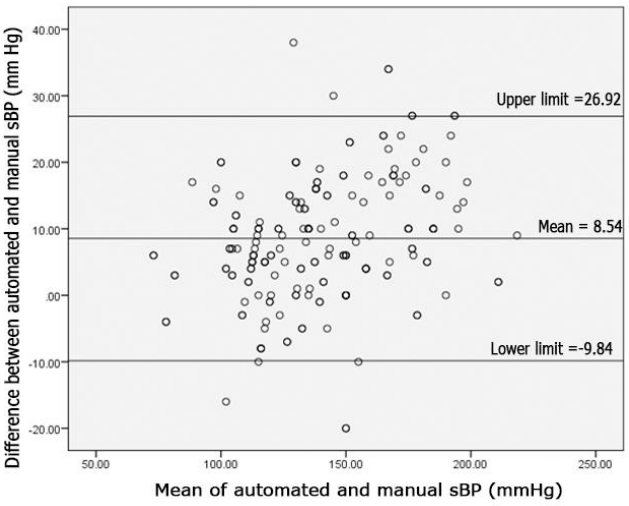

Figure 1: Variation in a Bland – Altman plot for difference between automated and manual systolic blood pressure when compared to the mean of diastolic blood pressure

This range of 36.76 demonstrates that automated blood pressure monitors lack precision for use at the emergency department. The 95% CI for diastolic blood pressure lies in between +19.66 to -11.24, this range of 30.9 is yet again not precise enough to yield a reliable diastolic blood pressure reading in the ED. The plots in Figure 1 and Figure 2 show that there is significant disagreement between automated and manual devices, again certifying that automated devices should not be recommended for use in the ED. Looking at the Bland-Altman plots for

systolic blood pressure readings, there appears to be a positive linear trend – as the average blood pressure is rising, the difference between manual and electronic readings (subsequently called the error of electronic measurement) is also rising.

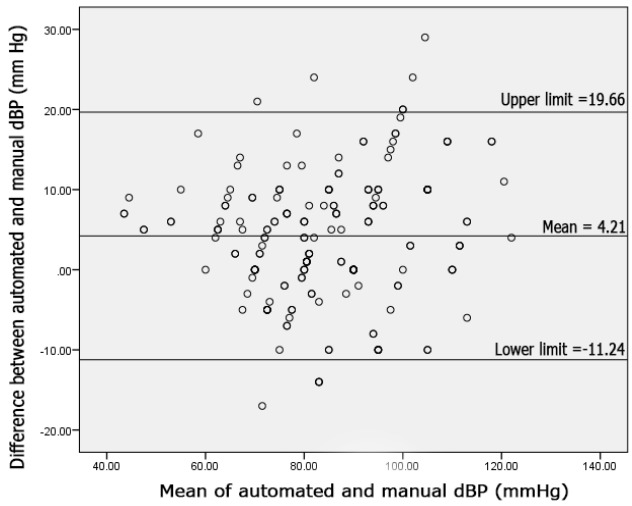

Figure 2: Variation in a Bland – Altman plot for difference between automated and manual diastolic blood pressure when compared to the mean of systolic blood pressure

Exploring this further, we divided the patients into three groups based on their BP readings: patients who had a manual systolic reading of below 120 mmHg, those who had readings between 120 mmHg and 150 mmHg and those who had readings above 150 mmHg. The differences between manual and electronic readings for the three groups are given as follows.

Table 1: Differences between manual and electronic readings for the three groups

Systolic BP	Mean Difference Electronic and Manual	N	Std. Deviation	p-values
<= 120	6.27	73	8.39	0.175
>120 & <=150	8.91	70	8.96	0.004
>150	10.98	57	10.49	0.001
Total	8.54	200	9.38	

A one-tailed t-test was done to see whether the observed error of electronic equipment was statistically different from the recommended AAMI criteria: mean < 5 mmHg and a standard deviation < 8 mmHg. Based on the p-values, patients who had systolic blood pressure less than 120 mmHg fell within acceptable range.

We also observed differences in measurements between the left arm and the right arm, particularly for Systolic readings. SBP measured on the right arm had less error and less standard deviation as compared to measurements on the left arm.

Analysing this further, we checked whether the error component of the electronic device was varying based on the arm of measurement and the actual (manual) blood pressure readings. One-tailed t-tests were done to check if each group was statistically different from the recommended AAMI criteria (mean of < 5 mmHg and standard deviation of < 8 mmHg).

Table 2: Differences in measurements between the left arm and the right arm

Right Arm	N	Mean	Std. Deviation
Difference in Systolic	102	7.3333	0.87017	8.78831
Difference in Diastolic	102	3.6275	0.79541	8.03325
Left Arm	N	Mean	Std. Deviation
Difference in Systolic	98	9.7959	0.99409	9.84096
Difference in Diastolic	98	4.8163	0.77927	7.71442

The null hypothesis was that the observed error is not different from the AAMI criteria. Where the null hypothesis was rejected, the p-values have been marked in red, which indicate that there is a difference between the observed error and the AAMI criteria.

Table 3: Differences in systolic and diastolic blood pressure

		Difference Systolic				Difference Diastolic			
Blood Pressure	Arm	Count	Mean	Standard Deviation	p-value	Count	Mean	Standard Deviation	p-value
<= 120	Right	42	4.02	8.24	0.71	42	3.38	6.58	0.84
	Left	31	9.32	7.73	0.02	31	6.16	5.56	0.25
>120 & <=150	Right	36	7.83	6.91	0.06	36	2.64	8.94	0.88
	Left	34	10.06	10.71	0.02	34	5.06	9.46	0.49
>150	Right	24	12.38	9.94	0.00	24	5.54	8.89	0.41
	Left	33	9.97	10.91	0.02	33	3.30	7.41	0.81

From this table (Table 3) it can be seen that the error difference of diastolic readings is falling within the acceptable range under all conditions. However, the error in systolic readings is only acceptable when taken on the right arm and when the measurement is below 150 mmHg.

Discussion

While some studies clearly favour oscillometric devices [17], others argue that auscultatory measurements are comparatively more accurate [18]. The inaccuracy of automated over manual monitoring has been reported with regards to the failure of automated monitoring to reliably detect orthostatic hypotension in patients at the ER in triage [31]. In our study, out of the sample of 200 patients, 89 had diagnosed hypertension. In these patients, the mean of difference of systolic blood pressure was 9.43 with a standard deviation of ± 9.89 (*p-value 0.000*), while the mean of difference of diastolic blood pressure was 4.26 with a standard deviation of ± 7.35 (*p-value 0.000*), this shows that there is greater variability of the measurements when automated devices are used on hypertensive patients. Van Popele et al reported that increased arterial stiffness causes higher SBP and DBP readings on oscillometric devices although the underlying mechanism of why it occurs is not clear [32]. In our study, both systolic and diastolic readings were overstated majority of the

times in comparison to the popular notion that automated devices underestimate readings [33].

An automated device is only recommended if AAMI criteria are fulfilled; that is both systolic and diastolic measurements should not have a mean difference > 5 mmHg or a standard deviation > 8 mmHg. The British Hypertensive Society denotes a grade of A or B if a device is approved [34, 35]. The device we used was the Dinamap Procare 100, which has been validated using both protocols and is on the list of approved devices. Despite this fact, when tested in the emergency setting, it failed to reproduce the same results as was expected for systolic readings. However, it can be stated that this device is capable of measuring systolic and diastolic blood pressure when used on the right arm of non-hypertensive patients. A review by Wan *et al* showed that most of the devices approved by the BHS and AAMI are able to reproduce the results during protocol testing (i.e. artificial settings) with 60-86% of the measurements collected within the reference range of 5 mmHg. However, in devices tested in community/clinic-based studies only 35-46% values were within 5 mmHg of the observed value [36]. Wan *et al* stated that the poor performance in community settings is because the devices do not perform up to par when they are tested outside the validation setting (ideal setting). This is an evident finding in our study, where only 33% of systolic measurements and 44.5% of diastolic measurements were within the range. Skirton *et al* suggested that hypertensive patients, patients with arrhythmias and trauma should be monitored with manual meters as opposed to automated. In our study, in 89 patients with diagnosed HTN, 24.7% of systolic measurements and 39.3% of diastolic measurements were within reference range [19]. The decrease in the validation percentage for hypertensive patients was in accordance with Skirton *et al* [19].

Health care is a rising burden on governments across the world. This is further magnified when developing nations are taken into consideration. There are 28 recommended devices that are considered to be reliable by the British Hypertension Society, which roughly range from 136 GBP to 2000 GBP [28]. Out of these, the prices of 15 devices are listed. The average price is 1258.4 GBP, which equates to PKR 198409.15. The cost is significantly high for countries like Pakistan where 12.7% of the population is below the poverty line [37]. In Pakistan, most of the healthcare is provided by the private sector leading to patients possibly being confronted by catastrophic expenses. More devices are needed which are accurate but also cost effective. Banning mercury sphygmomanometers in third world countries seems a remote possibility until and unless cheaper alternative devices are developed. Another alternative is the use of aneroid BP apparatus, but they need regular calibration, which also at times require mercury sphygmomanometers.

In conclusion, mercury is an environmental hazard and will probably face a worldwide ban as more awareness spreads, thus urgent research for developing new and accurate devices is warranted. Furthermore, separate protocols and criteria have to be established for the use of automated devices in different departments, as the ideal conditions in which these devices are tested won't be available when these devices are put to test on the field. Our study shows that systolic readings from a previously validated device are not reliable when used in the ED. Secondly, if the blood pressure is measured for the right arm then there is a higher chance of an accurate reading. Lastly, even validated devices show great variability and low precision when they are used outside validation setting. Thus the role of automated blood pressure monitors should be evaluated for their use in the emergency departments.

Awareness needs to be spread amongst physicians that there is a higher level of discrepancy when using these devices on hypertensive patients. Researchers are required to test aneroid blood pressure monitors and there is an urgent need for developing low cost but reliable automated blood pressure devices so that the transition of banning mercury sphygmomanometers is smooth.

References

1. Kaczorowski J, Dawes M, Gelfer M. Measurement of blood pressure: new developments and challenges. Blood pressure. 2007:7.

2. Abboud H, Henrich WL. Stage IV chronic kidney disease. N Engl J Med. 2010;362:56-65. http://dx.doi.org/10.1056/NEJMcp0906797 PMid:20054047

3. Staessen JA, Richart T, Birkenhäger WH. Less atherosclerosis and lower blood pressure for a meaningful life perspective with more brain. Hypertension. 2007; 49:389-400. http://dx.doi.org/10.1161/01.HYP.0000258151.00728.d8 PMid:17283254

4. Faruqui A. Hypertension in Pakistan: What are we going to do about it? Pakistan Heart Journal. 1997;30:3-4.

5. Wilkins K, Campbell NRC, Joffres MR, et al. Blood pressure in Canadian adults. Health Rep. 2010;21:37-46. PMid:20426225

6. Lawes CM, Vander Hoorn S, Rodgers A. International Society of Hypertension. Global burden of blood-pressure-related disease, 2001. Lancet. 2008;371(9623):1513-1518. http://dx.doi.org/10.1016/S0140-6736(08)60655-8

7. Reeves RA. Does this patient have hypertension? How to measure blood pressure. JAMA. 1995;273:1211–1216. http://dx.doi.org/10.1001/jama.1995.03520390071036 PMid:7707630

8. Ogedegbe G, Pickering T. Principles and techniques of blood pressure measurement. Cardiology Clinics. 2010;28(4):571-586. http://dx.doi.org/10.1016/j.ccl.2010.07.006 PMid:20937442 PMCid:PMC3639494

9. O'Brien E. Has conventional sphygmomanometry ended with the banning of mercury? Blood Press Monit. 2002;7:37–40. http://dx.doi.org/10.1097/00126097-200202000-00007 PMid:12040241

10. O'Brien E, Asmar R, Beilin L, Imai Y, Mallion J-M, Mancia G, Mengden T, Myers M, Padfield P, Palatini P, Parati G, Pickering T, Redon J, Staessen J, Stergiou G, Verdecchia P. European Society

of Hypertension recommendations for conventional, ambulatory and home blood pressure measurement. Journal of Hypertension. 2003;21:821–848. http://dx.doi.org/10.1097/00004872-200305000-00001 PMid:12714851

11. Le Pailleur C, Helft G, Landais P, et al. The effects of talking, reading, and silence on the "white coat" phenomenon in hypertensive patients. Am J Hypertens. 1998;11:203–207. http://dx.doi.org/10.1016/S0895-7061(97)00403-2

12. Mancia G, Bertinieri G, Grassi G, Parati G, Pomidossi G, Ferrari et al. Effects of blood pressure measurement by the doctor blood pressure and heart rate. Lancet. 1983; ii:695–698. http://dx.doi.org/10.1016/S0140-6736(83)92244-4

13. Mancia G, Ferrari A, Gregorini L, Parati G, Pomidossi G, Bertet al. Blood pressure and heart rate variabilities in normotensive hypertensive human beings. Circ Res. 1983; 53:96–104. http://dx.doi.org/10.1161/01.RES.53.1.96 PMid:6861300

14. Parati G, Ulian L, Santucciu C, Omboni S, Mancia G. Blood pressure variability, cardiovascular risk and antihypertensive treatment. J Hypertens Suppl. 1995;13(4): S27–S34. http://dx.doi.org/10.1097/00004872-199512002-00005 PMid:8824684

15. Parati G, Ulian L, Santucciu C, Omboni S, Mancia G. Difference between clinic and daytime blood pressure is not a measure white coat effect. Hypertension. 1998;31(5):1185–1189. http://dx.doi.org/10.1161/01.HYP.31.5.1185 PMid:9576133

16. Myers MG, Valdivieso M, Kiss A. Use of automated office blood pressure measurement to reduce the white coat response. J Hypertens. 2009;27:280–286. http://dx.doi.org/10.1097/HJH.0b013e32831b9e6b PMid:19155785

17. Myers, M. G. Why Automated Office Blood Pressure Should Now Replace the Mercury Sphygmomanometer. The Journal of Clinical Hypertension. 2010;12: 478–480. http://dx.doi.org/10.1111/j.1751-7176.2010.00301.x

18. Myers MG, Godwin M, Dawes M, Kiss A, Tobe SW, Curry F, Grant, Kaczorowski J. Conventional versus automated measurement of blood pressure in primary care patients with systolic hypertension: randomised parallel design controlled trial. BMJ. 2011; 342:d286. http://dx.doi.org/10.1136/bmj.d286 PMid:21300709 PMCid:PMC3034423

19. Skirton H, Chamberlain W, Lawson C, Ryan H, Young E. A systematic review of variability and reliability of manual and automated blood pressure readings. Journal of Clinical Nursing. 2011; 20: 602–614. http://dx.doi.org/10.1111/j.1365-2702.2010.03528.x PMid:21320189

20. World Health Organization. Department of Protection of the Human Environment Water, Sanitation and Health. Mercury in Health Care. August 2005.

21. Hospitals for a Healthy Environment. Regulated Medical Waste Reduction. http://www.h2e-online.org.

22. Health Care Without Harm. End of An Era. The phase-out of mercury-based blood pressure measurement devices in the United States and its implications for Europe and the rest of the world. 2008. http://noharm.org/lib/downloads/mercury/End_of_an_Era_Mercury.pdf

23. Myers MG, McInnis NH, Fodor GJ, Leenen FHH. Comparison between and Automated and manual sphygmomanometer in a population survey. Am J Hypertens. 2008;21(3):280-283. http://dx.doi.org/10.1038/ajh.2007.54 PMid:18219304

24. O'Brien E, Petrie J, Littler WA, de Swiet M, Padfield PL, Altman D, et al. The British Hypertension Society protocol for the evaluation of blood pressure measuring devices. J Hypertens. 1993;11(Suppl 2):S43–S63.

25. Heinemann M, Sellick K, Rickard C, Reynolds P, McGrail M. Automated versus Manual blood pressure measurement: A randomized crossover trial. Int J Nurs Pract. 2008;14:296-302. http://dx.doi.org/10.1111/j.1440-172X.2008.00696.x PMid:18715391

26. O'Brien E, Waeber B, Parati G, Staessen J, Myers MG. Blood pressure measuring devices: recommendations of the European Society of Hypertension. Brit Med J. 2001;322:531-536. http://dx.doi.org/10.1136/bmj.322.7285.531 PMid:11230071 PMCid:PMC1119736

27. Pickering TG, Hall JE, Appel LJ, Falkner B, Graves J, Hill M, et al. Recommendations for blood pressure measurement in humans and experimental animals, part 1: blood pressure measurement in humans: a statement for professionals from the Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research. Hypertension. 2005;45:142-161. http://dx.doi.org/10.1161/01.HYP.0000150859.47929.8e PMid:15611362

28. British Hypertension Society http://www.bhsoc.org/bp-monitors/bp-monitors/

29. Hutton P, Dye J, Prys-Roberts C. An assessment of the Dinamap 845. Anaesthesia. 1984;39:261–267. http://dx.doi.org/10.1111/j.1365-2044.1984.tb07240.x PMid:6703297

30. Rose KM, Arnett DK, Ellison RC, Heiss G. Skip patterns in DINAMAP measured blood pressure in 3 epidemiological studies. Hypertension. 2000;35:1032–1036. http://dx.doi.org/10.1161/01.HYP.35.5.1032 PMid:10818059

31. Dind A, Short A, Ekholm J, Holdgate A. The inaccuracy of automatic devices taking postural measurements in the emergency department. International Journal of Nursing Practice. 2011; 17: 525–533. http://dx.doi.org/10.1111/j.1440-172X.2011.01958.x PMid:21939485

32. van Popele NM, Bos WJ, de Beer NA, van Der Kuip DA, Hofman A, Grobbee DE, Witteman JC. Arterial stiffness as underlying mechanism of disagreement between an oscillometric blood pressure monitor and a sphygmomanometer. Hypertension. 2000;36(4):484-8. http://dx.doi.org/10.1161/01.HYP.36.4.484 PMid:11040223

33. Braam RL, Thien T. Is the accuracy of blood pressure measuring devices underestimated at increasing blood pressure levels? Blood Press Monit. 2005;10(5):283-9. http://dx.doi.org/10.1097/01.mbp.0000180671.76279.c7

34. Association for the Advancement of Medical Instrumentation. American National Standard. Manual, electronic or automated sphygmomanometers ANSI/AAMI SP10-2002/A1. Arlington, VA 22201-4598, USA: AAMI, 2003.

35. O'Brien E, Atkins N. A comparison of the British Hypertension Society and Association for the Advancement of Medical Instrumentation protocols for validating blood pressure measuring devices: can the two be reconciled? J Hypertens. 1994; 12(9):1089-94. http://dx.doi.org/10.1097/00004872-199409000-00015

36. Wan Y, Heneghan C, Stevens R, et al. Determining which automatic digital blood pressure device performs adequately: A systematic review. Journal of Human Hypertension. 2010; 24: 431–438. http://dx.doi.org/10.1038/jhh.2010.37 PMid:20376077 PMCid:PMC2897978

37. World Bank – Poverty Data; http://povertydata.worldbank.org/poverty/country/PAK

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):410-412.
http://dx.doi.org/10.3889/oamjms.2016.077
eISSN: 1857-9655
Clinical Science

Role and Importance of *Chlamydia Trachomatis* in Pregnant Patients

Mariya Angelova[1*], Emil Kovachev[2], Veselina Tsankova[1], Iliana Koleva[1], Silvia Mangarova[1]

[1]Department of Obstetrics and Gynecology, Trakia University, Faculty of Medicine, Stara Zagora, Bulgaria; [2]Department of Obstetrics and Gynecology, Paraskev Stoyanov Medical University, Varna, Bulgaria

Citation: Angelova M, Kovachev E, Tsankova V, Koleva I, Mangarova S. Role and Importance of *Chlamydia Trachomatis* in Pregnant Patients. Open Access Maced J Med Sci. 2016 Sep 15; 4(3):410-412. http://dx.doi.org/10.3889/oamjms.2016.077

Keywords: *Chlamydia trachomatis*; pregnancy; neonatal infections; respiratory symptoms; polymerase chain reaction.

***Correspondence:** Mariya Angelova. Department of Obstetrics and Gynecology, Trakia University, Faculty of Medicine, Stara Zagora, Bulgaria. E-mail: angelova70@abv.bg

Received: 27-Apr-2016; **Revised:** 25-May-2016; **Accepted:** 05-Jul-2016; **Online first:** 05-Aug-2016

Competing Interests: The authors have declared that no competing interests exist.

Abstract

AIM: The aim of this study was to assess the prevalence of chlamydial infection among pregnant women and to determine the role of this infection in the fetus.

MATERIAL AND METHODS: In the first phase of this study were reported 58 pregnant women with a positive test for active chlamydial infection by applying immunofluorescence. In the second phase of the study were reported pregnant with premature burst membranes (PBM), postnatal complications associated with chlamydial infection as puerperal endometritis, and newborns are monitored for low birth weight and growth retardation at birth.

RESULTS: With a positive test are 58 patients in the first trimester or pregnancy registration in our consultation. After regimen with Sumamed (2 x 500 mg for three days and after 10 days again same scheme for them and their partner) at the beginning of the third trimester, the PCR test was made again. Of these, 5 were positive again, participants are between 20 and 30 years old. With premature rupture of OM are 20 patients. There was no increased incidence of premature births. Infants born to infected mothers have a higher risk of developing respiratory symptoms in the first 60 days of life. 3 of them have low for his age bodyweight.

CONCLUSIONS: The scarcity of data on manifestations of chlamydial infection during pregnancy and neonatal outcomes justifies this study. Early diagnosis for registration of pregnancy and timely treatment of chlamydial infection as well as scrutinising the infection during the third trimester of pregnancy can prevent infection of the newborn. Therefore, preventive examinations should be considered as a priority for early detection of asymptomatic chlamydial infection in the conduct of antenatal care.

Introduction

According to SZO genital chlamydiosis, today is considered the most common sexually transmissible infection. Various studies have been used for diagnosis of chlamydial infection in pregnant women; the frequency varies in different countries. Chlamydia Trachomatis during pregnancy can lead to miscarriage, premature birth, and premature rupture of membranes, intrauterine growth retardation, and puerperal endometritis [1-5].

Infection with *Chlamydia trachomatis* with the mother is associated with increased morbidity in newborns and infants up to three months. Approximately two-thirds of infants born vaginally to infected mothers will be infected at birth. These infections may lead to conjunctivitis, otitis media, pharyngitis and pneumonia in neonates. Moreover, neonatal infection with *Chlamydia trachomatis* may cause long-term consequences such as chronic obstructive pulmonary disease.

The scarcity of data on manifestations of chlamydial infection during pregnancy and neonatal

outcomes justifies this study. Early diagnosis for registration of pregnancy and timely treatment of chlamydial infection as well as scrutinising the infection during the third trimester of pregnancy can prevent infection of the newborn [6]. Therefore, preventive examinations should be considered as a priority for early detection of asymptomatic chlamydial infection in the conduct of antenatal care.

The aim of this study was to assess the prevalence of chlamydial infection among pregnant women and to determine the role of this infection in the fetus, the results associated with miscarriage, premature birth, premature rupture of membranes and low birth weight.

Materials and Methods

Phase I

Endocervical samples were collected from 90 pregnant women. In the first phase of this study were reported 58 pregnant women with a positive test for active chlamydial infection by applying immunofluorescence.

Phase II study - maternal and perinatal data

In the second phase of the study were reported pregnant with premature rupture of membranes (PROM), postnatal complications associated with chlamydial infection as puerperal endometritis and infants were observed for low weight and hypotrophy at birth.

Pregnant women included in this study meet the following requirements for forming the record: information about age, marital status, the number of sexual partners in life and socio-economic status. Family income and level of education are the criteria used to determine the socio-economic status. Patients were excluded if the gestational age when less than 28 weeks and six days, having other infections that require antibiotic treatment or a history of use of antibiotics in the previous 30 days.

During the second stage of the study 45, pregnant women who meet the eligibility criteria were observed for 60 days after birth. From a total of 58 participants in the first phase of the study 13 were excluded from the study: four cannot be contacted and nine due to insufficient follow-up time (less than 40 days of birth).

A study was conducted in 45 infants who were evaluated within 24 hours and monitored thereafter every 15 days to 60 days of life. However, 10 newborns were excluded due to loss of contact.

Results

Phase I - study

Of 58 patients positive for the registration of pregnancy and treated with Sumamed (2 x 500 mg for three days and then 10 days later again the same scheme for them and their partner) at the beginning of the third trimester was made the immunofluorescence test again. Of these, 5 are positive again. The average age of the positive patients was 23.5 years with a standard deviation of ± 4.5 years.

Although we are looking for associations between *Chlamydia trachomatis* infection with family income (45.5%) and level of education (45.5%), no statistically significant relationship between them was found.

Phase II study - maternal and perinatal data

No statistically significant relationship between positivity for *Chlamydia trachomatous* infection and premature rupture of OM was found (Table 2). No correlation was seen for premature birth, and low birth weight (weight less than 2500 g) in chlamydia -positive women.

Table 1: Table for the presence of cervicitis or endocervical secretion in *Chlamydia trachomatis* positive patients

Vaginal examination	Chlamydia positive		Chlamydia negative	
Cervicitis	n	%	n	%
Yes	2	3.45%	4	6.90%
No	3	5.17%	49	84.48%
Endocervical secretion				
Yes	3	5.17%	16	27.59%
No	2	3.45%	37	63.79%

Caesarian section is recommended for chlamydia positive patients. During surgery and three days after it is recommended antibiotic, due to the risk of developing endometritis.

Table 2: Early rupture membranes in pregnant women with a positive test for *Chlamydia trachomatis*

Premature rupture of membranes(PROM)	Immunofluorescence test for *Chlamidia trachomatis*			
	Positive		Negative	
	n	%	n	%
Yes	3	5.17%	17	29.31%
No	2	3.45%	36	62.07%
Total	5	8.62%	53	91.38%

Study of neonatal outcome

Of 58 newborns, 8 (13.79%), exhibit respiratory symptoms. Of these five infants (62.5%) were born of chlamydia -positive mothers. Moreover, infants born to infected mothers are more likely to develop respiratory symptoms such as nasal congestion, rhinitis, cough, and dyspnea.

Table 3: Respiratory events in 58 infants born by mothers suffered *Chlamydia trachomatis*

At risk of *Chlamydia trachomatis*	Respiratory symptoms					
	Yes		No		Total	
	n	%	n	%	n	%
Yes	3	5.17%	2	3.45%	5	8.62%
No	5	8.62%	48	82.76%	53	91.38%
Total	8	13.89%	50	86.21%	58	100%

Discussion

Studies have shown that infection by *Chlamydia trachomatis* during pregnancy can lead to serious complications [7]. Prenatal screening still in the registration of pregnancy would be beneficial to reduce morbidity among patients themselves, but also as prevention of transmission to the newborn, and the partner.

The prevalence of *Chlamydia trachomatis* infection in this study was 8.62%. Although we found that the majority (45.5%) of patients with chlamydia - positive results had a low-income family, in addition, low level of education (45.5 percent) and were not aware of the existence of *Chlamydia trachomatis* and its impact on reproductive function. A total of 88% of positive on *Chlamydia trachomatis* in this study are married or live in cohabitation with their partner. Only one sexual partner was reported by 98% of women; 75% of chlamydia - positive patients were under 25 years of age. The risk of introduction of chlamydial infection is twice as high at 25 years young women. These findings indicate the need for sharing information about the risk factors for *Chlamydia trachomatis*. Given that 40 % of the chlamydia-positive patients are identified in cervicitis gynaecological examination, this is an important clinical finding, since in a subsequent pregnancy infection can ascend and lead to premature birth and neonatal infections.

Respiratory symptoms are more common in infants born by Chlamydia-infected mothers. It is, therefore, clear that the clinical findings in this study in infants born by Chlamydia positive pregnant women were associated with prenatal chlamydial infection of their mothers.

Our findings indicate the real need for prenatal screening for *Chlamydia trachomatis*. It is, therefore, necessary introduction and implementation of prenatal screening programs in laboratories and offices for outpatient care for detecting *Chlamydia Trachomatis*.

References

1. Alencar AAF, Ferreira LCL, Loureiro JAS. Detecção de Chlamydia trachomatis por imunofluorescência direta em esfregaços endocervicais. J Bras Ginecol. 1993;103:199–203.

2. Ward BJ, Plourde P. Travel and sexually transmitted infection. J Travel Med. 2006;13 :300–317. http://dx.doi.org/10.1111/j.1708-8305.2006.00061.x PMid:16987130

3. Santos C, Teixeira F, Vicente A, Astolfi-Filho S. Detection of Chlamydia trachomatis in endocervical smears of sexually active women in Manaus-AM, by PCR. Braz J Infect Dis. 2003;2:91–95.

4. Jalil EM, Pinto VM, Benzaken, et al AS. Prevalence of Chlamydia and Neisseria gonorrhoeae infections in pregnant women in six Brazilian cities. Rev Bras Ginecol Obstet. 2008;30:614–619. PMid:19219343

5. Fenton KA, Lowndes CM. Recent trends in the epidemiology of sexually transmitted infections in the European Union. Sex Transm Infect. 2004;80:255–263. http://dx.doi.org/10.1136/sti.2004.009415 PMid:15295121 PMCid:PMC1744866

6. Urdea M, Penny LA, Olmsted SS, Giovanni MY, Kaspar P, Shepherd A, Wilson P, Dahl CA, Buchsbaum S, Moeller G, Hay Burgess DC. Requirements for high impact diagnostics in the developing world. Nature. 2006;444(Suppl 1):73-9. http://dx.doi.org/10.1038/nature05448 PMid:17159896

7. Ilieva V, Nikolova G, Gadjeva V. Lipid Peroxidation and Catalase Activities in Patients with Chronic Obstructive Pulmonary Disease: A Comparative Study with Other Pulmonary Disease. Trakia Journal of Sciences. 2014;2:177-181.

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):413-419.
http://dx.doi.org/10.3889/oamjms.2016.081
eISSN: 1857-9655
Clinical Science

The Impact of the Nasal Trauma in Childhood on the Development of the Nose in Future

Gabriela Kopacheva-Barsova[1*], Slavica Arsova[2]

[1]University Clinic for Ear, Nose and Throat, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Vodnjanska 17, Skopje 1109, Republic of Macedonia; [2]University Clinic of Psychiatry, Ss Cyril and Methodius University of Skopje, Vodnjanska 17, Skopje 1109, Republic of Macedonia

Citation: Kopacheva-Barsova G, Arsova S. The Impact of the Nasal Trauma in Childhood on the Development of the Nose in Future. Open Access Maced J Med Sci. 2016 Sep 15; 4(3):413-419. http://dx.doi.org/10.3889/oamjms.2016.081

Keywords: nasal fractures in children; nasal growth; treatment of recent traumatic deformities; rhino surgery in children and adolescents; psychological behaviour.

***Correspondence:** Gabriela Kopacheva Barsova, PhD. University Clinic for Ear, Nose and Throat, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Vodnjanska 17, Skopje, MK 1109, Republic of Macedonia. E-mail: gabrielak70@yahoo.com

Received: 01-Jul-2016; **Revised:** 19-Jul-2016; **Accepted:** 20-Jul-2016; **Online first:** 02-Aug-2016

Competing Interests: The authors have declared that no competing interests exist.

Abstract

AIM: To prevent and to treat nasal trauma in children properly, because it can lead to displacement or depression of the nasal bones or septum. Second, our aim was, for the patient to recognise and create a mature decision for eventual nose changes which will be made with the operative intervention or they are not mature enough and the decisions were made by their parents.

MATERIAL AND METHODS: Our retrospective study was made at University Clinic for Ear, Nose and Throat, Faculty of Medicine, Ss Cyril and Methodius University of Skopje in the period of 6 years (2005 -2016). Seventy-three patients were admitted with recent or previous nasal trauma or nasal deformity. The first group of 32 were children and adolescents from 6-14 years old who were admitted to our hospital because of recent nasal trauma. The second group of 41 children and adolescents from 6-14 years old were admitted to our hospital because of previous nasal trauma, which was not treated on time, or it was not treated properly. They were admitted to our clinic for surgical intervention septo/rhinoplasty. The second group of patients fills the brief psychological questioner prepared by Clinical psychiatrist from University Clinic of Psychiatry, in Skopje, and their psychological reactions were taken into consideration.

RESULTS: Eleven of the children and adolescents who had nasal fracture without dislocation, who have no symptoms, minimal swelling, and no septal deviation or hematoma, were observed with a specific follow-up: 3 days after nasal fracture, then every week in the first month, after 1 month, and after 3 months period. Sixteen of children and adolescents who had a nasal fracture with subluxation of nasal septum were operated with closed reduction (repositio nasi) under general anaesthesia. The others with septal hematomas and subperichondrial abscess were treated as in adults' patients. The second group of 41 children and adolescents from 6-14 years old consisted with with the previous nasal trauma which was not treated on time or it was improperly treated. In 24 (58.54%) of these patients septoplasty was performed and in 17 (41.46%) was performed rhino septoplasty.

CONCLUSION: Often, difficult septal deformations in children are followed with deformation of the nasal pyramid (rhino scoliosis, rhino lordosis). In those cases, we cannot solve septal pathology without nasal pyramid intervention in the same time and opposite. Clinical reports have not produced solid evidence for the statement that septal surgery has no negative effect on nasal growth or can serve for correcting abnormal growth. The functional and esthetic problems of the patient, however, mean a continuous stimulus for further clinical and experimental investigations.

Introduction

Nasal fractures have been reported as 1 of the 3 most commonly encountered paediatric facial bone fractures. The nasal bones and mandible are the facial bones most commonly fractured in children. Nasal fractures occur more commonly than do mandibular fractures because they require less force to produce.

The most common causes of nasal fractures in this age group are auto accidents, usually involving bicyclists or pedestrians (40%), sports injuries (25%), intended injuries such as weight lifting (15%), and home injuries (10%). Child abuse also must be considered.

Fractures of the bony nasal pyramid do not occur as frequently as in adults, because of the lesser prominence of the nasal bones during childhood because the greater part of the nasal skeleton is

cartilaginous. Therefore, fractures of the facial bones are uncommon occurrences in children younger than five years of age, but the incidence increases with increasing age and peaks between 16 and 20 years. Most of these injuries are minor (almost in 85%) [1-4].

Anatomy

The sutures bordering the nasal bones have not been yet ossified and can "split off" in a case of trauma. The most frequent lesion is the septal fracture with dislocation. The upper one-third of the nose is supported by the paired nasal bones and the frontal processes of the maxilla; the lower two-thirds are maintained by cartilaginous structures. The superior portion of the nasal bones is thick and relatively resistant to fracture. In contrast, the inferior portion is thin and weak. The bony nasal septum articulates with the undersurface of the nasal bones to provide support to the nasal dorsum. The lacrimal bones and ethmoid labyrinth lie deep to this bony pyramid [5-7].

Blood supply of the nose, derived from the internal and external carotid arteries. Kiesselbach plexus, the vascular watershed area of the anteroinferior nasal septum, is the site of origin of most nosebleeds. Because of the rich nose blood supply frequency and intensity of epistaxis and swelling in patients with nasal trauma are very common.

Pathogenesis

Direct blows to the nose can fracture the nasal skeleton and lead to displacement or depression of the nasal bones or septum. Because the nasal pyramid is variably cartilaginous during childhood, greenstick fractures are more common.

Rupture of a triangular (dorsolateral) cartilage from the piriform aperture is a difficult diagnosis. It appears with dorsum hematoma, caused by rupture of the external brunch of the anterior ethmoid artery, On the outside, the hematoma is often connected with facial oedema. Expansion of the hematoma separates the cartilage from the mucoperichondrium, obstructing blood flow to the nasal cartilage and causing pressure-induced avascular necrosis of the nasal cartilage. Septal perforation and irreversible damage result after three to four days of ischemia. The accumulated blood and necrotic tissue become a “good” place for infection of the nasal mucosa [8, 9].

The replacement of necrotic tissue by fibrous tissue, retraction of scar tissue, and loss of support to the lower nose may lead to facial deformity, including saddle nose, displacement of the maxilla, retraction of the anterior nasal septum (columella), widening of the nasal base, and diminished size of the nasal cavity [10, 11].

Naso-orbito-ethmoid fractures

Naso-orbito-ethmoid fractures occur with high impact to the central midface and involve complete separation of the nasal bones and the medial walls of the orbits from the frontal bone and the infraorbital rim. The bones typically are fragmented and displaced posteriorly into the ethmoid region. The medial canthal tendons, which are attached to the medial walls of the orbits, are shifted laterally with the fracture segments, resulting in increased inner canthal distance (traumatic telecanthus) [12, 13].

Clinical manifestation of nasal fractures in children

Unfortunately, most of the nasal fractures in children are not refers to the ENT - specialist immediately. Evaluation of nasal symptoms following nasal trauma includes nasal obstruction, nasal bleeding, pain, anosmia, and cosmetic deformity. The typical symptoms of nasal septal hematoma and/or abscess are a progressive nasal obstruction (95 percent), persistent or worsening pain (50 percent), rhinorrhea (25 percent). All children with facial trauma should be assessed for associated injuries to the cervical spine, central nervous system, chest, orbits, and teeth [14-16] (Fig. 1).

If symptoms present with the change in visual acuity, diplopia, and sensory deficits should have a prompt evaluation for neurologic or ophthalmologic trauma.

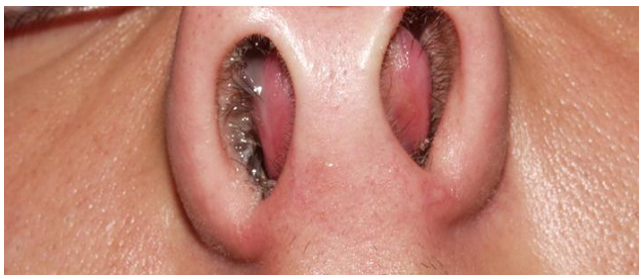

Figure 1: Septal hematoma after previous nasal trauma

Patient physical examination and evaluation

The septum should be examined for fracture, displacement, laceration, discoloration, and abnormal swelling. The intranasal cavity should be evaluated using an appropriate lighting in all children with nasal trauma. The signs and symptoms of nasal septal injury may evolve during the 24 to 72 hours after injury.

A correct diagnosis of fracture and dislocation of the bony and cartilaginous nasal pyramid is more difficult in children than in adults, because of the smaller dimensions and abundant swelling which is

very often due to oedema and or hematoma and less cooperation of the patient. The examination should be repeated after two or three days, when the soft tissue swelling has diminished [17, 18].

The examination of children with nasal trauma should include inspection of the nose and facial structures, palpation of the facial and nasal bones, and internal examination of the nose. Periorbital ecchymoses in the absence of other orbital findings are suggestive of a nasal fracture.

External nasal deformity, epistaxis, oedema, and ecchymosis suggest septal injury. A flattened, broad nose with increased inner canthal distance and vertical orbital displacement suggest a nasal-orbito-ethmoid fracture.

Tenderness, deformity, mobility, crepitus, or step should be evident on the nose palpation. Tenderness over the frontal sinus may indicate frontal sinus fractures. Tenderness to palpation of the tip of the nose is suggestive of septal hematoma. Malocclusion and instability of the palate are indicative of a midfacial LeFort fracture.

In addition, nasal bleeding and swelling, which occur soon after trauma, can impair the evaluation [19-21]. Applying local pressure or topical vasoconstricting agents (e.g., phenylephrine nasal spray, two drops in each nostril) or nasal packing may be necessary to improve the ability to assess the septum for hematoma or deviation.

Imaging Evaluation

Modern computed tomography (CT) is the gold standard for viewing craniofacial and nose fractures. CT images provide excellent detail of the cranium, midfacial structures, and the mandibular condyle. In addition to sagittal and coronal views, reformatting images into a three-dimensional reconstruction provides an improved perspective in complex injuries.

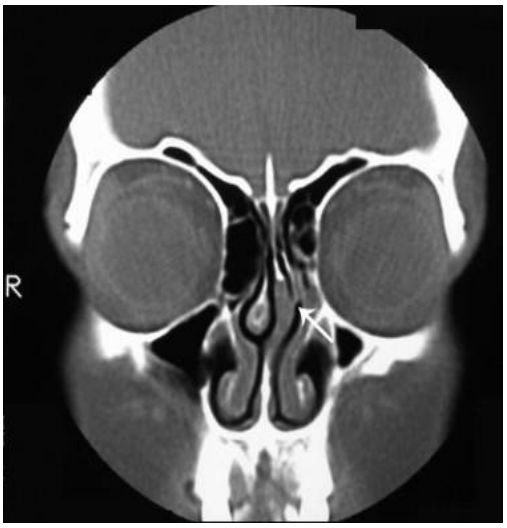

Figure 2: CT tomography of nasal fracture

Plain radiographs are not helpful in the diagnosis of nasal fractures in children and should not be substituted for a complete external and internal examination. The nasal bones of children are poorly visualised on plain radiography because they are not fused and are composed primarily of cartilage [22, 23] (Fig. 2).

Material and Methods

In our retrospective study which was made at University Clinic for Ear, Nose and Throat, Faculty of Medicine, Ss Cyril and Methodius University of Skopje in the period of 6 years (2005 -2016), Seventy-three patients were admitted with recent or previous nasal trauma or nasal deformity.

The first group of 32 were children and adolescents from 6-14 years old who were admitted to our hospital because of recent nasal trauma (nasal bone fracture, or together with consecutive complications caused by recent nasal trauma) were observed. All of them were examined by rhinoscopy, plain radiography or CT scan of nose and paranasal sinuses depending on the severity of the nasal fracture.

The second group of 41 children and adolescents from 6-14 years old were admitted to our hospital because of previous nasal trauma, which was not treated on time, or it was not treated properly. They were admitted to our clinic for surgical intervention septo/rhinoplasty. They had the first nasal trauma in early childhood or several years before they came for surgery.

Results

The first group consisted of 32 children and adolescents, 11 were with nasal fracture without dislocation, 15 with nasal fracture with septal subluxation, in 4 of the children septal hematoma were observed and in 2 cases subperichondrial abscess appeared (Fig. 3).

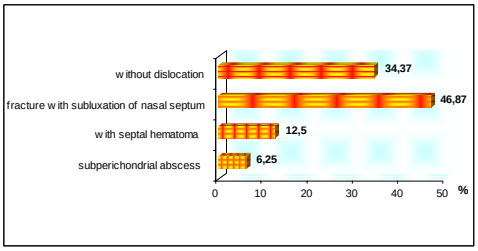

Figure 3: Distribution of recent nasal fracture and associated symptoms in children and adolescents

Eleven of the children and adolescents who had nasal fracture without dislocation, who have no symptoms, minimal swelling, and no septal deviation or hematoma, were observed with a specific follow-up: 3 days after nasal fracture, then every week in the first month, after 1 month, and after 3 months period. Fortunately, after the observation, surgery, reposition on the nose bones or septoplasty was not performed on any of them.

Sixteen of children and adolescents who had a nasal fracture with subluxation of nasal septum were operated with closed reduction (repositio nasi) under general anaesthesia. The others with septal hematomas and subperichondrial abscess were treated as in adults' patients (Table 1).

Table 1: Treatment of recent nasal trauma in children in adolescents

Treatment of recent nasal trauma in children in adolescents		N (%)
Nasal fracture without dislocation	Without intervention	11 (34.37)
Nasal fracture with subluxation	Repositio nasi	15 (46.87)
Septal hematoma	Conservative treatment	4 (12.5)
Septal abscess	Conservative treatment	2 (6.25)

The second group of 41 children and adolescents from 6-14 years old were admitted to our hospital because of previous nasal trauma which was not treated on time or it was improperly treated. They were admitted to our clinic for surgery septo/rhinoplasty. They had the first nasal trauma in early childhood or several years before they came for surgery. In 24 (58.54%) of these patients septoplasty was performed and in 17 (41.46%) was performed rhino septoplasty.

The second group of patients fills the brief psychological questioner prepared by Clinical psychiatrist from University Clinic of Psychiatry, University Campus "St. Mother Theresa" in Skopje, and their psychological reactions were taken into consideration. The aim was, for the patient to recognise and create a mature decision for eventual nose changes which will be made with the operative intervention or they are not mature enough and the decisions were made by their parents. We ask them whether the surgery will change their emotional and psychological way of living.

Table 2: Patients self-concerning for their nasal deformities

Variable	N (%)
How big is their self-concern?	
Little concern	6 (14.63)
Middle concern	26 (63.41)
Very concern	9 (21.95)
Their expectations?	
Real	31 (75.61)
Unreal	7 (17.07)
Impossible	3 (7.32)
Why is the operation important for them?	
Aesthetic moment	10 (24.39)
Breathing disturbances	11 (26.83)
Both	21 (51.22)

About their self-concerning, the result shows that most of them 63.9% were middle concerned, dominant of them, 75%, have real expectations from the operation. Their Answer to the question "Why you are doing this operation", 24.39% thought that the intervention will make to look aesthetically better, both (51.22%) the breathing will be better too (Table 2).

During the testing, their psychological maturity and their decision for the operation were observed. Positive decision for operation has 32 (78.05 %) of course with parental permission, 2 (4.8%) of them were still thinking even their parents allowed them, and in 7 (17.07%) decision for rhino/septoplasty were made by parental permission and opinion because they were not mature enough to conclude the right decision (Fig. 4).

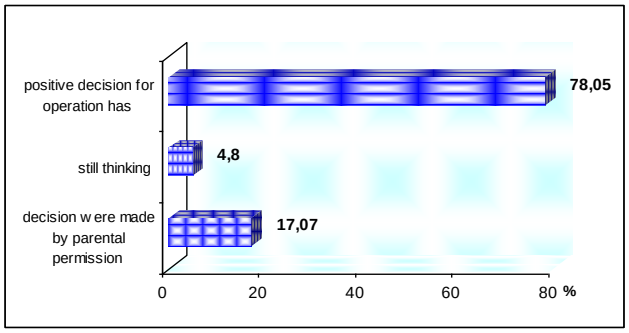

Figure 4: Psychological maturity and their decision for operation

Case reports

Case 1: Four years old boy with nasal fracture without dislocation caused by hitting his head on a table (Fig. 5).

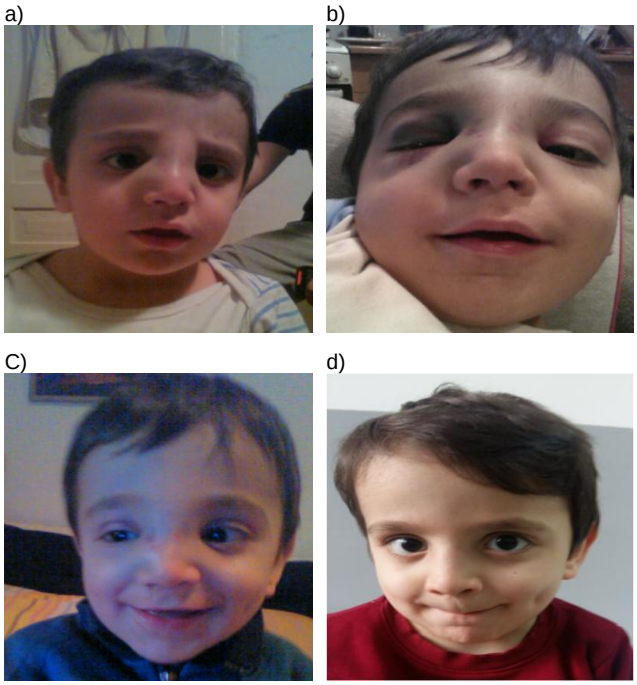

Figure 5: Four years old boy with nasal fracture without dislocation caused by hitting his head on a table. *a) 2 days after the injury; b) 7 days after the injury; c) 14 days after the injury; d) 1 month after the injury (with the permission of parents)*

Case 2: a) Six and a half-year-old boy with saddle deformity after septum abscess at the age of 4 years; b) Twelve years old girl with nasal septal subluxation after a fall. Five days after injury; c) Ten years old boy with nasal septal subluxation after a fall. Three months after injury (Fig. 6).

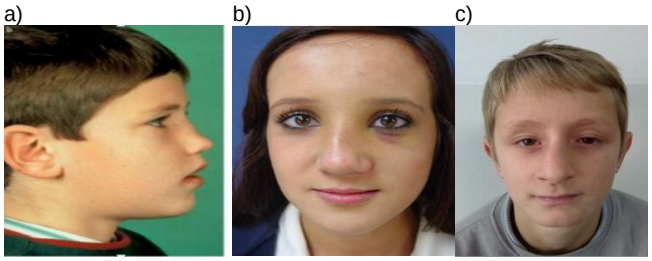

Figure 6: a) Six and a half-year-old boy with saddle deformity after septum abscess at the age of 4 years; b) Twelve years old girl with nasal septal subluxation after a fall. Five days after injury; c) Ten years old boy with nasal septal subluxation after a fall. Three months after injury (with the permission of parents)

Case 3: Eight years old boy with nasal septal subluxation after a fall (Fig. 7).

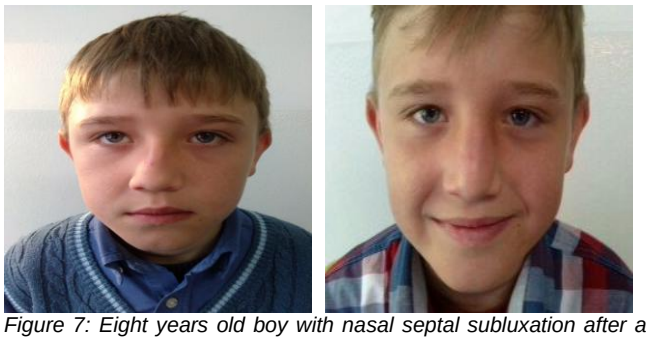

Figure 7: Eight years old boy with nasal septal subluxation after a fall. a) Three days after injury; b) Seven days after reposition (with the permission of parents)

Case 4: Fourteen years old boy with subluxation of nasal septum after nasal trauma in a previous car accident. Septoplasty was prepared. Septal media position (Fig. 8).

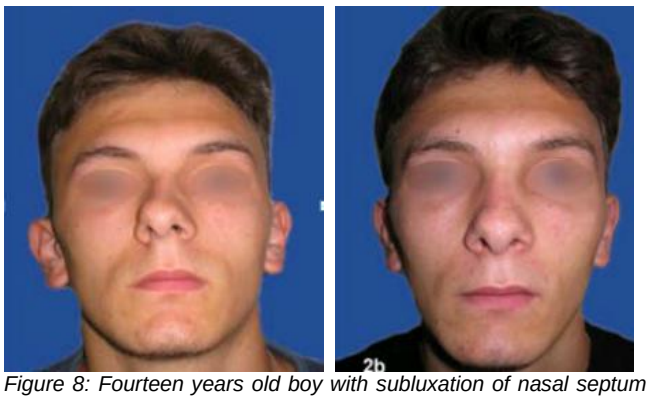

Figure 8: Fourteen years old boy with subluxation of nasal septum after nasal trauma in a previous car accident. a) Preoperative results; b) post-operative results

Case 5: Fourteen years old girl with rhino scoliosis after nasal trauma in early childhood. Septo/rhinoplasty with closed approach was performed. Medial and lateral osteotomy. Septal media position (Fig. 9).

Figure 9: Fourteen years old girl with rhino scoliosis after nasal trauma in early childhood. a) Preoperative results; b) postoperative results (with the permission of parents)

Case 6: Twelve years old girl with rhino kyphosis after nasal trauma in early childhood. Septo/rhinoplasty with closed approach was performed. Medial and lateral osteotomy. Septal media position. Hump reduction (Fig. 10).

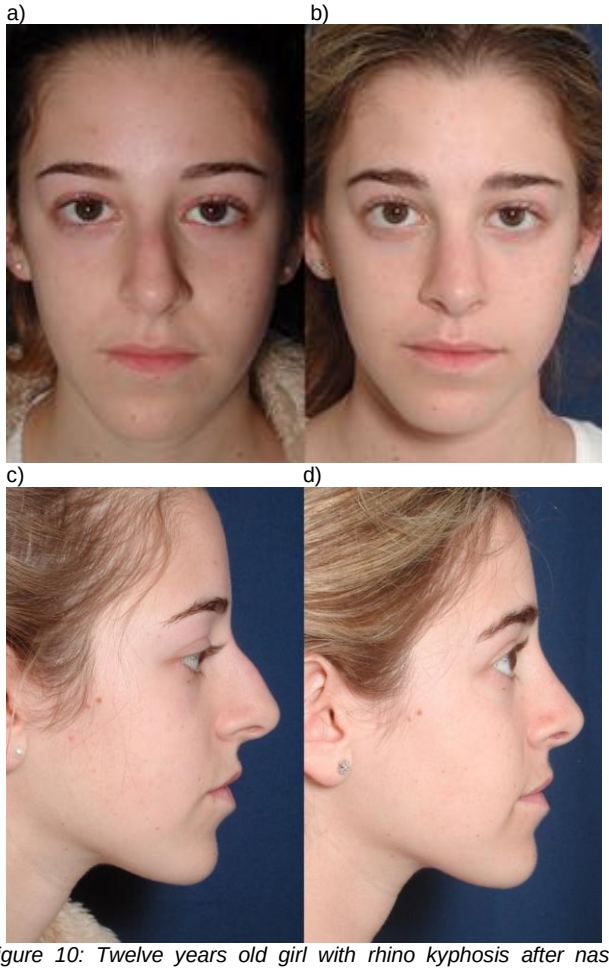

Figure 10: Twelve years old girl with rhino kyphosis after nasal trauma in early childhood. a) Preoperative; b) postoperative; c) pre-operative; d) postoperative (with the permission of parents)

Case 7: Fourteen years old girl with rhino kyphosis after nasal trauma in early childhood.

Septo/rhinoplasty with closed approach was performed. Medial and lateral osteotomy. Septal media position. Hump reduction (Fig. 11).

Figure 11: Fourteen years old girl with rhino kyphosis after nasal trauma in early childhood. a) Preoperative; b) postoperative; c) pre-operative; d) postoperative (with the permission of parents)

Discussion

The management of nasal trauma in children and adolescents depends on upon their age, the degree of nasal obstruction, and associated injuries. A correct diagnosis of the fracture of the nasal pyramid (bony or cartilaginous) is more difficult in children than in adults. Patients who have no symptoms, minimal swelling, and no septal deviation or hematoma do not need specific follow-up. Patients with a nasal fracture but no septal hematoma may be referred to the otorhinolaryngologist within three to five days. The examination should be repeated after 3 days and after one week when the soft tissue swelling has diminished. Most nasal fractures in children can be managed with closed reduction under general anaesthesia. Fractures with splayed nasal bones and no impactions can be reduced with bilateral digital compression on the dorsum for 10 to 15 minutes. Use of intranasal instrumentation may be necessary if digital compression alone is not successful.

Open reduction may need to be performed if significant dislocations are present; the injury is more than two weeks old, or closed and intranasal instrumentation fail. In exceptional cases, it is necessary to use a 2mm osteotome to produce a satisfactory alignment of the nasal bones. Children with septal hematomas or abscesses warrant prompt paediatric otolaryngology consultation. Septal hematoma and abscesses are treated with incision, drainage, and nasal packing. Nasal packing for a few days is only tolerated by older children. In addition, if parents are instructed to bring a recent photograph of the child to the appointment, the otorhinolaryngologist can compare the nasal contours before and after the trauma [24-26].

After the nasal bones reposition, the nasal bones remain mobile for approximately two weeks and can be depressed by force for up to six weeks. Thus, recommendation for the children and the adolescents are to avoid all sports activities for two weeks and some contact sports (karate, wrestling) for six weeks. Most athletes successfully return to their sport after a nasal fracture. However, if these nasal fractures are not treated in time, or if they are not treated at all, they can cause difficult septal deformations, followed by deformation of the nasal pyramid (rhino scoliosis, rhino lordosis, rhino kyphosis) later in life. This happens because the childhood trauma caused a negative effect on nasal growth and serious damage on the nasal growth spurts and the development and ossification of the nose. These deformities can later cause changes in the emotional development of the young adolescents, making them the next candidates for septal/rhinoplasty [27, 28].

Nasal trauma in children can fracture the nasal skeleton and lead to displacement or depression of the nasal bones or septum. Because the nasal pyramid is variable cartilaginous during childhood, and the midline suture is no fused, greenstick fractures are more common. Evaluation of nasal symptoms following nasal trauma includes assessment of nasal obstruction, nasal bleeding, pain, anosmia, and cosmetic deformity. Symptoms of nasal septal hematoma and/or abscess consist of progressive nasal obstruction, persistent or worsening pain after trauma, rhinorrhea, and fever. The clinician should perform a complete external and internal examination of the nose with attention to signs of associated injuries, including cervical spine injury, cerebrospinal fluid leak, naso-orbito-ethmoid fractures, and LeFort fractures. Plain radiographs are not helpful in the diagnosis of nasal fractures in children and should not be performed solely for this purpose. Nasal fractures with a deviation of the nasal septum should be reduced by an otorhinolaryngologist within seven days.

With advances in prevention, imaging evaluation, and bone fixation technology, the management of paediatric facial fractures continues to evolve at a rapid pace. Although often complex, effective management of fractures within this

challenging population is directly dependent upon thorough initial evaluation, correct injury assessment, and timely initiation of chosen therapy. Although facial fractures in this group are uncommon relative to their adult counterparts, a thorough understanding of issues relevant to paediatric facial fractures is critical in providing ideal acute management and optimising long-term success [29-31].

In summary, the growth centres of the nose have to be avoided if possible; long-term nasal issues will theoretically be minimised. If the surgeon replaces it, the cartilage of the nose becomes straighter but still intact.

References

1. Fattahi T, Steinberg B, Fernandes R, Mohan M, Reitter. Repair of nasal complex fractures and the need for secondary septo-rhinoplasty. E J Oral Maxillofac Surg. 2006; 64(12):1785-9. http://dx.doi.org/10.1016/j.joms.2006.03.053 PMid:17113446

2. Desrosiers AE 3rd, Thaller SR. Pediatric nasal fractures: evaluation and management. J Craniofac Surg. 2011;22(4):1327-9. http://dx.doi.org/10.1097/SCS.0b013e31821c932d PMid:21772190

3. Atighechi S, Karimi G. Serial nasal bone reduction: a new approach to the management of nasal bone fracture. J Craniofac Surg. 2009; 20(1):49-52. http://dx.doi.org/10.1097/SCS.0b013e318190def5 PMid:19164988

4. Yabe T, Ozawa T. Treatment of nasoethmoid-orbital fractures using Kirschner wire fixation of the nasal septum. J Craniofac Surg. 2011; 22(4):1510-2. http://dx.doi.org/10.1097/SCS.0b013e31821d4dc9 PMid:21778850

5. Bae JS, Kim ES, Jang YJ. Treatment outcomes of pediatric rhinoplasty: the Asan Medical Center experience. Int J Pediatr Otorhinolaryngol. 2013;77(10):1701-10. http://dx.doi.org/10.1016/j.ijporl.2013.07.030 PMid:23958392

6. Wright RJ, Murakami CS, Ambro BT. Pediatric nasal injuries and management. Facial Plast Surg. 2011;27(5):483-90. http://dx.doi.org/10.1055/s-0031-1288931 PMid:22028012

7. Stucker FJ Jr, Bryarly RC, Shockley WW. Management of nasal trauma in children. Arch Otolaryngol. 1984; 110(3):190-2. http://dx.doi.org/10.1001/archotol.1984.00800290054011

8. Rettinger G. Interventions on the nose--management of acute nasal injuries; interventions of the nasal septum. Laryngorhinootologie. 2008; 87(3):217-23. PMid:18415963

9. Frodel JL. Revision of severe nasal trauma. Facial Plast Surg. 2012; 28(4):454-64. http://dx.doi.org/10.1055/s-0032-1319839 PMid:22872562

10. Rohrich RJ, Adams WP. Nasal fracture management: minimizing secondary nasal deformities. J Plast Reconstr Surg. 2000; 106(2):266-73. http://dx.doi.org/10.1097/00006534-200008000-00003

11. Cole P, Kaufman Y, Hollier LH Jr. Managing the pediatric facial fracture. Craniomaxillofac Trauma Reconstr. 2009;2(2):77-83. http://dx.doi.org/10.1055/s-0029-1202592 PMid:22110800 PMCid:PMC3052668

12. Hatef DA, Cole PD, Hollier LH Jr. Contemporary management of pediatric facial trauma. Curr Opin Otolaryngol Head Neck Surg. 2009;17(4):308-14. http://dx.doi.org/10.1097/MOO.0b013e32832d95a0 PMid:19528801

13. Chao MT, Losee JE. Complications in pediatric facial fractures. Craniomaxillofac Trauma Reconstr. 2009;2(2):103-12. http://dx.doi.org/10.1055/s-0029-1215873 PMid:22110803 PMCid:PMC3052670

14. Siy RW, Brown RH, Koshy JC, Stal S, Hollier LH Jr. General management considerations in pediatric facial fractures. J Craniofac Surg. 2011;22(4):1190-5. http://dx.doi.org/10.1097/SCS.0b013e31821c0cf9 PMid:21772218

15. Meier JD, Tollefson TT. Pediatric facial trauma. Curr Opin Otolaryngol Head Neck Surg. 2008;16(6):555-61. http://dx.doi.org/10.1097/MOO.0b013e3283169321 PMid:19005327

16. Sanyaolu LN, Farmer SE, Cuddihy PJ. Nasal septal haematoma. BMJ. 2014;349:g6075. http://dx.doi.org/10.1136/bmj.g6075 PMid:25370844

17. Wilson SW, Milward TM. Delayed diagnosis of septal haematoma and consequent nasal deformity. Injury. 1994; 25:685. http://dx.doi.org/10.1016/0020-1383(94)90017-5

18. Olsen KD, Carpenter RJ 3rd, Kern EB. Nasal septal injury in children. Diagnosis and management. Arch Otolaryngol. 1980; 106:317. http://dx.doi.org/10.1001/archotol.1980.00790300005002 PMid:7378017

19. Ginsburg CM, Leach JL. Infected nasal septal hematoma. Pediatr Infect Dis J. 1995;14:1012. http://dx.doi.org/10.1097/00006454-199511000-00022

20. Stucker FJ Jr, Bryarly RC, Shockley WW. Management of nasal trauma in children. Arch Otolaryngol. 1984; 110:190. http://dx.doi.org/10.1001/archotol.1984.00800290054011

21. Grymer LF, Bosch C. The nasal septum and the development of the midface. A longitudinal study of a pair of monozygotic twins. Rhinology. 1997; 35:6. PMid:9200255

22. Jalaludin MA. Nasal septal abscess--retrospective analysis of 14 cases from University Hospital, Kuala Lumpur. Singapore Med J. 1993; 34:435.

23. Neuman MI, Eriksson E. Facial trauma. In: Textbook of Pediatric Emergency Medicine, Fleisher GR, Ludwig S, Henretig FM (Eds), Lippincott Williams & Wilkins, Philadelphia, 2006: p.1475.

24. Mathog RH, Bauer W. Posttraumatic pseudohypertelorism. (Telecanthus). Arch Otolaryngol. 1979; 105:81. http://dx.doi.org/10.1001/archotol.1979.00790140027005 PMid:760717

25. Holt GR, Holt JE. Incidence of eye injuries in facial fractures: an analysis of 727 cases. Otolaryngol Head Neck Surg. 1983; 91:276. http://dx.doi.org/10.1177/019459988309100313

26. Gussack G S, Luterman A, Powell R W, Rodgers K, Ramenofsky M L. Pediatric maxillofacial trauma: unique features in diagnosis and treatment. Laryngoscope. 1987;97:925–930. http://dx.doi.org/10.1288/00005537-198708000-00008 PMid:3613792

27. Haug R H, Foss J. Maxillofacial injuries in the pediatric patient. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2000;90:126–134. http://dx.doi.org/10.1067/moe.2000.107974 PMid:10936829

28. Baumann A, Troulis M J, Kaban L B. Facial trauma I: midfacial fractures. In: In: Kaban LB, Troulis MJ, editor. Pediatric Oral and Maxillofacial Surgery. Philadelphia, PA: Saunders, 2004:pp. 425–440. PMid:15093849

29. Adams CD, Januszkiewcz JS, Judson J. Changing patterns of severe craniomaxillofacial trauma in Auckland over eight years. Aust N Z J Surg. 2000;70:401–404. http://dx.doi.org/10.1046/j.1440-1622.2000.01836.x PMid:10843392

30. Dodson TB, Kaban LB. California mandatory seat belt law: the effect of recent legislation on motor vehicle accident related maxillofacial injuries. J Oral Maxillofac Surg. 1988;46:875–880. http://dx.doi.org/10.1016/0278-2391(88)90054-7

31. Kaban LB. Diagnosis and treatment of fractures of the facial bones in children. J Oral Maxillofac Surg. 1993;51:722–729. http://dx.doi.org/10.1016/S0278-2391(10)80409-4

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):420-422.
http://dx.doi.org/10.3889/oamjms.2016.065
eISSN: 1857-9655
Clinical Science

Early Prognostic Factors for the Progress of Preeclampsia – Our Experience in the Period 2010-2011

Mariya Angelova[1*], Ivan Todorov[1], Emil Kovachev[2]

[1]Department of Obstetrics and Gynecology, Trakia University, Faculty of Medicine, Stara Zagora, Bulgaria; [2]Department of Obstetrics and Gynecology, Paraskev Stoyanov Medical University, Varna, Bulgaria

Citation: Angelova M, Todorov I, Kovachev E. Early Prognostic Factors for the Progress of Preeclampsia – Our Experience in the Period 2010-2011. Open Access Maced J Med Sci. 2016 Sep 15; 4(3):420-422. http://dx.doi.org/10.3889/oamjms.2016.065

Keywords: Preeclampsia; Doppler test; Pregnancy-associated plasma protein A; Body Mass Index.

***Correspondence:** Mariya Angelova. Department of Obstetrics and Gynecology, Trakia University, Faculty of Medicine, Stara Zagora, Bulgaria. E-mail: angelova70@abv.bg

Received: 27-Apr-2016; **Revised:** 20-May-2016; **Accepted:** 25-May-2016; **Online first:** 06-Aug-2016

Competing Interests: The authors have declared that no competing interests exist.

Abstract

AIM: To determine the prognostic value of the low Pregnancy-associated plasma protein A (PAPP-A) levels in the early stages of pregnancy (11–13 weeks GA) independently and in combination with a Doppler test of the uterine arteries during the second half of pregnancy (22–23 weeks GA).

MATERIAL AND METHODS: The study covered the period 2010–2011 and included 106 pregnant women, aged 35–40, with a single child pregnancy. The research excluded pregnant women with anomalies of the fetus, smokers and women taking prophylactically low doses of aspirin.

RESULTS: Thirty-six pregnant women had PAPP-A level below 0.4 MoM, whereas 20 of them developed preeclampsia and 7 – early preeclampsia. The combination of the low PAPP-A values and the abnormal Doppler test of the uterine arteries is with a considerably better prognostic value in regards to the risk of developing preeclampsia.

CONCLUSION: The Doppler test is a non-invasive, quick and easy method for assessment of the uterine-placental blood flow.

Introduction

Preeclampsia (PE) is one of the most serious complications during the second half of pregnancy [1]. The high perinatal maternal mortality and the children's pathology make it a priority in the research of a number of scientists and researchers [2].

Our main goal was on the grounds of separate factors or a group of factors to determine who of the pregnant women display a high risk of developing preeclampsia with a view to the subsequent antenatal cares for them. In addition, we aimed to select markers that are comparatively easy, accessible and cost-effective for us and for our patients.

Our task was to determine the prognostic value of the low PAPP – A levels in the early stages of pregnancy (11 – 13 weeks GA) independently and in combination with a Doppler test of the uterine arteries during the second half of pregnancy (22 – 23 weeks GA).

Material and Methods

The study covered the period 2010 – 2011 and included 106 pregnant women, aged 35 – 40, with a single child pregnancy. The research excluded

pregnant women with anomalies of the fetus, smokers and women taking prophylactically low doses of aspirin.

Out of all pregnant women observed, 26 developed preeclampsia and 7 of them – early preeclampsia (before 34 weeks GA). In the beginning of the pregnancy, 27 of them were registered for increased Body Mass Index (BMI – 26 kg/m^2). Thirteen out of all 26 women with preeclampsia had it during their previous pregnancies as well (Table 1).

Table 1: Investigated parameters in the control group and in the patients with early preeclampsia and preeclampsia

	Preeclampsia	Early preeclampsia before 34 week GA	Control group
Combination of increased PI, RI, presence of incisures and reduced PAPP – A	26 / 28%	7 / 7%	33 / 35%
Presence of incisures in the early diastolic phase	22 / 23%	6 / 6%	30 / 32%
Increased PI values	19 / 20%	6 / 6%	38 / 40%
Increased RI values	17 / 18%	5 / 5%	31 / 33%
Reduced levels of PAPP – A	14 / 15%	2 / 2%	36 / 38%
Anamnesis of previous preeclampsia	13 / 14%	4 / 4%	19 / 20%
Miscarriages in the anamnesis	5 / 5%	2 / 2%	29 / 31%
Increased BMI	12 / 13%	4 / 4%	27 / 29%
Increased RR values before pregnancy	27 / 29%	2 / 2%	32 / 34%
Accompanying diseases	6 / 6%	1 / 1%	22 / 23%

To examine the blood flow in the uterine arteries, MEDISON – AQUVIX V 10 and SONOACE X 8 devices were used. The blood flow velocity was registered by means of 3.5 or 5 MHz transabdominal sector array transducers. The test was performed in a standard manner, the patient lying down on her back, and a colour Doppler was used to chartering the uterine artery while a pulsating Power Doppler and a transducer, parallel to the uterine artery (from 15^o – to 30^o) were employed to determine pulsatility index (PI) and resistive index (RI).

The vascular resistance changes in the course of pregnancy. With its advance, the curves usually become low resistant – with low PI and RI and without diastolic incisures. The waveform was considered abnormal if there were bilateral notches with a mean RI of > 0.55, if there was a unilateral notch and a mean PI of > 0.65, or if the mean RI was 0.70.

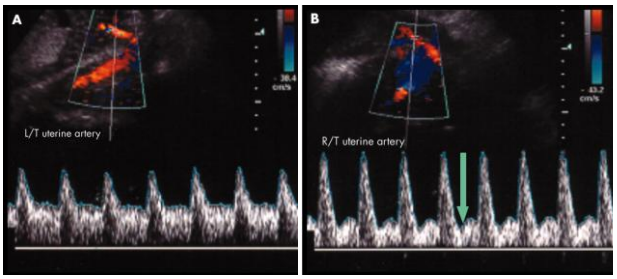

Figure 1: A) Normal blood flow; B) Abnormal blood flow

The quantitative measurement data of Pregnancy-associated plasma protein A (PAPP-A) were taken by the biochemical screening performed to these pregnant women at 11-13 week GA, employing the Delfia system for their laboratory determination.

The low PAPP-A levels in the first trimester of pregnant women without chromosome anomalies are connected with an unfavourable perinatal outcome – intrauterine growth retardation (IUGR), preeclampsia, miscarries. These namely were the pregnant women subject mandatory to a Doppler test of their umbilical artery. On the grounds of this survey, we drew the conclusion that women with increased PI of the umbilical artery develop more frequently IUGR. In the control group of 33 women with reduced PAPP-A, 16 pregnant women had IUGR.

The Doppler test of the two uterine arteries was also performed to all pregnant women in the control group.

Results

The frequency of developing preeclampsia increases with the age of the mother with 35% which is above the average generally mentioned percentage.

To determine the prognostic value of PAPP-A in the first trimester, the following data have been collected: 36 pregnant women had PAPP-A level below 0.4 MoM, whereas 20 of them developed preeclampsia and 7 – early preeclampsia.

The combination of the low PAPP-A values and the abnormal Doppler test of the uterine arteries is with a considerably better prognostic value in regards to the risk of developing preeclampsia (Fig. 1).

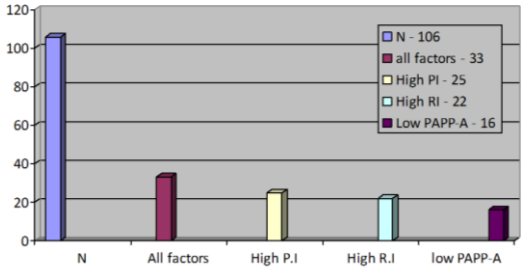

Figure 1: The combination of the low PAPP-A values and the abnormal Doppler test of the uterine arteries

Discussion

The increased PI in both uterine arteries is a good and reliable prognostic indicator for preeclampsia. Besides, RI in one or in both arteries

also plays a role [3]. Especially sensitive turned out the factor with which we observed subjectively the presence of incisures in the early diastolic phase.

Low serum PAPP-A levels are associated with a higher incidence of PE. Increased maternal serum PAPP-A levels have been observed in established PE [4-6]. A multicenter study of 8839 women demonstrated a significant relationship between PAPP-A levels at or below the 5th percentile and IUGR preterm delivery, PE, and stillbirth [7].

Alteration in plasma levels of angiogenic factors is more pronounced in early preeclampsia during clinical disease compared to late-onset preeclampsia [8-11]. Wikström et al. demonstrate 43 times higher median plasma concentrations of sFlt-1 in early onset compared to a 3-fold increase in late-onset preeclampsia and 21 times lower median plasma concentrations of PlGF in early onset disease compared to a 5-fold decrease in late-onset disease [8].

Although there are multiple potential biomarkers for PE their efficacy has been inconsistent and comparisons are difficult because of heterogeneity between different studies. Two papers with meta-analysis on biomarkers in preeclampsia were published recently (2015). Wu et al. found low predictive values using individual biomarkers which included a disintegrin and metalloprotease 12 (ADAM-12), inhibin-A, pregnancy-associated plasma protein A (PAPP-A), placental growth factor (PlGF) and placental protein 13 (PP-13) [12]. In the second meta-analysis the objective was to investigate the accuracy of serum biochemical markers(Pregnancy- Associated Plasma Protein-A (PAPP-A), human Chorionic Gonadotropin (hCG), Placental Growth Factor (PlGF), Placental Protein 13 (PP13) used in first trimester serum screening in predicting preeclampsia, small for gestational age (SGA) and preterm delivery [13]. The results showed low predictive accuracy overall. For preeclampsia, the best predictor was PlGF. The predictive value of serum markers for early preeclampsia was better than that of late preeclampsia. For SGA the best predictor was PP13. For preterm delivery, the best predictor was PP13 [13].

The predictive value of all these factors, when combined, is significantly enhanced.

In conclusion, the Doppler test is a non-invasive, quick and easy method for assessment of the uterine-placental blood flow.

References

1. Roberts JM, Gammill HS. Preeclampsia: Recent insights. Hypertension. 2005;46:1243–1249. http://dx.doi.org/10.1161/01.HYP.0000188408.49896.c5 PMid:16230510

2. Gathiram P, Moodley J. Pre-eclampsia: its pathogenesis and pathophysiolgy. Cardiovasc J Afr. 2016;27(2):71-8. http://dx.doi.org/10.5830/CVJA-2016-009 PMid:27213853 PMCid:PMC4928171

3. Khong SL, Kane SC, Brennecke SP, da Silva Costa F. First-trimester uterine artery Doppler analysis in the prediction of later pregnancy complications. Dis Markers. 2015;2015:679730. http://dx.doi.org/10.1155/2015/679730 PMid:25972623 PMCid:PMC4418013

4. Deveci K, Sogut E, Evliyaoglu O, Duras N. Pregnancy-associated plasma protein-A and C-reactive protein levels in pre-eclamptic and normotensive pregnant women at third trimester. J Obstet Gynaecol Res. 2009;35:94–98. http://dx.doi.org/10.1111/j.1447-0756.2008.00835.x PMid:19215554

5. Bersinger NA, Odegard RA. Second- and third-trimester serum levels of placental proteins in pre-eclampsia and small-for-gestational age pregnancies. Acta Obstet Gynecol Scand. 2004;83:37–45. http://dx.doi.org/10.1111/j.1600-0412.2004.00277.x

6. Bersinger NA, Smarason AK, Muttukrishna S, Groome NP, Redman CW. Women with pre-eclampsia have increased serum levels of pregnancy-associated plasma protein A (PAPP-A), inhibin-A, activin-A and soluble E-selectin. Hypertens Pregnancy. 2003;22:45–55. http://dx.doi.org/10.1081/PRG-120016794 PMid:12648442

7. Smith GC, Stenhouse EJ, Crossley JA, Aitken DA, Cameron AD, Connor JM. Early pregnancy levels of pregnancy-associated plasma protein a and the risk of intrauterine growth restriction, premature birth, pre-eclampsia, and stillbirth. J Clin Endocrinol Metab. 2002;87:1762–1767. http://dx.doi.org/10.1210/jcem.87.4.8430 PMid:11932314

8. Wikstrom AK, Larsson A, Eriksson UJ, Nash P, Norden-Lindeberg S, Olovsson M. Placental growth factor and soluble fms-like tyrosine kinase-1 in early-onset and late-onset preeclampsia. Obstet Gynecol 2007;109:1368–74. http://dx.doi.org/10.1097/01.AOG.0000264552.85436.a1 PMid:17540809

9. Chaiworapongsa T, Romero R, Tarca A, Kusanovic JP, Mittal P, Kim SK, Gotsch F, et al. A subset of patients destined to develop spontaneous preterm labor has an abnormal angiogenic/anti-angiogenic profile in maternal plasma: Evidence in support of pathophysiologic heterogeneity of preterm labor derived from a longitudinal study. J Matern Fetal Neonatal Med. 2009;22:1122–1139. http://dx.doi.org/10.3109/14767050902994838 PMid:19916710 PMCid:PMC3437777

10. Molvarec A, Szarka A, Walentin S, Szucs E, Nagy B, Rigó J Jr. Circulating angiogenic factors determined by electrochemiluminescence immunoassay in relation to the clinical features and laboratory parameters in women with pre-eclampsia. Hypertens Res. 2010;33:892–898. http://dx.doi.org/10.1038/hr.2010.92 PMid:20535121

11. Mijal RS, Holzman CB, Rana S, Karumanchi SA, Wang J, Sikorskii A. Midpregnancy levels of angiogenic markers in relation to maternal characteristics. Am J Obstet Gynecol. 2011;204:244.e1–244.12.

12. Wu P, van den Berg C, Alfirevic Z, O'Brien S, Röthlisberger M, Baker PN, Kenny LC, Kublickiene K, Duvekot JJ. Early pregnancy biomarkers in pre-eclampsia: a systematic review and meta-analysis. International journal of molecular sciences. 2015 Sep 23;16(9):23035-56. http://dx.doi.org/10.3390/ijms160923035 PMid:26404264 PMCid:PMC4613350

13. Conde-Agudelo A, Papageorghiou AT, Kennedy SH, Villar J. Novel biomarkers for predicting intrauterine growth restriction: a systematic review and meta-analysis. BJOG: An International Journal of Obstetrics & Gynaecology. 2013 May 1;120(6):681-94. http://dx.doi.org/10.1111/1471-0528.12172 PMid:23398929

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):423-427.
http://dx.doi.org/10.3889/oamjms.2016.094
eISSN: 1857-9655
Clinical Science

The Association between Malnutrition and Pressure Ulcers in Elderly in Long-Term Care Facility

Lenche Neloska[1]*, Katerina Damevska[2], Andjelka Nikolchev[1], Lidija Pavleska[1], Biljana Petreska-Zovic[1], Milenko Kostov[3]

[1]Gerontology Institute "13 November Skopje", Skopje, Republic of Macedonia; [2]University Clinic of Dermatology, Medical Faculty, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia; [3]University Clinic of Neurosurgery, Medical Faculty, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia

Citation: Neloska L, Damevska K, Nikolchev A, Pavleska L, Petreska-Zovic B, Kostov M. The Association between Malnutrition and Pressure Ulcers in Elderly in Long-Term Care Facility. Open Access Maced J Med Sci. 2016 Sep 15; 4(3):423-427. http://dx.doi.org/10.3889/oamjms.2016.094

Keywords: malnutrition; pressure ulcers; geriatric patients; palliative patients; long-term care facility.

***Correspondence:** Neloska Lenche, MD, MSc. Gerontology Institute "13 November Skopje", Skopje, Republic of Macedonia. E-mail: neloskal@gmail.com

Received: 09-Jul-2016; **Revised:** 12-Jul-2016; **Accepted:** 14-Jul-2016; **Online first:** 22-Aug-2016

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: Malnutrition is common in elderly and is a risk factor for pressure ulcers.

AIM: The aim of the present study was to determine the prevalence of malnutrition in geriatric and palliative patients hospitalised in long-term care facility, and to examine the influence of nutritional status on the prevalence of pressure ulcers (PU).

MATERIAL AND METHODS: Descriptive, observational and cross-sectional study including 2099 patients admitted to the Hospital during a 24 month period (January 2013 to December 2014). We recorded: demographic data, body mass index (BMI), Braden score, laboratory parameters of interest (albumin, total protein, RBC count, haemoglobin and iron levels) and presence or absence of malnutrition and pressure ulcers.

RESULTS: The pressure ulcer prevalence was 12.9% (256 out of 2099). Based on the BMI classification, 61.7% of patients had a good nutritional status, 27.4% were undernourished, and 2.1% were considered malnourished. Nutritional status was statistically significantly different between patients with and without PU ($p < 0.0001$). This study also showed that hypoproteinemia, hypoalbuminemia, low RBC was positively associated with PU prevalence.

CONCLUSION: The results highlight the impact of nutritional status on the prevalence of pressure ulcers in hospitalised geriatric and palliative population. It is of paramount importance to correctly evaluate the presence of malnutrition in patients at risk of pressure ulcers.

Introduction

A pressure ulcer (PU) is localised injury to the skin and/or underlying tissue usually over a bony prominence, as a result of pressure or pressure in combination with shear. It leads to ischemia, progressive destruction and necrosis of the underlying soft tissues [1]. Pressure ulcers are debilitating chronic wounds, which occur in people with advanced age, physical or cognitive impairments, and multiple comorbidities. It is a common problem among older adults in all health care settings [2].

The prevalence of PUs in long-term settings ranges from 11 to 29% [3-6]. Development of PU in hospitalised elderly patients is complex and multifactorial. Predisposing factors are classified as intrinsic (e.g. activity or mobility limitation, altered consciousness, abnormalities in nutritional status, comorbidities, ageing skin) or extrinsic (e.g., pressure, friction, shear, incontinence).

Malnutrition is a very common problem that affects approximately 30-50% of hospitalised patients. It is estimated that at least one-third of patients has some degree of malnutrition upon admission to the hospital (7). Additionally, among patients who are not malnourished upon admission, about one third may become malnourished while in the hospital (8). Among the elderly residents of long-term care institutions, the prevalence of in-hospital malnutrition has been estimated to be between 12.5 and 78.9% in different

studies [9-11]. Overall, 50% of the residents require an individualised nutritional care plan [9].

Hospital malnutrition is associated with an increase in morbidity, mortality, a higher readmission rate, functional disabilities and physical complications, and, therefore, higher healthcare costs. Malnutrition is closely related to frailty, a clinical syndrome characterised by increased vulnerability to adverse health outcomes including acute illness, the decline in physiological reserve, and increased risk for disability, falls, hospitalisation, need for long-term care, and death [12].

The principal objective of this study was to assess the prevalence of malnutrition in elderly patients hospitalised in the long-term care facility. The secondary objective was to examine the influence of malnutrition on the prevalence of pressure ulcers.

Material and Methods

This observational study was carried out at the Geriatric and Palliative Care Hospital "13 November" in Skopje, Macedonia, the largest specialised geriatric and palliative hospital in the country that attends to patients through the Public Health System. The study design was approved by the Hospital Ethics Committee. Informed consent was waived because of the study's observational nature.

Consecutive patients admitted to the hospital were enrolled from January 2013 to December 2014. We collected data using a case report form and recorded information about demographics, main diagnosis, BMI, laboratory findings, the presence of PU and PU characteristics.

Body mass index (BMI) was calculated using the formula: weight in kilogrammes/height in meters2. Using BMI classification, patients were classified as severely underweight, underweight, normal weight, overweight, severely obese or morbidly obese.

The Braden scale was used to assess the risk of developing PUs. The total score can range from 6 to 23 with a lower score indicating a higher risk [13].

The risk for PU development was assigned according to the stratification determined by the scale, into four groups; according to the Braden score [13]. The risk of PUs increases in patients with a score ≤ 12 points. The grading system of the EPUAP (European Pressure Ulcer Advisory Panel) was used [1].

Descriptive statistics was used to describe the study population, with continuous outcomes summarised as a mean and range, and categorical outcomes presented as a percentage. Chi-square test was used to analyse categorical variables. Independent t-test was used to analyse continuous variables. P-values < 0.05 were considered statistically significant.

Results

During the study period, two thousand and ninety-nine patients were consecutively admitted. Baseline characteristics of the study population are shown in Table 1. One thousand eight hundred and forty-three patients without PU (724 male and 1119 female, mean age 76.32 years, SD 11.192, range 22-103 years), and 256 patients with PU (86 male and 170 female, mean age 76.38, SD 11.296, range 37-97 years) participated in the study. No statistically significant difference was noted regarding age between the groups (p = 0.80).

Table 1: Baseline characteristics of patient population, according to the presence/absence of PU

	GENDER		AGE		
		N (%)	x̄	SD	Min.-Max.
PU absent	Male	724 (39.28)	74.63	11.697	25-103
	Female	1119 (60.71)	77.41	10.717	22-101
	Total	1843 (87.8)	76.32	11.192	22-103
PU present	Male	86 (33.59)	74.50	12.234	38-97
	Female	170 (66.4)	77.34	10.702	37-95
	Total	256 (12.19)	76.38	11.296	37-97
Total		2099 (100)	76.32	11.202	22-103

Braden score ranged from 6 to 23 (mean 13.64, SD 3.247) and 1948 (92.8%) had a risk for pressure ulcers (Table 2).

Table 2: Patient's level of risk for development of PUs according to the Braden Scale (BS)

Level of risk	N	%
High (BS ≤ 12)	913	43.5
Moderate (BS 13-14)	478	22.8
Low risk (BS 15-19)	557	26.5
No risk (BS ≥ 20)	151	7.2
Total	2099	100

Table 3 shows the distribution of patients according to the nutritional status. Based on the BMI classification, 61.7% of patients had a good nutritional status, 27.4% were undernourished, and 2.1% were considered malnourished. Nutritional status was statistically significantly different between patients with and without PU ($\chi 2 = 25.350$; $p < 0.0001$).

Table 3: Differences in nutritional status between patients with (n = 256) and without a pressure ulcer (n = n=1843)

BMI classification	BMI	PU absent N (%)	PU present N (%)	Total N (%)
Severely underweight	15-16	29 (1.57)	16 (6.25)	45 (2.1)
Underweight	16-18.5	501 (27.18)	74 (28.9)	575 (27.4)
Normal weight	18.5–24.9	1151 (62.45)	144 (56.25)	1295 (61.7)
Overweight	25.0–29.9	102 (5.53)	12 (4.68)	114 (5.4)
Severely obese	30.0–34.9	59 (3.2)	10 (3.9)	69 (3.3)
Morbidly obese	>35	1 (0.05)	0	1 (0.05)
Total		1843 (87.81)	256 (12.19)	2099

Table 4 shows nutrition-related laboratory values, according to the presence of PU. We detected a significant difference in presence of hypoalbuminemia ($p < 0.0001$), hypoproteinemia ($p = 0.019$), low RBC ($p = 0.004$) and low hemoglobin levels ($p < 0.0001$) in patients with PU, compared to patients without PU. Iron and triglyceride levels were not related to the presence of PU.

Table 4: Nutrition-related laboratory values

	PU absent N (%)	PU present N (%)	$\chi 2$	p
↓ RBC count	755 (40.96)	131 (51.17)	11.124	0.004
↓ Hemoglobin level	1050 (57.36)	186 (72.94)	27.156	<0.0001
↓ Iron (serum)	1 (0.07)	1 (0.5)	2.48	0.289
↓ Total protein	1194 (71.71)	197 (78.8)	5.495	0.019
↓ Albumin	704 (48.55)	175 (70.28)	40.184	<0.0001
↑Triglyceride level	220 (14.58)	34 (14.78)	0.006	0.938

Discussion

In this retrospective study involving 2099 geriatric and palliative patients in a long-term setting, the following variables were significantly more frequently documented in patients with PU compared to those without PU: malnutrition measured as BMI, hypoproteinemia, hypoalbuminemia and anaemia ($p < 0.05$ for all).

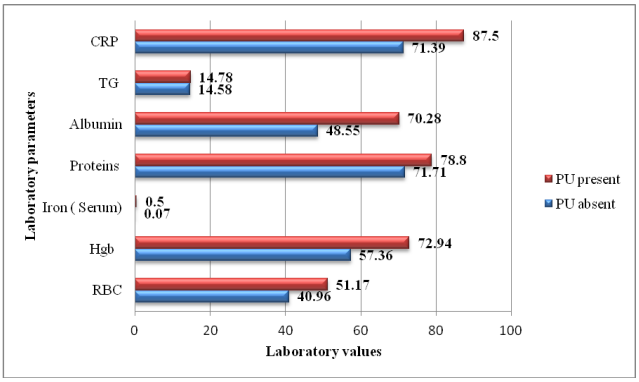

Figure 1: Percentage of patients with altered nutrition-related lab values

Malnutrition is defined as a state of nutrition in which a deficiency or excess of energy, protein and other nutrients causes measurable effects both on tissue/body structure and function [14].

The causes of malnutrition are multiple and complex [15]. In elderly patients, malnourishment may result from a combination of factors, including cardiac failure, difficulty chewing, dysphagia inflammatory illness, respiratory problems, reduced sense of smell and taste, and medications [16]. Furthermore, chronic diseases and cancer cause inflammation and increased cytokine production, which suppresses appetite. Acute and chronic infections, wounds, and hypermetabolism cause increased the need for energy and protein [17, 18]. Monotony of diet in institutional care, the timing of meals, decreased taste thresholds and a loss of olfactory distinction also impact nutritional wellness [19].

Malnutrition has numerous effects, including impaired collagen synthesis, and immune function. Wound healing refers to the complex and dynamic process of restoring cellular structures and tissue layers. In each phase of wound healing, poor nutritional status can delay the healing process or cause inadequate healing when nutritional deficiencies are not corrected [20, 21].

A number of studies have demonstrated that the relationship between malnutrition and pressure ulcers is bidirectional [22-24]. A direct correlation between malnutrition severity and the pressure ulcers has been reported [24].

Patients with chronic PUs - wounds that remain unhealed for more than six weeks - experience a continuous cycle in which they lose protein through excess exudate, resulting in delayed wound healing.

Our results revealed marked hypoproteinemia and hypoalbuminemia in patients with PUs. Protein-energy malnutrition reduces fibroblastic cellular activity and delays angiogenesis in the proliferative stage and reduced collagen synthesis and maturation in the remodelling stage, leading to increased wound dehiscence. Furthermore, proteins are lost in wound exudates, which can contain as much as 100 g of protein per day [25]. A deficiency in serum albumin causes impaired wound perfusion, reducing the osmotic pressure in the intravascular space. This causes interstitial oedema, reducing tissue oxygenation and tissue tolerance to the forces of pressure. Oedema also may be a factor in unidentified malnutrition by masking muscle and fat loss [26].

Older people are at risk of malnutrition if they have a BMI below 24, and malnutrition is indicated by a BMI of less than 20 [27]. The World Health Organization categorises underweight as BMI < 18.5. However, BMI may be unreliable in the presence of confounding factors such as oedema or ascites, and may not identify significant unintentional weight loss if used as a single assessment. Reliable measurement of height can be difficult in the elderly because of vertebral compression, loss of muscle tone and postural changes [28].

Malnutrition in a hospital setting may be prevented by using strategies that include assisting patients into a position conducive to swallowing safely and comfortably; implementing food charts to ensure accurate documentation of intake; and using fluid charts to record input and output to calculate fluid balance [21].

Maintaining nutritional status and preventing malnutrition aims to ensure that energy, protein and other micronutrients are available to prevent pressure ulcer development. No biochemical marker on its own offers a satisfactory screening test for malnutrition.

Serum proteins synthesised by the liver have been used as markers of nutrition—albumin, transferrin, retinol-binding protein and thyroxine-binding albumin. Serum albumin has been most widely adopted; however, the long half-life of albumin means that serum albumin does not respond to short-term changes in protein and energy intake.

Nutrition and hydration play an important role in preserving skin and tissue viability and in supporting tissue repair for PU healing [29].

In the older population, undernutrition rather than overnutrition is the main cause for concern [30]. Malnourished older people are at increased risk of falls, lengthy hospital stays and rehabilitation, institutionalisation, postoperative complications, infections, pressure ulcers, poor wound healing, impaired muscle and respiratory function and death [31]. Unfortunately, malnutrition continues to be under-recognized in many hospitals [32].

Pressure ulcers are debilitating chronic wounds. Prevention of PUs in hospitalised elderly patients is an important health priority, one that requires clear identification of risk factors. Effective management of malnutrition requires collaboration among multiple clinical disciplines and all members of the clinical team [33].

In conclusion, malnutrition is a problem of high prevalence and impact in geriatric and palliative patients. Given the high prevalence of malnutrition among patients with pressure ulcers, performing a routine nutritional screening should result in early identification of residents with the risk of development of pressure ulcer.

References

1. European Pressure Ulcer Advisory Panel, National Pressure Ulcer Advisory Panel, Pan Pacific Pressure Injury Alliance. Prevention and Treatment of Pressure Ulcers: Quick Reference Guide. Washington, DC: National Pressure Ulcer Advisory Panel, 2009.

2. Allman RM. Pressure ulcer prevalence, incidence, risk factors, and impact. Clin Geriatr Med. 1997;13(3):421-36. PMid:9227937

3. Horn SD, Bender SA, Ferguson ML, Smout RJ, Bergstrom N, Taler G, Cook AS, Sharkey SS, Voss AC. The National Pressure Ulcer Long-Term Care Study: pressure ulcer development in long-term care residents. J Am Geriatr Soc. 2004;52(3):359-67. http://dx.doi.org/10.1111/j.1532-5415.2004.52106.x PMid:14962149

4. Donini LM, De Felice MR, Tagliaccica A, De Bernardini L, Cannella C. Comorbidity, frailty, and evolution of pressure ulcers in geriatrics. Med Sci Monit. 2005;11(7):CR326-36. PMid:15990690

5. Lahmann NA, Halfens RJ, Dassen T. Pressure ulcers in German nursing homes and acute care hospitals: prevalence, frequency, and ulcer characteristics. Ostomy Wound Manage. 2006;52(2):20-33. PMid:16464992

6. Neloska L, Damevska K, Nikolchev A, Pavleska L, Petreska-Zovic B, Kostov M. The influence of comorbidity on the prevalence of pressure ulcers in geriatric patients. Glob Dermatol. 2016; 3(3): 319-322. http://dx.doi.org/10.15761/GOD.1000183

7. Barker LA, Gout BS, Crowe TC. Hospital malnutrition: Prevalence, identification and impact on patients and the healthcare system. Int J Environ Res Public Health. 2011;8(2):514-527. http://dx.doi.org/10.3390/ijerph8020514 PMid:21556200 PMCid:PMC3084475

8. Braunschweig C, Gomez S, Sheean PM. Impact of declines in nutritional status on outcomes in adult patients hospitalized for more than 7 days. J Am Diet Assoc. 2000;100(11):1316-22. http://dx.doi.org/10.1016/S0002-8223(00)00373-4

9. Pezzana A, Cereda E, Avagnina P, Malfi G, Paiola E, Frighi Z, Capizzi I, Sgnaolin E, Amerio ML. Nutritional Care Needs in Elderly Residents of Long-Term Care Institutions: Potential Implications for Policies. J Nutr Health Aging. 2015;19(9):947-54. http://dx.doi.org/10.1007/s12603-015-0537-5 PMid:26482698

10. Lelovics Z. [Nutritional status and nutritional rehabilitation of elderly people living in long-term care institutions]. Orv Hetil. 2009;150(44):2028-36. http://dx.doi.org/10.1556/OH.2009.28723 PMid:19861289

11. Lou MF, Dai YT, Huang GS, Yu PJ. Nutritional status and health outcomes for older people with dementia living in institutions. J Adv Nurs. 2007;60(5):470-7. http://dx.doi.org/10.1111/j.1365-2648.2007.04442.x PMid:17973710

12. Gielen E, Verschueren S, O'Neill TW, Pye SR, O'Connell MD, Lee DM, et al. Musculoskeletal frailty: a geriatric syndrome at the core of fracture occurrence in older age. Calcif Tissue Int. 2012;91(3):161–77. http://dx.doi.org/10.1007/s00223-012-9622-5 PMid:22797855

13. Bergstrom N, Braden B, Kemp M, Champage M, Ruby E. Predicting pressure ulcer risk: a multisite study of the predictive validity of the Braden Scale. Nurs Res.1998; 47:261-9. http://dx.doi.org/10.1097/00006199-199809000-00005 PMid:9766454

14. Abbasi AA, Rudman D. Observations on the prevalence of protein-calorie undernutrition in VA nursing homes. J Am Geriatr Soc. 1993;41(2):117-21. http://dx.doi.org/10.1111/j.1532-5415.1993.tb02043.x

15. Saunders J, Smith T. Malnutrition: causes and consequences. Clin Med (Lond). 2010;10(6):624-7. http://dx.doi.org/10.7861/clinmedicine.10-6-624

16. Brownie S. Why are elderly individuals at risk of nutritional deficiency? Int J Nurs Pract. 2006;12(2):110-8. http://dx.doi.org/10.1111/j.1440-172X.2006.00557.x PMid:16529597

17. Norman K, Pichard C, Lochs H, Pirlich M. Prognostic impact of disease-related malnutrition. Clin Nutr. 2008;27(1):5-15. http://dx.doi.org/10.1016/j.clnu.2007.10.007 PMid:18061312

18. Donini LM, Savina C, Cannella C. Eating habits and appetite control in the elderly: the anorexia of aging. Int Psychogeriatr. 2003;15(1):73–87. http://dx.doi.org/10.1017/S1041610203008779 PMid:12834202

19. Harris CL, Fraser C. Malnutrition in the institutionalized elderly: the effects on wound healing. Ostomy Wound Manage. 2004;50(10):54-63. PMid:15509882

20. Todorovic V. Food and wounds: nutritional factors in wound formation and healing. Br J Community Nurs. 2002;43-4, 46, 48 passim. http://dx.doi.org/10.12968/bjcn.2002.7.Sup2.12981 PMid:12362158

21. Smoliner C, Norman K, Scheufele R, Hartig W, Pirlich M, Lochs H. Effects of food fortification on nutritional and functional status in frail elderly nursing home residents at risk of malnutrition Nutrition. 2008;24(11-12):1139-44. http://dx.doi.org/10.1016/j.nut.2008.06.024 PMid:18789649

22. Günes ÜY, Efteli E. Predictive validity and reliability of the Turkish version of the risk assessment pressure sore scale in intensive care patients: results of a prospective study. Ostomy

Wound Manage. 2015;61(4):58-62. PMid:25853378

23. Fossum M, Ehnfors M, Svensson E, Hansen LM, Ehrenberg A. Effects of a computerized decision support system on care planning for pressure ulcers and malnutrition in nursing homes: an intervention study. Int J Med Inform. 2013;82(10):911-21. http://dx.doi.org/10.1016/j.ijmedinf.2013.05.009 PMid:23827767

24. Park KH. A retrospective study using the pressure ulcer scale for healing (PUSH) tool to examine factors affecting stage II pressure ulcer healing in a Korean acute care hospital. Ostomy Wound Manage. 2014;60(9):40-51. PMid:25211606

25. Russell L. The importance of patient's nutritional status in wound healing. B J Nurs. 2001:10(6S): S42–49. http://dx.doi.org/10.12968/bjon.2001.10.Sup1.5336 PMid:12070399

26. James J. Optimal wound healing: a comprehensive approach through metabolic, anabolic and nutritional interventions. Involuntary weight loss and its effect on the body to heal. WOUNDS. 2002;14(9):4–8.

27. Mathus-Vliegen EM. Old age, malnutrition, and pressure sores: an ill-fated alliance. J Gerontol A Biol Sci Med Sci. 2004;59(4):355-60. http://dx.doi.org/10.1093/gerona/59.4.M355 PMid:15071079

28. Balcombe NR, Ferry PG, Saweirs WM. Nutritional status and well being. Is there a relationship between body mass index and the well-being of older people? Curr Med Res Opin. 2001;17: 1-7. http://dx.doi.org/10.1185/03007990152005397 PMid:11464440

29. Posthauer ME, Banks M, Dorner B, Schols JM. The role of nutrition for pressure ulcer management: national pressure ulcer advisory panel, European pressure ulcer advisory panel, and pan pacific pressure injury alliance white paper. Adv Skin Wound Care. 2015;28(4):175-88. http://dx.doi.org/10.1097/01.ASW.0000461911.31139.62 PMid:25775201

30. Elia M, editor. Screening for Malnutrition: A Multidisciplinary Responsibility. Development and use of the 'Malnutrition Universal Screening Tool' ('MUST') for Adults. BAPEN, 2003.

31. Milne AC, Potter J, Avenell A. Protein and energy supplementation in elderly people at risk from malnutrition. Cochrane Database Syst Rev. 2005 Apr 18;(2):CD003288. Review. Update in: Cochrane Database Syst Rev. 2009;(2):CD003288. PMid:19370584

32. Kirkland LL, Kashiwagi DT, Brantley S, Scheurer D, Varkey P. Nutrition in the hospitalized patient. J Hosp Med. 2013;8(1):52-58. http://dx.doi.org/10.1002/jhm.1969 PMid:23065968

33. Tappenden KA, Quatrara B, Parkhurst ML, Malone AM, Fanjiang G, Ziegler TR. Critical role of nutrition in improving quality of care: an interdisciplinary call to action to address adult hospital malnutrition. J Parenter Enteral Nutr. 2013;37(4):482-97. http://dx.doi.org/10.1177/0148607113484066 PMid:23736864

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):428-434.
http://dx.doi.org/10.3889/oamjms.2016.091
eISSN: 1857-9655
Clinical Science

Clinical, Laboratory and Radiographic Features of Patients with Pneumonia and Parapneumonic Effusions

Sanja Petrusevska-Marinkovic[1*], Irena Kondova-Topuzovska[1], Zvonko Milenkovic[1], Goran Kondov[2], Ankica Anastasovska[1]

[1]University Infectious Diseases Clinic, Medical Faculty, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia; [2]University Thoracocardiovascular Surgery Clinic, Medical Faculty, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia

Citation: Petrusevska-Marinkovic S, Kondova-Topuzovska I, Milenkovic Z, Kondov G, Anastasovska A. Clinical, Laboratory and Radiographic Features of Patients with Pneumonia and Parapneumonic Effusions. Open Access Maced J Med Sci. 2016 Sep 15; 4(3):428-434. http://dx.doi.org/10.3889/oamjms.2016.091

Keywords: community-acquired pneumonia (CAP); parapneumonic effusion; empyema; clinical features; laboratory features; radiographic features.

***Correspondence:** Sanja Petrusevska Marinkovic, MD. University Infectious Diseases Clinic, Medical Faculty, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia. Tel.: 00389 72 206 661. E-mail: sanjapm@yahoo.com

Received: 22-Jul-2016; **Revised:** 08-Aug-2016; **Accepted:** 22-Aug-2016; **Online first:** 23-Aug-2016

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: Parapneumonic effusions complicating pneumonia are associated with increased morbidity and mortality.

AIM: To determine the role of the clinical, laboratory and radiographic features to the differential diagnosis of patients with community- acquired pneumonia (CAP) without effusion, uncomplicated parapneumonic effusion (UCPPE) and complicated parapneumonic effusion (CPPE).

MATERIAL AND METHODS: We analysed 148 patients with CAP without effusion, 50 with UCPPE and 44 with CPPE. In three groups of patients, the majority was male patients (58.11%, 58%, 61.36%) consequently.

RESULTS: The chronic heart failure was the most common comorbidity in a group with CAP (28; 18.92%) and UCPPE (7; 14%), alcoholism (12;12.77%) in a group with CPPE. Patients with CPPE had significantly longer fever compared to patients with CAP without effusion (p = 0.003). Pleuritic chest pain (86.36%) and dyspnea (88.64%) were the most common symptoms in CPPE, then to group with UCPPE (60%; 52%), and in CAP without effusion (25.68%; 47,97%). Diffuse pulmonary changes were detected more frequently in the group with CAP without effusion compared with the group with CPPE (64.86 % vs. 27.27 %), while the segment lung changes were more common in patients with CPPE (50% vs. 20.27%). Patients with CPPE were significant with higher erythrocytes sedimentation rate (ESR), white blood cells (WBC) and serum C- reactive protein (CRP) than it the other two groups (p = 0.00090, p = 0.01, p= 0.000065).

CONCLUSION: Proper analysis of clinical, laboratory and radiographic features of patients with CAP and parapneumonic effusion can prevent mismanagement in these patients and will reduce morbidity and mortality.

Introduction

Pleural effusion represents a common complication of community- acquired pneumonia. Parapneumonic effusions occur in 20- 40% of patients who are hospitalised with pneumonia [1]. The mortality rate in patients with parapneumonic effusions is higher than that in patients with pneumonia without parapneumonic effusion [1]. Some of the excess mortality is due to the mismanagement of pneumonia and parapneumonic effusion [1, 2]. The clinical classification of parapneumonic effusions identifies three groups with a distinct prognosis [1-3]. First, there is the simple or uncomplicated parapneumonic effusion (UCPPE), which should resolve with appropriate antibiotic therapy without any consequences within the pleural space. Second, there is a complicated parapneumonic effusion (CPPE) denoting the presence of bacterial infection in the pleural space with an associated inflammatory response. A complicated effusion sometimes requires pleural drainage to resolve and without treatment may progress to an empyema [1-4]. CPPE occurs in 10% of all patients hospitalised with effusion [4].

Finally, empyema represents frank pus in the pleural space requires pleural drainage and may also require surgical treatment [3-5]. About 60% of empyemas are related to a primary pneumonic

process; therefore risk factors for pleural infection are similar to those for pneumonia [1, 6]. Independent risk factors for the development of empyema include diabetes, alcohol and intravenous abuse, immunosuppression, gastro- oesophagal reflux disease, aspiration and poor oral hygiene [4, 7, 8]. After a variable time interval, pleural infection enters an organizing' stage characterised by fibroblast proliferation and the development of solid fibrous peel. This inhibits lung reexpansion and usually necessitates surgical thoracotomy and decortication [7, 8].

There is considerable variation in the course and aggressiveness of parapneumonic effusions; therefore an understanding of its progression is important [4]. Along out with increased mortality, complicated parapneumonic effusion and empyema often necessitate prolonged treatment, longer hospital stay and interventions. Thus, identification of these patients and prompt management is critical.

Recent studies have shown that relying on clinical findings alone do not predict the clinical course of illness in individual patients [9-11]. The dilemma of differentiating between this three diagnosis, using history, physical examination, radiographic findings and markers of inflammation, is challenging [9-11].

In this study, we want to see the possibility of using standard examinations that are available in daily practice to prevent possible complications and to understand the relationship of parapneumonic effusion with pneumonia.

Material and Methods

Of 1059 patients with CAP, 304 patients were excluded from the study according to the criteria for exclusion, and because it had realised ultrasound of the pleura and lung as a more sensitive method for the diagnosis of parapneumonic effusion of lung X-ray. Excluding criteria were: cancer and malignant effusion, hospital- acquired pneumonia, solid organ transplanting, transudate effusion, vasculitis, pulmonary emboli (PE), pulmonary tuberculosis and age less than 18 years. From 755 patients, 175 (23.18%), had parapneumonic effusion. Thoracentesis was performed in 94 (53.71%) patients, 50 patients were with uncomplicated parapneumonic effusions (UCPPE) and 44 with complicated parapneumonic effusions (CPPE). Of 580 patients with CAP without effusion, we analysed 148 patients with CAP without effusion because other patients did not have all the necessary laboratory and radiographic parameters to be analysed in this study. The patients were diagnosed and treated in University Infectious Diseases Clinic, Faculty of Medicine, Skopje in the Department of Respiratory Diseases in the period from September 2011 to June 2015.

The demographic characteristics, physical examination findings, laboratory and microbiological findings of all study participants were monitored regularly on University Infectious Diseases Clinic. Initial lung X- rays were taken for all patients at the Institute of Radiology, Medical Faculty in Skopje. After admission, all the patients underwent an ultrasound of the pleura and lung with a three- dimensional echo at the University Infectious Diseases Clinic for diagnosis of pleural effusions and implementation of diagnostic thoracocentesis if the size of effusion was more than 10 mm. After verification of pneumonia and pleural effusion, the distinction between transudation and exudates was done according to Light's criteria. Exudative pleural effusion is one that meets at least one of the criteria of Light. The transudate if the effusion that meets all three criteria at the same time: 1) to have intercourse protein p/s below 0.5; 2) intercourse LDH p/s below 0.6, and 3) LDH in the pleural fluid under 282 U/L, which is the lowest limit in our laboratory. Than exudative pleural effusion according to their evolution and on the basis of pH, glucose and LDH value in the pleural fluid are division: - Uncomplicated parapneumonic effusions: pH > 7.2, glucose >60 mg/dl, LDH< 1000UI/ml; - Complicated parapneumonic effusions: pH<7.2, glucose <60 mg/dl, LDH>/= 1000 UI/ml.

ERS was determined in all three groups of patients at admission on a clinic. ERS measures the distance through which erythrocytes fall within 1 hour in a vertical tube of anticoagulated blood, and is measured in millimetres/hour. The blood is drawn into a vertical tube anticoagulated with sodium citrate. Leukocyte count (WBC) was also determined in the clinic laboratory by the white blood cell counter (number per microliter).The amount of protein is determined in the same biochemical laboratory with the standard method. C- reactive protein (CRP) was measured by quantitative methods in biochemistry laboratory of the same clinic with quantitative sandwich enzyme heterogeneous test, Ektahem Clinical Chemistry test, an automated biochemical analyser Vitros 250.

Statistical analysis

Statistical analysis was conducted using SPSS 17 for Windows. The testing of normality in the distribution of the data was used Kolmogorov-Smirnov and Shapiro-Wilk's W tests. Categorical traits of displayed by absolute and relative representation with quantitative traits mean SD, median, minimum, maximum, 25-75 percentiles. To compare three groups of subjects in relation to the variables analysed were used Kruskal-Wallis ANOVA and Mann-Whitney U тест (Z). The level of significance or importance was taken the value of $p < 0.05$, a significantly higher value of $p < 0.01$.

Results

In three groups of patients, majority was male patients (58.11%, 58%, and 61.36% consequently). It was insignificant the difference in the distribution of patients with CAP without effusion, UCPPE and CPPE in terms of their sex (p = 0.9). The average age of patients only with CAP was 54.58 ± 17.5 years, in UCPPE 55.5 ± 16.6 and in a group with CPPE was 51.91 ± 18.4 and there was no statistical difference (p = 0.58) (Table 1).

Table 1: Demographic characteristics of three groups of patients

Variable	CAP N = 148	UCPPE N = 50	CPPE N = 44	p value
Sex n (%)				
Male	86 (58.11)	29 (58)	27 (61.36)	[a]p = 0.9
Female	62 (41.89)	21 (42)	17 (38.64)	
Age (years) mean ± SD, min-max				
	54.58 ± 17.5	55.5 ± 16.6	51.91 ± 18.4	[b]p = 0.58
	18-89	21-83	18-93	

[a]p (Chi-square test); [b]p (Analysis of variance).

Analysis of the results regarding the presence of comorbidity showed a significant difference between the three groups of subjects (p = 0.028). Usually with accompanying diseases were CPPE patients (77%), compared with patients with UCPPE (56%) and CAP without effusion (52.7%).

The chronic heart failure was the most common comorbidity in a group with CAP (28; 18.92%) and UCPPE(7; 14%), alcoholism (12;12.77%) in a group with CPPE. The structure of morbidity among the three groups of respondents is presented in Table 2.

Table 2: Comorbidity of the three patient groups

Type of comorbidity n (%)	CAP without effusion N= 78 (52.7%)	UCPPE N=28 (56%)	CPPE N=34 (77%)
COPD	8 (5.41)	2 (4)	2 (4.55)
Chronic heart failure	28 (18.92)	7 (14)	2 (4.55)
Diabetes melitus	13 (8.78)	7 (14)	3 (6.82)
Chronic liver disease	2 (1.35)	1 (2)	1 (2.27)
Renal failure	2 (1.35)	1 (2)	0
Alcoholism	4 (2.7)	1 (2)	11 (25)
Malignancy	3 (2.03)	4 (8)	2 (4.55)
Chronic systemic disease	3 (2.03)	1 (2)	2 (4.55)
Poor dental hygiene	0	0	2 (4.55)
Drug users	0	0	2 (4.55)
Neurological disease	3 (2.03)	0	2 (4.55)
Haematological disease	2 (1.35)	0	0
Two or three comorbidities	35 (23.65)	3 (6)	5 (11.36)
Other diseases	7 (4.73)	1 (2)	0

The three groups of participants significantly differ in terms of frequency of occurrence of dyspnea (p = 0.000009). Very high percentage of patients with CPPE had dyspnea (88.64 %), despite the significantly lower percentage of patients with CAP without effusion (47.97%), and significantly lower percent patients with UCPPE (52%). Pleuritic chest pain (86.36%) was the most common symptom and significantly more often in a group with CPPE, then to group with UCPPE (60%), and group with CAP without effusion (25.68%) (Table 3).

Table 3: Clinical characteristics of the three groups of patients

Variable	CAP without effusion N = 148 N (%)	UCPPE N = 50 N (%)	CPPE N = 44 N (%)	p-value
	1	2	3	
Fever	107 (72.3)	30 (60)	29 (65.91)	[a]p = 0.2
Catarrhal symptoms	51 (34.46)	16 (32)	11 (25)	[a]p = 0.5
Sore throat	61 (41.22)	15 (30)	10 (22.73)	[a]p = 0.05
Hyperemia of the tonsils and pharynx	78 (52.7)	21 (42)	19 (43.18)	[a]p = 0.3
Cough type				
Productive	43 (29.05)	12 (24)	20 (45.45)	[a]p = 0.049*
Serous sputum	29 (19.59)	15 (30)	3 (6.82)	2vs3 p = 0.005**
Haemoptisis	14 (9.46)	2 (4)	6 (13.64)	
Dry cough	40 (27.03)	15 (30)	7 (15.91)	
Without cough	22 (14.86)	6 (12)	8 (18.18)	
Headache	95 (64.19)	33 (66)	23 (52.27)	[a]p = 0.3
Malaise	138 (93.24)	45 (90)	44 (100)	2vs3 [a]p = 0.038*
Myalgia	81 (54.73)	23 (46)	24 (54.55)	[a]p = 0.5
Arthralgia	66 (44.59)	19 (38)	17 (38.64)	[a]p = 0.6
Weight loss	76 (51.35)	30 (60)	38 (86.36)	[a]p = 0.0002** 1vs3 p = 0.00003** 2vs3 p = 0.004**
Dispnea	71 (47.97)	26 (52)	39 (88.64)	[a]p = 0.000009** 1vs3 p = 0.00002** 2vs3 p = 0.0001**
Days of disease before hospitalisation				
1 – 4 days	62 (41.89)	23 (46)	9 (20.45)	[a]p < 0.001
5 – 10 days	70 (47.3)	20 (40)	21 (47.73)	1vs3 p = 0.00006
> 10 days	16 (10.81)	7 (14)	14 (31.82)	1vs3 p = 0.001** 2vs3 p = 0.017*
Days of disease before hospitalisation (mean ± SD) median (25-75[th]quartiles)				
	6.39 ± 6.0	6.24 ± 4.7	10.59 ± 8.8	[c]p = 0.0002**
	5 (3-7)	4 (3-7)	10 (5-13.5)	1vs3 p = 0.00006**
Length of hospitalisation (mean±SD) median (25-75[th]quartiles)				
	12.39 ± 2.7	15.78 ± 4.3	20.75 ± 18	[c]p < 0.0001
	12 (10-14)	15 (13-16)	21(18-23)	1vs2 p < 0.0001 1vs3 p < 0.0001

[a]p (Chi-square test); [c]p (Kruskal-Wallis test); *p < 0.05; **p < 0.01.

Participants from all three groups had a significantly different duration of disease symptoms before hospitalisation (p = 0.0002). This difference was due to a significantly longer duration of symptoms in the group with CPPE compared with the group wit CAP without effusion (p = 0.00006).

The length of hospitalisation significantly differed among the three groups of patients (p < 0.0001). The average time of hospital days shows that half of the patients with CPPE were in the hospital more than 21 days, half of the patients with CAP without effusion were hospitalised more than 12 days, 50% of patients with UCPPE more than 15 days. The results are shown in Table 3.

The values of body temperature before hospitalisation insignificant differed between patients of the three groups (p = 0.07). Again along the duration of fever before hospitalisation was significant among the three groups of subjects (p = 0.0006).

In patients with CPPE average period of the fever duration during hospitalisation is 4 days, in a group with CAP without effusion is 2 days, and in patients with UCPPE is 3 days. Average value of temperature during hospital treatment in patients with CPPE was 39^0C (38.25-39.8^{0}C), in patients with UCPPE was 38.4^0C (37.5-39.6^{0}C) and the lowest values were noted in the patient with CAP without effusion 38.3^0C (37.4-39.2^{0}C) (Table 4).

Table 4: Value and duration of temperature in the three patients groups

Variable	CAP without effusion N = 148	UCPPE N = 50	CPPE N = 44	p-value
	1	2	3	
Values of temperatures before hospitalisation, °C [n (%)]				
< 37.1°C	8 (5.41)	0	0	
37.1 – 37.7°C	7 (4.73)	9 (18)	1 (2.27)	
37.8 – 39°C	70 (47.3)	24 (48)	24 (54.55)	
39.1 – 40°C	39 (26.35)	14 (28)	4 (9.09)	
40°C >	24 (16.22)	3 (6)	15 (34.09)	
Values of temperatures before hospitalisation, (mean ± SD) median (25-75thquartiles)				
	38.81 ± 3.1	38.87 ± 0.9	39.37 ± 1.0	^{c}p = 0.07
	39 (38.5-40)	39 (38.3-39.8)	39 (38.5-40.5)	
Duration of temperatures before hospitalisation (days) (mean ± SD) median (25-75thquartiles)				
	4.36 ± 4.2	4.0 ± 2.9	7.45 ± 6.4	^{c}p = 0.0006**
	4 (2-5.5)	3.5 (3-5)	6 (3-10)	1вс3 p = 0.0003**
				2вс3 p = 0.001*
Duration of temperatures during hospitalisation, °C (days) [n (%)]				
Without fever	92 (62.16)	18 (36)	20 (45.45)	
1 – 2 days	22 (14.86)	18 (36)	6 (13.64)	
3 – 4 days	13 (8.78)	11 (22)	13 (29.55)	
5 – 7 days	9 (6.08)	0	5 (11.36)	
> 7 days	12 (8.11)	3 (6)	0	
Duration of temperatures during hospitalisation (days) mean ± SD median (25-75thquartiles)				
	2.7 ± 3.1	3.3 ± 2.0	4.77 ± 5.0	^{c}p = 0.0005**
	2 (1-3)	3 (2-4)	4 (1.5-5)	1вс3 p = 0.003**
Values of temperatures during hospitalisation, °C [n (%)]				
< 37.1°C	23 (15.54)	6 (12)	1 (2.27)	
37.1 – 37.7°C	27 (18.24)	9 (18)	7 (15.91)	
37.8 – 39°C	53 (35.81)	17 (34)	15 (34.09)	
39.1 – 40°C	41 (27.7)	17 (34)	17 (38.64)	
40°C >	4 (2.7)	1 (2)	4 (9.09)	
Values of temperatures during hospitalisation mean ± SD median (25-75thquartiles)				
	38.38 ± 1.1	38.55 ± 1.1	38.9 ± 1.1	^{c}p = 0.02*
	38.3 (37.4-39.2)	38.4 (37.5-39.6)	39(38.25-39.8)	1вс3 p = 0.006**

^{c}p (Kruskal-Wallis test); *p < 0.05; **p < 0.01.

In the admission to the clinic, patients with CAP without effusion, with UCPPE and CPPE were significantly different ERS rate (p = 0.00090). Patients with CPPE had average ERS in the first hour of 74.77 ± 27.3 mm/h and is significantly higher than the average value in the group with pneumonia without effusion (56.35 ± 28.6), and the average value of the group with UCPPE (60.02 ± 27.7). In the admission, WBC was significantly higher in the group with CPPE versus the group with CAP without effusion (median 12.6 vs. 10.6, p = 0.01). Normal values of WBC had more patients with CAP without effusion (21.62 % vs. 34.09 %), while the values of WBC higher than 20x10^{9}/L had more patients with CPPE (25% vs. 10.14%). The values of the inflammatory marker CRP at the admission and discharge on the clinic significantly dependent on the form of manifestation of community-acquired pneumonia (CAP) or its manifestation without effusion, with UCPPE or CPPE (p < 0.0001 and p = 0.04 respectively). The mean values of CRP (231.79 ± 112.2 mg/L) were significantly higher in the group with CPPE compared with the group wit CAP without effusion (139.48 ± 105.7 mg/L) (p = 0.000004), and compared with the group with UCPPE (163.8 ± 147.9 mg/L) (p = 0.000065) (Table 5).

Radiographic findings regarding the type of infiltration were significantly different between patients with CAP without effusion and CPPE (p = 0.00019), and between the two patient groups with effusion (p = 0.035). Alveolar infiltrates often had as radiographic finding patients with CPPE (65.91 %). Interstitial infiltration was the most common radiographic finding in the group with CAP without effusion (14.86 %), this finding was no patient with CPPE, and the mixed finding was most common in the group with CAP without effusion (51.35%).

Table 5: Markers of inflammation in the three clinical groups of patients

Variable	CAP N = 148	UCPPE N = 50	CPPE N = 44	p value
	1	2	3	
ERS mm/h - admission n (%)				
≤ 20	20 (13.51)	4 (8)	2 (4.55)	
21 – 40	36 (24.32)	8 (16)	6 (13.64)	
41 – 60	37 (25)	19 (38)	8 (18.18)	
61 – 100	52 (35.14)	17 (34)	24 (54.55)	
> 100	3 (2.03)	2 (4)	4 (9.09)	
ERS mm/h mean ± SD median (25-75thquartiles)				
Admission	56.35 ± 28.6	60.02 ± 27.7	74.77 ± 27.3	^{b}p = 0.0009**
	60 (33.5-80)	55 (45-80)	76.5 (60-97)	1вс3 p = 0.0004**
Dischagre	34.8 ± 24.0	36.12 ± 22.2	41.73 ± 26.8	2вс3 p = 0.03*
	31 (11-50)	40 (15-52)	40 (20-60)	^{c}p = 0.3
WBC x 10^{9}/L n (%)				
≤ 4	13 (8.78)	3 (6)	0	
4 – 9	48 (32.43)	15 (30)	7 (15.91)	
9.1 – 12	32 (21.62)	14 (28)	15 (34.09)	
12.1 – 15	26 (17.57)	3 (6)	6 (13.64)	
15.1 – 20	14 (9.46)	10 (20)	5 (11.36)	
> 20	15 (10.14)	5 (10)	11 (25)	
WBC x 10^{9}/L mean ± SD median (25-75thquartiles)				
Admission	11.56 ± 6.8	12.19 ± 6.0	14.68 ± 6.6	^{c}p = 0.013*
	10.6 (6.4-14.1)	11.1 (8.1-16.7)	12.6 (10.2-20.2)	1вс3 ^{d}p = 0.01*
Dischagre	7.91 ± 2.6	7.26 ± 1.9	7.62 ± 2.8	^{b}p = 0.26
	7.6 (6.2-9.3)	7.5 (5.8-8.6)	6.8 (6-8.8)	
CRP serum means ± SD median (25-75thquartiles)				
Admission	139.48 ± 105.7	163.8 ± 147.9	231.79± 112.2	^{c}p < 0.0001
	120 (48-228)	120.5 (63-204)	218 (162.5-313)	1вс3 ^{d}p = 0.000004**
Dischagre	13.82 ± 22.9	12.6 ± 16.1	32.43 ± 47.3	2вс3 ^{d}p = 0.00028**
	6 (3-15)	7 (3-14)	17 (4.5-33.5)	^{c}p = 0.04*
				1вс3 ^{d}p = 0.014*

^{b}p (Analisys of variance); ^{c}p (Kruskal-Wallis test); ^{d}p (Mann-Whitney test); *p < 0.05; **p < 0.01.

Regarding the other two radiographic parameters analysed, distribution by extensiveness and prevalence of pulmonary changes, comparative analysis of the results confirmed the only significant difference between the group with CPPE and the group with CAP without effusion (p = 0.0001, p = 0.00005). Diffuse pulmonary changes were detected more frequently in the group with CAP without effusion compared with the group with CPPE (64.86% vs. 27.27%), while the segment distribution was more common finding in the group with CPPE (50 % vs. stands at 20.27%).

Unilateral localization of the inflammatory process was common finding in the group with CPPE compared with the group with CAP without effusion (79.55% vs. 44.59%), while patients with CAP without effusion were more likely than those with CPPE had radiographic finding of both lungs (55.41% vs. 20:45%) (Table 6).

Discussion

Pneumonia can be complicated by the development of parapneumonic effusion, which has increased morbidity and mortality. Aside from inflammation in the lung and pleural space from direct

invasion of bacteria and bacteriologic virulence features contributing to parapneumonic effusion, patient's factors and comorbidities also contribute to the pathophysiology of parapneumonic effusion development.

Table 6: Radiographic characteristics of the three groups of patients

Variable	CAP without effusion N = 148	UCPPE N = 50	CPPE N = 44	p-value
	1	2	3	
Type of pulmonary infiltrates n (%)				
Alveolar infiltrates	50 (33.78)	22 (44)	29 (65.91)	[a]p = 0.0012** 1vs3 p = 0.00019**
Interstitial infiltrate	22 (14.86)	4 (8)	0	2vs3 p = 0.035*
Mixed (alveo-interstitial infiltrate)	76 (51.35)	24 (48)	15 (34.09)	
Distribution according to infiltrate extensiveness n (%)				
Diffuse	96 (64.86)	25 (50)	12 (27.27)	[a]p = 0.0007**
Multilocular	3 (2.03)	1 (2)	2 (4.55)	1vs3 p = 0.0001**
Lobar	19 (12.84)	11 (22)	8 (18.18)	
Segmental	30 (20.27)	13 (26)	22 (50)	
Distribution of changes in lung n (%)				
Unilaterally	66 (44.59)	32 (64)	35 (79.55)	[a]p = 0.00008**
Bilaterally	82 (55.41)	18 (36)	9 (20.45)	1vs3 p = 0.00005**

[a]p (Chi-square test); [c]p (Kruskal-Wallis test); [d]p (Mann-Whitney test); *p < 0.05; **p < 0.01.

A recent study [11] analysed 4715 patients with CAP and 882 (19%) had pleural effusion, of which 261 (30%) had complicated parapneumonic effusion or empyema. In this study a multivariable analysis, no single baseline patient's characteristics distinguished patients without pleural effusion from those with uncomplicated parapneumonic effusion. However, five independent baseline characteristics could predict the development of complicated parapneumonic effusion or empyema: age < 60 years old, alcoholism, pleural pain, tachycardia and leucocytosis. These investigators and others have found a reduced prevalence of clinical manifestation in older patients, suggesting a possible age-related change in the immune response [11-13]. In our study patients with complicated parapneumonic effusion are younger compared with patients with CAP without effusion and those with UCPPE but there is no significant statistical difference regardless of age.

But when it comes to comorbidities, patients with complicated parapneumonic effusion have more comorbidities of patients with CAP without effusion and patients with UCPPE. As in the previously mentioned Falguera's study, and in Chalmer's study realised in 2011 [10], in our study also alcoholism was the most common comorbidities which were noted in patients with CPPE, then followed diabetes mellitus. In patients with CAP without effusion and patients with UCPPE most common accompanied disease in this research is chronic heart failure [4, 14]. Having more than one comorbidity proved significant in our study in the development of CPPE [14].

In an earlier study as the most common comorbidity when it comes to CPPE were reported diabetes mellitus, malignancy, and then alcoholism, cardiovascular disease, liver cirrhosis and immunosuppressive states (HIV infection) [5, 12, 15, 16]. In Chapman's study from 2004 [7] and Finish study from 2014 [3], it is generally accepted that diabetes mellitus increases susceptibility to infection and diabetes is typically included in the list for pleural infection and empyema. Perhaps the explanation for alcoholism, like as a significant risk factor is the existence of anaerobic infections in these patients, is associated with poor dental hygiene and aspiration. CPPE and empyema occur commonly, however, in the absence of any identifiable risk factors [7].

In daily practice, medical doctors first encounter with the clinical features of patients. Unfortunately, the symptoms of pneumonia involving parapneumonic effusion or empyema (i.e fever, malaise, cough, dyspnea and pleural chest pain) are similar to those of pneumonia without a parapneumonic effusion [1, 2, 7]. The symptoms with a parapneumonic effusion can be either acute or chronic [1, 7, 17]. Anaerobic pulmonary infections frequently have an associated pleural effusion and are characterised by a more chronic course [1, 2, 11, 17]. Weight loss and anaemia are common with anaerobic infection [1, 7]. Similarly, if the patients have a parapneumonic effusion, the clinical picture is similar whether or not the effusion is complicated [1].

Very high percentage of patients with CPPE had dyspnea (88.64%), despite the significantly lower percentage of patients with CAP without effusion (47.97%), and significantly lower percent patients with UCPPE (52%). Pleuritic chest pain (86.36%) was the most common symptom and significantly more often in a group with CPPE, then to group with UCPPE (60%), and group with CAP without effusion (25.68%). Weight loss is statistically significantly more frequent in patients with CPPE (86.36%) than in patients with UCPPE (60%), and patients with pneumonia without effusion (51.35%). These results correlate with findings from two major studies of Chalmers and Falguera that pleural chest pain correlated with other factors (clinical, laboratory and microbiological) can predict the development of complicated parapneumonic effusion or empyema [10, 11]. But, the absence of pleural chest pain does not exclude pleural infection [18].

Our results show that significantly different duration of disease symptoms before hospitalisation (p = 0.0002). This difference was due to a significantly longer duration of symptoms in the group with CPPE compared with the group with CAP without effusion (p = 0.00006). The length of illness > 5 days is more common in patients with CPPE, and we found the average length of illness of 10 days (5-13.5 days) in our patients with CPPE [11]. The length of hospitalisation significantly differed among the three groups of patients (p < 0.0001). The average time of hospital days shows that half of the patients with CPPE were in the hospital more than 21 days, half of the patients with CAP without effusion were

hospitalised more than 12 days, 50 % of patients with UCPPE more than 15 days. Significantly different length of hospitalisation was reported in several studies [1, 2, 7, 10, 11, 17, 18]. This is due to shortcomings in the management of patients with pneumonia and UCPPE. Early antibiotic treatment will prevent the development of UCPPE and progression to CPPE and empyema. In that case, effusion is associated with antimicrobial treatment failure [1-3], prolonged systematic sepsis, increased the length of hospital stay [2], higher financial costs [2] greater morbidity from invasive procedures [17, 18] and greater mortality [1-3, 7, 10, 11].

We found and that duration of elevated temperature before and after hospitalisation in patients with CPPE significantly different from the length and duration of the temperature in the remaining two groups of patients. Average value of temperature during hospital treatment in patients with CPPE was 39^0C (38.25-39.8^0C) in patients with UCPPE was 38.4^0C (37.5-39.6^0C) and the lowest values were noted in the patient with CAP without effusion 38.3^0C (37.4-39.2^0C). This suggests that the temperature as a clinical marker of inflammation is higher and longer lasting in patients with complicated effusion. A temperature that persists in patients with pneumonia suggests that the inflammatory process takes the adverse course and complicating [19-21].

We decided to see if the standard markers of inflammation, like ERS and WBC, which are widely used in everyday practice, may indicate what course shall take and whether pneumonia complicated by the development of effusion. WBC has an important role in the inflammatory response to infection and the release of pro- inflammatory cytokines [21]. In our study, there were significantly higher values of WBC in patients with effusion especially in patients with CPPE like in study of Kruger and associates [21]. We found that higher value of WBC than 20 x 10^9 at admission at the clinic are meet in 25% of patients with CPPE because the patients with CPPE have a more severe clinical picture unlike patients with CAP without effusion and patients with UCPPE [1, 8].

Patients with CPPE had average ERS in the first hour of 74.77 ± 27.3 mm/h and is significantly higher than the average value in the group with pneumonia without effusion (56.35 ± 28.6 mm/h), and the average value of the group with UCPPE (60.02 ± 27.7 mm/h). Our result is the same as in growing number of studies, but just like WBC and ESR is a nonspecific marker that indicates not only the severity of the infection but increases in other types of inflammation [22, 23]. ERS is helpful in monitoring chronic inflammatory condition but does not benefit at monitoring responses to therapy in acute inflammatory conditions, such as acute infections [22].

Unlike the previous two markers CRP is more beneficial in acute infections Our results show that the mean values of CRP (231.79 ± 112.2 mg/L) were significantly higher in the group with CPPE compared with the group with UCPPE (163.8 ± 147.9 mg/L) (p = 0.000065), and especially compared with the group with CAP without effusion (139.48 ± 105.7 mg/L) (p = 0.000004). Chalmers et al have reported an association of a low CRP level of >= 100 mg/l at the time of hospital admission with a reduced risk of 30-day mortality, need for mechanical ventilation and/or inotropic support, and complicated pneumonia [24]. Similar results are and Mira in their study published 2008 in a group of patients hospitalised in intensive care unit [25].

Eisenhut's study from 2008 suggests that a persistently elevated or rising CRP level in a patients on the ICU should, therefore, alert the clinician not only to a potentially poor prognosis but also prompt a reassessment of the patients with a chest X- ray and chest ultrasound for the presence of an empyema that may require surgical evacuation [26]. Alveolar infiltrates with segment distribution and unilateral distribution of changes in the lung are associated with complicated parapneumonic effusion in the conducted study. Unlike them in patients with pneumonia usually meet mixed (also- interstitial infiltrate), according to infiltrate extensiveness diffuse distribution, and bilaterally distribution of changes in the lung.

If you know that about 200 ml of pleural fluid is detectable on PA chest radiography whereas only 50 ml fluid is detectable on a lateral film [8]. Chest radiography with lateral decubitus film in everyday practice is not realised. Very often there is a pleural effusion that classical chest radiography not visualises.

Ultrasound is more accurate and more sensitive for estimable pleural fluid volume and aids thoracocentesis [8, 27, 28]. Ultrasound is also useful in showing separation and echogenicity (correlating with an exudate) and differentiates between the pleural fluid and thickening [8, 27, 28]. It is portable and position flexible [8]. So when we see segmental or lobar pneumonia without recording the effusion is necessary to consider and such a complication and make lung ultrasound.

We can summarise that if we have a patient aged 60 years with a fever that lasts longer, elevated leukocyte, persisting elevated CRP and radiographic findings of segmental or lobar pneumonia should suspect to a parapneumonic effusion as a possible complication, especially CPPE.

In conclusion, perhaps our study will help to clarify some contradictions that are associated with pneumonia and development of parapneumonic effusions, especially CPPE. But, proper analysis of clinical, laboratory and radiographic features, together, of patients with CAP, UCPPE and CPPE to prevent mismanagement of these patients, will reduce morbidity and mortality and help to define new diagnostic and therapeutic approaches.

Refferences

1. Light RW. Parapneumonic effusions and empiema. Proc Am Thorac Soc. 2006;3(1):75-80. http://dx.doi.org/10.1513/pats.200510-113JH PMid:16493154

2. Lim TK. Management of parapneumonic pleural effusion. Curr Opin In Pulm Med. 2001;7: 193-197. http://dx.doi.org/10.1097/00063198-200107000-00005

3. Finich S, Chalmers JD. Parapneumonic effusions: epidemiology and predictors of pleural infection. Curr Respir Care Rep. 2014;3:52-60. http://dx.doi.org/10.1007/s13665-014-0074-4

4. Mc Cauley L, Dean N. Pneumonia and empiema: causal, causal or unknown. J Thorac Dis. 2015;7(6):992-998.

5. Koegelenberg CFN, Diacon AH, Bolliger CT. Parapneumonic pleural effusin and empyema. Respiration. 2008;75:241-250. http://dx.doi.org/10.1159/000117172 PMid:18367849

6. Smith JA, Mulleroworth MH, Westlake GW, et al. Empyema thoracis: 14- year expirience in a teaching center. Ann Thorac Surg. 1991;51:39-42. http://dx.doi.org/10.1016/0003-4975(91)90443-T

7. Chapman SJ, Davies RJO. The management of pleural space infection. Respirology. 2004;9:4-11. http://dx.doi.org/10.1111/j.1440-1843.2003.00535.x PMid:14982595

8. Medford AR, Maskell N. Pleural effusion. Postgrad Med J. 2005;81:702e10.

9. Falk G, Fahey T. C-reactive protein and community-acquired pneumonia in ambulatory care: systematic review of diagnostic accuracy studies. Fam Pract. 2009;26(1):10-21. http://dx.doi.org/10.1093/fampra/cmn095 PMid:19074757

10. Chalmers JD, Singanayagam A, Murray MP, et al. Risk factors for complicated parapneumonic effusion and empyema on presentation to hospital with community-acquired pneumonia. Thorax. 2009;64(7):592-7. http://dx.doi.org/10.1136/thx.2008.105080 PMid:19131449

11. Falguera M, Carratalà J, Bielsa S, et al. Predictive factors, microbiology and outcome of patients with parapneumonic effusion. Eur Respir J. 2011; 38(5):1173-9. http://dx.doi.org/10.1183/09031936.00000211 PMid:21565916

12. Hasley PB, Albaum MN, Li Y-H, et al. Do pulmonary radiographic findings at presentation predict mortality in patients with community acquired pneumonia ? Arch Intern Med. 1996;156:2206- 2212. http://dx.doi.org/10.1001/archinte.1996.00440180068008 PMid:8885819

13. Metlay JP, Schulz R, li YH, et al. Influence of age on symptoms at presentation in patents with community- acquired pneumonia. Arch Intern Med. 1997;157:1453-9. http://dx.doi.org/10.1001/archinte.1997.00440340089009 PMid:9224224

14. Finich S, Chalmers JD. Brief clinical review: Non- responding pneumonia. EMJ Respir. 2014;104-111.

15. Huang H-C, Chang H-Y, Chen C-W, et al. Predicting factors for outcome of tube thoracostomy in complicated parapneumonic effusion or empiema. Chest. 1999;115:751-756. http://dx.doi.org/10.1378/chest.115.3.751

16. Tsang KY, Leung WS, Chan VL, et al. Complicated parapneumonic effusion and empiema thoracis: microbiology and predictors of adverse outcomes. Hong Kong Med J. 2007; 13:178-86. PMid:17548905

17. Sahn SA. Diagnosis and management of parapneumonic effusions and empyema. Clinical Infectious Diseases. 2007;45:1480-6. http://dx.doi.org/10.1086/522996 PMid:17990232

18. Rosenstengel A, Gary Lee YC. Pleural infection- current diagnosis and management J Thorac Dis. 2012;4(2):186-193. PMid:22833824 PMCid:PMC3378219

19. McGrath EE Anderson PB. Diagnosis of pleural effusion: A systematic approach. American Journal of Critical Care. 2011;20:119-128. http://dx.doi.org/10.4037/ajcc2011685 PMid:21362716

20. Chapman SJ, Davies RJO. The management of pleural space infection. Curr Opin Pulm Med. 2004;10:299-304. http://dx.doi.org/10.1097/01.mcp.0000129755.79467.67 PMid:15220756

21. Girdhar A, Shujaat A, Bajwa A. Management of infectious processes of the pleural space: a review. Pulmonary Medicine. 2012; 2012:816502. http://dx.doi.org/10.1155/2012/816502 PMid:22536502 PMCid:PMC3317076

22. Krüger S, Ewig S, Papassotiriou J, et al. Inflammatory parameters predict etiologic patterns but do not allow for individual prediction of etiology in patients with CAP: results from the German competence network CAPNETZ. Respir Res. 2009;10:65. http://dx.doi.org/10.1186/1465-9921-10-65 PMid:19594893 PMCid:PMC2714042

23. Brigden ML. Clinical utility of the erythrocyte sedimentation rate. Am Fam Physician. 1999;60(5):1443-1450. PMid:10524488

24. Chalmers JD, Singanayagam A, Hill AT. C-reactive protein is an independent predictor of severity in community-acquired pneumonia. Am J Med. 2008;121(3):219-25. http://dx.doi.org/10.1016/j.amjmed.2007.10.033 PMid:18328306

25. Mira JP, Max A, Burgel PR. The role of biomarkers in community-acquired pneumonia: predicting mortality and response to adjunctive therapy. Crit Care. 2008;12(Suppl 6):S5. http://dx.doi.org/10.1186/cc7028 PMid:19105798 PMCid:PMC2607115

26. Eisenhut M. A persistently elevatd C- reactive protein level in pneumonia may indicate empiema. Critical Care. 2008;12:409. http://dx.doi.org/10.1186/cc6204 PMid:18341702 PMCid:PMC2374620

27. Tsai TH, Yang PC. Ultrasound in the diagnosis and management of pleural disease. Curr Opin Pulm Med. 2003;9(4):282-90. http://dx.doi.org/10.1097/00063198-200307000-00007

28. Yang PC, Luh KT, Chang DB, Wu HD, Yu CJ, Kuo SH. Value of sonography in determining the nature of pleural effusion: analysis of 320 cases. AJR Am J Roentgenol. 1992;159(1):29-33. http://dx.doi.org/10.2214/ajr.159.1.1609716 PMid:1609716

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):435-438.
http://dx.doi.org/10.3889/oamjms.2016.101
eISSN: 1857-9655
Clinical Science

Heart Failure Predictors in a Group of Patients with Myocardial Infarction

Sokol Myftiu[1], Petrit Bara[1], Ilir Sharka[1], Artan Shkoza[2], Xhina Belshi[1], Edlira Rruci[2], Gentian Vyshka[2*]

[1]Service of Cardiology, University Hospital Centre "Mother Theresa", Tirana, Albania; [2]Biomedical and Experimental Department, Faculty of Medicine, University of Medicine, Tirana, Albania

Citation: Myftiu S, Bara P, Sharka I, Shkoza A, Belshi X, Rruci E, Vyshka G. Heart Failure Predictors in a Group of Patients with Myocardial Infarction. Open Access Maced J Med Sci. 2016 Sep 15; 4(3):435-438. http://dx.doi.org/10.3889/oamjms.2016.101

Keywords: Heart Failure (HF); Acute Myocardial Infarction (AMI); ejection fraction (EF); epidemiology; prognosis.

***Correspondence:** Gentian Vyshka. Biomedical and Experimental Department, Faculty of Medicine, University of Medicine, Tirana, Albania. Tel: +355 69 7566130. E-mail: gvyshka@gmail.com

Received: 30-Jun-2016; **Revised:** 11-Aug-2016; **Accepted:** 27-Aug-2016; **Online first:** 06-Sep-2016

Competing Interests: The authors have declared that no competing interests exist.

Abbreviations: AMI – acute myocardial infarction, MI – myocardial infarction, BP – blood pressure, HF – heart failure, EF – ejection fraction, STEMI – ST-Segment Elevation Myocardial Infarction, AF – atrial fibrillation.

Abstract

AIM: The present study considers of the prevalence of heart failure (HF) in patients suffering from acute myocardial infarction (AMI) in the University Hospital Centre of Tirana (UHCT) "Mother Theresa"; the demographic and clinical characteristics of the sample during hospitalization; and the main predictors of heart failure occurrence inside the group of patients suffering an AMI.

MATERIAL AND METHODS: During a period of study from 2013-2015 we studied demographic and clinical data from 587 consecutive patients presenting with AMI; Framingham criteria were adopted for classifying patients with HF upon admission.

RESULTS: A Killip class ≥ 2 was the main diagnostic criterion of HF during hospitalisation. HF was identified in 156 patients (26.6%). The subgroup with HF had significant differences when compared with the other patients with regard to age, sex (male), heart rate upon admission, systolic blood pressure on admission, previous episodes of AMI, glycemia on admission, previous antihypertensive treatment, previous revascularization procedures, peripheral vascular disease, chronic renal disease, ejection fraction (EF), anemia, and atrial fibrillation presence. Independent predictors for HF occurrence in the logistic regression model were EF, previous revascularization, peripheral vascular disease, age, sex, previous AMI, systolic blood pressure upon admission, and anaemia.

CONCLUSION: As a conclusion, HF seems to be a common occurrence after AMI, in spite of changes in the epidemiological profile of the acute coronary syndrome. An increase in the incidence is registered as well, parallel to a decrease in the mortality following AMI. Attention must be shown for highly risked subpopulations, aged persons, patients with the previous coronary disease, and concomitant conditions.

Introduction

Heart failure (HF) represents a syndrome due to structural or functional heart disorders, hampering the ability of this organ to sufficiently pump the blood. HF will be accompanied with a shorter life expectancy, as well as other important symptoms such as dyspnea, fluid retention, fatigue and malaise, that will severely worsen the quality of life [1]. Coronary disease is recognised as the main cause of mortality, approximating 20% of all deaths due to cardiovascular conditions in Europe [2]. An improvement in the treatment of AMI, especially following reperfusion therapies, and the increased mean longevity have contributed to an increase of survivals following AMI; the same is valid for patients with HF generally [3-7]. Almost half of the newly diagnosed patients with HF ageing less than 75 years are due to coronary artery disease; and 60% of the patients with HF have a positive history for AMI [8, 9].

HF is a common complication in patients suffering AMI, causing higher costs of treatment and a poor prognosis. Systolic dysfunction of the left ventricle is the main cause of HF following an AMI. The reasons for HF development in the majority of patients following an AMI include the myocardial necrosis and the structural remodelling of the ventricle that pursues. The process of remodelling produces a rapid appearance in the immediate period post-AMI, with the process slowing down thereafter. Studies suggest a bimodal form of HF appearance; the first peak coincides with the hospitalisation, and the

second peak the fourth day after admission [10].

HF symptoms are variable, and hardly might one foresee those that lead toward a “chronic” HF. Different factors such as recurrent ischemia, the extent of myocardial necrosis, the so-called “stunned myocardium”, ventricle remodelling, mechanical complications and “hibernating myocardium”, will all play a role in the emergence of systolic ventricular dysfunction post AMI, be it in the setting of a HF or lacking the clinical picture of a HF. In a US study of 2002, it resulted that 20% of AMI patients showed the clinical picture of HF, with 9% having a later appearance [11]. A French study suggested that 38% of AMI patients had the signs of HF within the first five days after the infarction [12]. Several sources suggest a higher HF risk in aged people, patients positive for cardiac diseases and diabetes mellitus [13]. Predictors of an early developing of HF include aged patients, diabetes, previous cardiac symptoms; whereas predictors of a later developing HF include hypertensive disease, male sex, tachycardia and higher values of cardiac enzymes. It seems that HF is more frequent in anterior AMI when compared to other regions of the heart.

There is not much information with regard to the profile of the coronary disease, risk factors, treatment methods and their influence on the prognosis of an AMI [14]. In Albania, a transitional south-eastern European country, only a few studies have approached the issue. A previously published paper aimed towards the evaluation of the incidence, and of the predictors of HF, in a sample population of AMI patients [15].

The present study aimed to assess the incidence of HF developing in patients with AMI admitted and treated in the Coronary Intensive Care Unit of the “Mother Theresa” University Hospital Centre of Tirana, Albania.

Materials and Methods

We studied the demographics and clinical characteristics of the AMI patients during their admission, as well as the predictors of HF. The present is a prospective study, including the demographic and clinical data of 587 consecutive patients suffering from AMI, from January 2013 till May 2015. Framingham criteria were applied for diagnosing patients with HF upon admission, and a Killip class ≥ 2 was the diagnostic criterion of HF during the period of hospitalisation, I a three-week follow-up period. Valvular, non-ischemic cardiopathy was excluded from the study group.

The data collection included age, gender, educational status, body height and weight, blood pressure, type of myocardial infarction (with ST elevation or in absence of the latter; STEMI vs. NSTEMI [ST Segment Elevation Myocardial Infarction]), localisation, HF complications and the medical treatment applied. Glycemia and lipid profile were performed during fasting. A thorough patient history regarding other risk factors for coronary disease (smoking, hypertension, diabetes, and dyslipidemia) and previous positive history for AMI was collected as well.

The study protocol was approved by the National Committee of Medical Ethics (No. of the protocol 092).

The statistical analysis of discrete (qualitative) variables was performed through presenting the variables in percentage; continuous variables were presented as a mean value ± standard deviation. Groups were compared to each other with a χ^2 test for discrete variables, and t-test for continuous variables. A logistic regression model was used to determine independent predictors of the HF developing in AMI patients.

Results

HF was identified in 156 patients (26.6%) of the entire sample group of AMI patients. Epidemiological and clinical characteristics of the group are summarised in Table 1.

Table 1: Clinical and epidemiological characteristics of the patients recruited in the present study

Variable	HF present	HF absent	P value
Socio-demographic profile			
Number of patients	156	431	
Age, years	69.4 ± 11.2	61.7 ± 11.6	0.001
Sex, male, n (%)	97 (62.3)	355 (82.4)	< 0.0001
BMI, kg/m^2	26.4±3.7	26.7±2.8	0.381
Clinical findings on admission			
Heart rate on admission, bpm	80.16 ± 19.35	70.64 ± 11.13	< 0.001
Systolic BP on admission, mmHg	119.6 ± 30.5	132.9 ± 24.2	0.001
Blood glucose on admission, mg/dl	168.5 ± 83.2	143 ± 77.4	0.007
AMI location			
Anterior, n (%)	112 (71.8)	190 (44.1)	<0.0001
Inferior, n (%)	38 (24.4)	192 (44.5)	<0.0001
Presence of STEMI, n (%)	114 (73)	338 (78.4)	0.141
LVEF (%)	36.9 ± 9.1	57.2 ± 5.7	<0 .0001
Previous MI, n (%)	34 (21.8)	29 (6.7)	<0 .0001
Previous HTN treatment, n (%)	55 (35.3)	278 (64.5%)	0.0031
Previous MI, n (%)	34 (21.8)	29 (6.7)	<0 .0001
Previous PCI or CABG, n (%)	16 (10.3)	15 (3.5%)	0.012
Coronary risk factors			
Smoking, n (%)	74 (47.4)	196 (45.5%)	0.054
Diabetes, n (%)	52 (33.4)	142 (32.9)	0.114
Dyslipidemia, n (%)	75 (48.1)	207 (48)	0.987
Hypertension, n (%)	125 (80.1)	343 (79.6)	0.923
Co-morbidity			
Chronic renal disease, n (%)	19 (12.2)	17 (4)	0.005
Anemia, n (%)	29 (18.6)	34 (7.9)	0.003
Peripheral vascular disease, n (%)	18 (11.5)	15 (3.5)	0.005
AF occurrence, n (%)	13 (8.4)	8 (1.8)	0.003
In-hospital medication			
Beta blockers, n (%)	56 (35.9)	292 (67.7)	<0.0001
ACEI or ARB, n (%)	113 (72.4)	315 (73.1)	0.916
Digoxin, n (%)	7 (4.5)	4 (0.9)	0.036
Statins, n (%)	134 (85.9)	380 (88.2)	0.876
Diuretics, n (%)	127 (81.4)	31 (7.2)	<0.0001

Plus – minus values are means ±SD. HF – heart failure; BMI – body mass index; BP – blood pressure; MI – myocardial infarction; HTN – systemic arterial hypertension; PCI – percutaneous coronary intervention; CABG – coronary artery bypass surgery; LVEF – left ventricular ejection fraction; AMI – acute myocardial infarction; STEMI – ST-segment elevation myocardial infarction; AF – atrial fibrillation; ACEI – angiotensin-converting enzyme inhibitor; ARB – angiotensin receptor blocker.

The subgroup of patients with HF showed significant differences from other patients when compared with regard to age, gender (male), heart rate on admission, systolic BP on admission, previous MI, glycemia on admission, previous antihypertensive treatment, previous coronary revascularization procedures, peripheral vascular disease, chronic renal disease, ejection fraction, as well as anemia and for the presence of atrial fibrillation. No significant differences were met with regard to the presence of hypertension (p = 0.923), diabetes (p = 0.114), dyslipidemia (p = 0.987), presence of STEMI (p = 0.141), smoking habits (p = 0.054).

Table 2: Multivariate analysis of the predictors of heart failure, in a group of patients with acute myocardial infarction

Variable	OR (95% CI)	P value
Age [years]	1.83 (1.25 – 3.16)	0.002
Sex [male]	0.42 (0.23 – 0.76)	0.047
Glycemia on admission	1.72 (0.78 – 3.71)	0.211
Systolic BP [mmHg] on admission	0.87 (0.63 – 0.91)	0.032
Ejection Fraction	4.78 (1.73 – 13.03)	0.001
Previous MI	1.89 (1.12 – 4.03)	0.013
Chronic renal disease	1.79 (0.82 – 3.94)	0.0384
Peripheral vascular disease	2.14 (1.57 – 4.36)	0.027
Previous PCI or CABG	3.84 (1.34 – 11.62)	0.0016
Anemia	1.69 (1.37 – 2.85)	0.046

CI – confidence interval; BP – blood pressure; MI – myocardial infarction; OR – odds ratio; PCI – percutaneous coronary intervention; CABG – coronary artery bypass surgery.

Significant differences in between the two subgroups (with / without HF) were seen with regard to the usage of diuretics, digoxin and beta-blockers. Table 2 represents the multivariate analysis for the predictors of the acute HF in AIM patients. Independent predictors for the HF developing in a logistic regression model were: ejection fraction, previous revascularization, peripheral vascular disease, age, gender, previous myocardial infarction, systolic BP on admission, and anaemia. The data are graphically represented in Figure 1 as well, focusing on the statistical significance of every independent predictor separately.

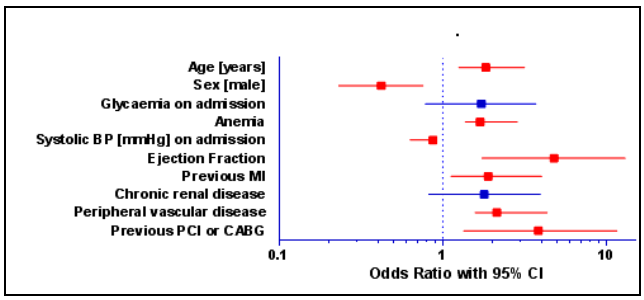

Figure 1: Multivariate analysis of predictors of heart failure in acute myocardial infarction patients

Discussion

HF incidence in our study resulted in 26.6%, whereas other large clinical studies have suggested somehow higher values up to 29.4% (GUSTO I, GUSTO IIb, GUSTO III and ASENT II) [16]. Registry values, including the VALIANT register, suggest figures from 20.4% up to 24.5% [11, 17]. Another large epidemiological study including the period 1970-1999, found that 24% of the AIM cases presented HF [18]. There are a few studies in Albania regarding the factors that might lead to HF developing in the acute phase of AMI [15, 19]. In our present paper, as well as in the Framingham analysis, HF patients were older, they showed a male preponderance, a higher heart rate on admission, higher systolic BP values on admission, positive history for previous MI as well as higher glycemic values on admission, together with more antihypertensive drug usage, an increased presence of previous attempts to coronary revascularization, peripheral vascular disease, chronic renal disease and anemia, a lower ejection fraction and an increased occurrence of atrial fibrillation.

With regard to risk factors, the HF subgroup of patients differed only for the smoking habits. As of the in-patient or intra-hospital treatment, our HF subgroup differed significantly with the subgroup without HF, vis-à-vis digoxin, diuretics and beta-blockers administration. Predictors for an early development of HF were older patients, diabetics, and people suffering from previous cardiac symptoms; whereas predictors for a late-onset HF were hypertensive disease, male gender, tachycardia and higher value of cardiac enzymes. HF was more frequent following anterior AMI when compared with other anatomic cardiac areas.

In our study, independent predictors of the HF developing in a logistic regression model were: ejection fraction value, previous revascularization procedures, peripheral vascular disease, age, gender, previous MI, systolic BP on admission and anaemia. The simultaneous presence of different risk factors and the clinical severity of AMI, together with a suboptimal medical care, will strongly influence the prognosis of the coronary disease. In Albania, there is as well a need for rehabilitation programs, let alone the necessity of a standardisation of interventions aiming at secondary prevention. Obviously, other studies are needed to clarify the importance of factors influencing the clinical profile of Albanian patients suffering an AMI, in order to optimise the quality of care within a coronary intensive care unit.

In conclusion, in spite of a falling trend of mortality linked to myocardial infarction, HF still remains an important complication of the everyday cardiologic practice. In order to better organise prevention and optimal treatment for the HF, an increased attention is needed towards highly risked populations, especially aged persons, those with the previous coronary disease, and other concomitant medical conditions. As a conclusion, we might say that HF is a common occurrence following AMI, notwithstanding changes in the epidemiology of the acute coronary syndrome. In fact, an increased incidence of HF is being reported, parallel to a

decrease in the mortality from AMI. Further observations are needed to demonstrate the impact of newly marketed drugs in the HF frequency after an AMI episode so that all requirements of an evidence-based medicine might be accomplished.

References

1. Cowie MR, Lacey L, Tabberer M. Heart Failure After Myocardial Infarction: A Neglected Problem? Br J Cardiol. 2005;12(3):205-208.

2. Nichols M, Townsend N, Scarborough P, Rayner M. Cardiovascular disease in Europe: epidemiological update. Eur Heart J. 2013; 34: 3028-3034. http://dx.doi.org/10.1093/eurheartj/eht356 PMid:24014390

3. Nabel EG, Braunwald E. A tale of coronary artery disease and myocardial infarction. N Engl J Med. 2012; 366: 54-63. http://dx.doi.org/10.1056/NEJMra1112570 PMid:22216842

4. Sulo E, Vollset SE, Nygard O, Sulo G, Igland J, Egeland GM, Ebbing M, Tell GS. Trends in 28-day and 1-year mortality rates in patients hospitalized for a first acute myocardial infarction in Norway during 2001-2009: a "Cardiovascular disease in Norway" (CVDNOR) project. J Intern Med. 2015; 277: 353-361. http://dx.doi.org/10.1111/joim.12266 PMid:24815825

5. Koopman C, Bots ML, van Oeffelen AA, van Dis I, Verschuren WM, Engelfriet PM, Capewell S, Vaartjes I. Population trends and inequalities in incidence and short-term outcome of acute myocardial infarction between 1998 and 2007. Int J Cardiol. 2013; 168: 993-998. http://dx.doi.org/10.1016/j.ijcard.2012.10.036 PMid:23168007

6. Kostis WJ, Deng Y, Pantazopoulos JS, Moreyra AE, Kostis JB, Myocardial Infarction Data Acquisition System Study G. Trends in mortality of acute myocardial infarction after discharge from the hospital. Circ Cardiovasc Qual Outcomes. 2010; 3: 581-589. http://dx.doi.org/10.1161/CIRCOUTCOMES.110.957803 PMid:20923995

7. Bucholz EM, Butala NM, Rathore SS, Dreyer RP, Lansky AJ, Krumholz HM. Sex differences in long-term mortality after myocardial infarction: a systematic review. Circulation. 2014; 130: 757-767. http://dx.doi.org/10.1161/CIRCULATIONAHA.114.009480 PMid:25052403 PMCid:PMC4415526

8. Cleland JG, McGowan J. Heart failure due to ischaemic heart disease: epidemiology, pathophysiology and progression. J Cardiovasc Pharmacol. 1999;33(suppl 3):S17-S29. PMid:10442681

9. Fox KF, Cowie MR, Wood DA et al. Coronary artery disease as the cause of incident heart failure in the population. Eur Heart J. 2001;22:228-36. http://dx.doi.org/10.1053/euhj.2000.2289 PMid:11161934

10. British Heart Foundation. Coronary Heart Disease Statistics Database. London: British Heart Foundation, 2003. Available at www.bhf.org.uk

11. Spencer FA, Meyer TE, Gore JM, Goldberg RJ. Heterogeneity in the management and outcomes of patients with acute myocardial infarction complicated by heart failure: the National Registry of Myocardial Infarction. Circulation. 2002; 105: 2605–2610. http://dx.doi.org/10.1161/01.CIR.0000017861.00991.2F PMid:12045165

12. Vaur L, Danchin N, Genes N et al. Epidemiology of myocardial infarction in France: therapeutic and prognostic implications of heart failure during the acute phase. Am Heart J. 1999;137:49-58. http://dx.doi.org/10.1016/S0002-8703(99)70459-X

13. Hellermann JP, Jacobsen SJ, Gersh BJ et al. Heart failure after myocardial infarction: a review. Am J Med. 2002;113:324-30. http://dx.doi.org/10.1016/S0002-9343(02)01185-3

14. Hung J, Teng TH, Finn J, Knuiman M, Briffa T, Stewart S, Sanfilippo FM, Ridout S, Hobbs M. Trends from 1996 to 2007 in incidence and mortality outcomes of heart failure after acute myocardial infarction: a population-based study of 20,812 patients with first acute myocardial infarction in Western Australia. J Am Heart Assoc. 2013; 2: e000172. http://dx.doi.org/10.1161/JAHA.113.000172 PMCid:PMC3835218

15. Burazeri G, Goda A, Sulo G, Stefa J, Roshi E, Kark JD. Conventional risk factors and acute coronary syndrome during a period of socioeconomic transition: population-based case-control study in Tirana, Albania. Croat Med J. 2007; 48: 225-233. PMid:17436387 PMCid:PMC2080510

16. Hasdai D, Topol EJ, Kilaru R, Battler A, Harrington RA, Vahanian A, Ohman EM, Granger CB, Van de Werf F, Simoons ML, O'Connor CM, Holmes DR Jr. Frequency, patient characteristics, and outcomes of mild-to-moderate heart failure complicating ST-segment elevation acute myocardial infarction: lessons from 4 international fibrinolytic therapy trials. Am Heart J. 2003; 145: 73–79. http://dx.doi.org/10.1067/mhj.2003.53 PMid:12514657

17. Velazquez EJ, Francis GS, Armstrong PW, Aylward PE, Diaz R, O'Connor CM, White HD, Henis M, Rittenhouse LM, Kilaru R, van Gilst W, Ertl G, Maggioni AP, Spac J, Weaver WD, Rouleau JL, McMurray JJ, Pfeffer MA, Califf RM; VALIANT registry. An international perspective on heart failure and left ventricular systolic dysfunction complicating myocardial infarction: the VALIANT registry. Eur Heart J. 2004; 25: 1911–1919. http://dx.doi.org/10.1016/j.ehj.2004.08.006 PMid:15522470

18. Velagaleti RS, Pencina MJ, Murabito JM, Wang TJ, Parikh NI, D'Agostino RB, Levy D, Kannel WB, Vasan RS. Long-term trends in the incidence of heart failure after myocardial infarction. Circulation. 2008; 118: 2057–2062. http://dx.doi.org/10.1161/CIRCULATIONAHA.108.784215 PMid:18955667 PMCid:PMC2729712

19. Myftiu S, Sulo E, Burazeri G, Sharka I, Shkoza A, Sulo G. A higher burden of metabolic risk factors and underutilization of therapy among women compared to men might influence a poorer prognosis: a study among acute myocardial patients in Albania, a transitional country in Southeastern Europe. Croat Med J. 2015 Dec 31;56(6):542-9. http://dx.doi.org/10.3325/cmj.2015.56.542 PMid:26718760 PMCid:PMC4707925

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):439-442.
http://dx.doi.org/10.3889/oamjms.2016.073
eISSN: 1857-9655
Case Report

Two Pregnancies with a Different Outcome in a Patient with Alport Syndrome

Biljana Gerasimovska Kitanovska[1*], Vesna Gerasimovska[1], Vesna Livrinova[2]

[1]University Clinic of Nephrology, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia; [2]University Clinic of Gynecology and Obstetrics, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia

Citation: Gerasimovska Kitanovska B, Gerasimovska V, Livrinova V. Two Pregnancies with a Different Outcome in a Patient with Alport Syndrome. Open Access Maced J Med Sci. 2016 Sep 15; 4(3):439-442. http://dx.doi.org/10.3889/oamjms.2016.073

Keywords: Alport syndrome; chronic kidney disease; pregnancy.

***Correspondence:** Assoc. Prof. Biljana Gerasimovska Kitanovska. Clinic of Nephrology, Medical Faculty, Vodnjanska 17, Skopje 1000, Republic of Macedonia. E-mail: bgerasimovska@yahoo.com

Received: 07-Jun-2016; **Revised:** 24-Jun-2016; **Accepted:** 25-Jun-2016; **Online first:** 02-Jul-2016

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: Alport syndrome is a genetic disease that progresses to chronic kidney failure, with X-linked, autosomal dominant or autosomal recessive type of inheritance. Women are generally carriers of the mutation and have a milder form of the disease. During pregnancy, they have an increased risk of impaired kidney function and preeclampsia.

CASE PRESENTATION: A 27-year old woman, gravida 1, para 0, in her 23rd gestational week came to the outpatient unit of the University Clinic of Nephrology for the first time because of slowly progressing proteinuria and Alport syndrome. She was admitted to the gynaecological ward in her 29th gw for proteinuria which increased from 3.8 g/day up to 20 g/day and the serum creatinine increased to 120- 150 micromol/l. She was delivered in the 30th gestational week due to obstetrical indications with a cesarian section and delivered a baby with a birth weight of 880 g. After delivery, proteinuria decreased to 2 g/d within 2 months and an angiotensin-converting enzyme inhibitor (ACEI) was started. Her second pregnancy, after 2 years, had an uneventful course and she delivered a healthy baby weighing 3000 g in the 39th week. Six months after the second delivery, her renal function remained normal and her proteinuria was 2 g/d.

CONCLUSIONS: Pre-pregnancy counselling and frequent controls during pregnancy are necessary for women with Alport syndrome, as well as regular monitoring after delivery. Recent reports are more in favour of good pregnancy and nephrological outcomes in women with Alport syndrome when renal disease is not advanced.

Introduction

Alport syndrome is a genetic disease that progresses to chronic kidney failure and is characterised by persistent hematuria, proteinuria and progressive renal disease. It may be associated with hearing loss and ocular abnormalities' but not all clinical characteristics are found in all.patients with Alport syndrome [1].

It is associated with mutations in type IV collagen gene and may have an X-linked (mutation on *COL4A5*), autosomal dominant (mutation on *COL4A3* or *COL4A4*) or autosomal recessive type of inheritance (mutation on *COL4A3* or *COL4A4*) [2].

Women are generally carriers of the mutation and have a milder form of the disease, with slow progression of the renal disease [3]. During pregnancy, they have an increased risk of impaired kidney function and preeclampsia [4]. There are only several case reports found in the literature on Alport syndrome and pregnancy.

Case Presentation

A 27-year old woman, gravida 1, para 0, in her 23rd gestational week consulted at the outpatient

unit of the University Clinic of Nephrology in Skopje, Macedonia, for the first time, because of slowly progressive proteinuria.

Her father was diagnosed with Alport syndrome with renal biopsy, had chronic kidney failure, was on a hemodialysis and died at the age of 57. Her uncle also had Alport syndrome confirmed by biopsy and was on hemodialysis. The patient and her two sisters were diagnosed with Alport and renal biopsy was not done because of their positive family history. They were followed since their childhood for erythrocyturia and different levels of proteinuria, but with normal serum creatinine and no hypertension. Her older sister, who has only erythrocyturia and no proteinuria, had a successful pregnancy with a term birth of a child with slightly lower birthweight. Her younger sister has erythrocyturia and proteinuria above 4 g/d. Unfortunately, genetic analysis is not available in the country and was not done in our patient.

Proteinuria was first registered in our patient 5 years before her first pregnancy, at her age of 22 and was in the range of 0.4 – 0.8 g/d.

First pregnancy

When the patient consulted in the Department of Nephrology in the 23rd gw, she had higher serum creatinine for pregnancy, 89 micromol/l, and in the urine, there were 20-25 erythrocytes in the sediment - 17% of which were dysmorphic. Her renal ultrasound revealed an ectopically placed right kidney and hypertrophy of her left kidney. She was started on Aspirin 100 mg from the onset of the pregnancy. The 24-hour proteinuria was 0.6 g/dU and progressed over time, and hypoalbuminemia worsened. Her D-dimers in the 23rd gw were slightly increased and low-molecular-weight heparin was started at a dose of 40 g.

In 26 the gw, intrauterine growth retardation (IUGR) was registered and the fetus was bio-metrically equal to 23 rd gestational week. There was already an increased resistance index (RI) at the umbilical artery up to 0.71 and a verified notch in the left uterine artery. In the 29th gw, the fetus was bio-metrically equal to 25th gw, with a notch in the right umbilical artery. Blood pressure was within normal.

She was admitted to the gynaecological ward in her 29th gw for proteinuria which increased from 3.8 g/day up to 20 g/day and the serum creatinine increased to 120- 150 micromoles/l. Her albumin was 29-22 g/l and uric acid was 332 micromoles/l. Blood pressures increased up to 140/80 mm Hg and she was started on Methyldopa 500 mg and continued with 250 mg. She was started on a small dose of corticosteroids in order to stimulate lung maturation.

She was delivered in the 30th gestational week due to obstetrical indications with a cesarian section and delivered a baby with a birth weight of 880 g. The baby died within 7 days and autopsy showed that respiratory distress was the cause of death.

After delivery, proteinuria decreased to 2 g/d within 2 months and angiotensin-converting enzyme inhibitor (ACEI) was started. Because of a persistent cough, she was switched to an angiotensin-receptor blocker (ARB), Losartan 25 mg/d and her proteinuria decreased to 0.8 g/d. Her blood pressure remained normal and serum creatinine was 70-83 micromol/l at the follow-up two years after delivery.

Second pregnancy

After two years of her first pregnancy, the patient became pregnant again, with a baseline proteinuria of 2 g/dU and serum creatinine 89 micromol/l. Her second pregnancy was uneventful, with regular monthly checkups, her blood pressure was in normal range and her proteinuria increased from 2 g/d up to 3.5 g/d by the 39th gestational week. Her serum albumin was 29 g/l at the 39th gestational week and creatinine rose to 81 g/l. She delivered a healthy baby by cesarian section in her 39th gestational week. Six months after the second delivery, her renal function remained normal and her proteinuria was 2/d.

Discussion

Chronic kidney disease (CKD) used to be a contraindication for pregnancy. It is no longer considered to be so, yet if the kidney disease is in an advanced stage, it is more difficult for a patient to conceive and have a successful pregnancy [4]. In the management of CKD in pregnancy, it is more important to manage clinical features than to know the aetiology [5]. Intrauterine growth retardation and preterm birth, as well as the risk for worsened kidney disease, occur twice more often in pregnant women with the renal disease. The risk from decreased renal function in pregnancy should be considered separately from comorbidities that may aggravate the condition [6]. A case-control study has shown that when proteinuria is below 1 g/d, serum creatinine is below 110 micrograms/d, without hypertension, pregnancy did not have an adverse effect on long-term kidney function [7].

Alport syndrome in women is generally a mild disease, but there is a wide variability of renal outcomes. It is suggested that in heterozygous females with Alport syndrome, inactivation of the X chromosome may be important for the severity of the disease. Tissue-specific distribution of an alternatively spliced *COL4A5* isoform and non-random X

chromosome inactivation reflect phenotypic variation in heterozygous X-linked Alport syndrome [8].

In the first pregnancy, our patient had chronic kidney disease in stage 1 from the beginning of pregnancy (erythrocyturia, albuminuria). Her serum creatinine was within normal, proteinuria was below 1 g/d and her blood pressure was normal so that an uneventful pregnancy was expected. Yet, worsening proteinuria and hypoalbuminemia were associated with intrauterine growth retardation and biometrically smaller fetus, and despite corticosteroids for lung maturation, and low-molecular weight protein prophylaxis, the fetus had birthweight of 880 g and died due to respiratory distress. In the following period after delivery, the patient was followed at regular intervals and proteinuria decreased due to ACEI. In her next pregnancy, proteinuria started from 2 g and at the end of the pregnancy it increased to 3.5 g/dU, yet it was not the reason for a pre-term delivery nor intrauterine growth retardation. After delivery, the patient's renal function did not deteriorate further.

There are only several case reports on the outcomes of pregnancy in women with Alport syndrome. Previous reports showed that pregnancies in women with Alport syndrome ended with preeclampsia and worsened renal function. Matsuo described a case with higher proteinuria (1-2 g/d) and higher serum creatinine from the start of the pregnancy, which evolved into preeclampsia and worsened kidney function [9]. He speculated that preeclampsia is more frequent in women with Alport syndrome because type 4 collagen can be found in placental and renal vessels and in the case of a mutation in both sites, destruction may occur and a common antigen may be involved [10]. Still, the report by Matsubara showed that umbilical cord of a newborn from a mother with Alport had negative immunofluorescence staining for the alpha 5 chain of type IV collagen. A good pregnancy outcome is reported by Matsoubara [11, 12] and Alessi [13]. Recent expert guidelines on Alport syndrome state that ACEI should be used between pregnancies because they are nephroprotective and that they would be beneficial for proteinuria, and other risk factors for renal failure progression in patients with Alport syndrome [14].

The risk for preeclampsia and worsened renal disease is increased in pregnant women with Alport syndrome [15]. Recent reports present cases with more favourable outcomes in both autosomal dominant and recessive cases [16, 17].

Considering the outcomes of pregnancies in women with Alport syndrome, pre-pregnancy counselling and frequent controls during pregnancy are necessary, as well as regular monitoring after delivery. Prepregnancy counselling should include information for the parents on the risk for the syndrome in the child and offered a prenatal diagnosis in the fetus. Controls during pregnancy should be once monthly, once a week immediately after delivery and then once a month in the first six months, their frequency in the next period depending on the renal affection. Recent reports are more in favour of good outcomes of pregnancies in women with Alport syndrome when renal disease is not advanced.

References

1. Chapter in a book: Sessa A, Conte F, Meroni F, Battini G. Alport Syndrome: clinical and genetic correlation in a type IV collagen. Hereditary kidney diseases, Karger, 1996.

2. Barker DF, Hostikka SL, Zhou J et al. Identification of mutations in the COL4A5 collagen gene in Alport syndrome. Science. 1990;248(4960):1224–27. http://dx.doi.org/10.1126/science.2349482 PMid:2349482

3. Rheault NM. Women and Alport syndrome. Pediatr Nephrol. 2012;27:41–46. http://dx.doi.org/10.1007/s00467-011-1836-7 PMid:21380623 PMCid:PMC3223569

4. Fischer MJ, Lehnerz SD, Hebert JR, Parikh CR. Kidney disease is an independent risk factor for adverse fetal and maternal outcomes in pregnancy. Am J Kidney Dis. 2004;43:415-23. http://dx.doi.org/10.1053/j.ajkd.2003.10.041 PMid:14981599

5. Williams D, Davison J. Chronic kidney disease in pregnancy. BMJ. 2008; 336(7637):211-5. http://dx.doi.org/10.1136/bmj.39406.652986.BE PMid:18219043 PMCid:PMC2213870

6. Nevis IF, Reitsma A, Dominic A, McDonad S, Thabane L et al. Pregnancy Outcomes in Women with Chronic Kidney Disease: A Systematic Review. CJASN. 2011;6:112587-2598. http://dx.doi.org/10.2215/CJN.10841210 PMid:21940842 PMCid:PMC3359575

7. Jungers P, Houillier P, Forget D, Labrunie M, Skhiri H, Giatras I, et al. Influence of pregnancy on the course of primary chronic glomerulonephritis. Lancet. 1995;346:1122-4. http://dx.doi.org/10.1016/S0140-6736(95)91798-5

8. Shimizu Y, Nagata M, Usui J, Hirayama K, Yoh K, Yamagata K, et al. Nephrol Dial Transplant. 2006;21(6):1582-7. http://dx.doi.org/10.1093/ndt/gfl051 PMid:16517570

9. Matsuo K, Tudor EL, Baschat AA., Alport syndrome and pregnancy. Obstet Gynecol. 2007;109(2):531-2. http://dx.doi.org/10.1097/01.AOG.0000240141.26395.82 PMid:17267885

10. Crovetto F,Moroni G, Zaina B, Acaia B, Ossola MW, Fedele L. Pregnancy in women with Alport syndrome. J Obstet Gynaecol. 2014;34(1):98-100.

11. Matsubara S, Muto S. Good obstetric outcome of consecutive pregnancies in a woman with Alport syndrome. Arch Gynecol Obstet. 2012; 286(1):261-2. http://dx.doi.org/10.1007/s00404-012-2225-6 PMid:22262491

12. Matsubara S, Ueda Y, Takahashi H, Nagai T, Kuwata T, Muto S, et al. Pregnancy complicated with Alport syndrome: a good obstetric outcome and failure to diagnose an infant born to a mother with Alport syndrome by umbilical cord immunofluorescence staining. J Obstet Gynaecol Res. 2009;35(6):1109-14. http://dx.doi.org/10.1111/j.1447-0756.2009.01069.x PMid:20144175

13. Alessi M, Fabris A, Zambon A, Cremasco D, Muraro E, Dosa L, et al. Pregnancy in Alport syndrome: a report of two differently-evolving cases. J Obstet Gynaecol. 2014; 34(1):98-100. http://dx.doi.org/10.3109/01443615.2013.834299 PMid:24359068

14. Temme J, Peters F, Lange K, Pirson Y, Heidet L, Torra R et al.Incidence of renal failure and nephroprotection by RAAS inhibition in heterozygous carriers of X-chromosomal and autosomal recessive Alport mutations. Kidney Int. 2012; 81:779–

783. http://dx.doi.org/10.1038/ki.2011.452 PMid:22237748

15. Savige J, Gregory M, Gross O, Kashtan C, Ding J, Flinter F. Expert guidelines for the management of Alport syndrome and thin basement membrane nephropathy. J Am Soc Nephrol. 2013; 24(3):364-75. http://dx.doi.org/10.1681/ASN.2012020148 PMid:23349312

16. Nishizawa Y, Takei T, Miyaoka T, Kamei D, Mochizuki T, Nitta K. Alport syndrome and pregnancy: Good obstetric and nephrological outcomes in a pregnant woman with homozygous autosomal recessive Alport syndrome. J Obstet Gynaecol Res. 2016; 42(3):331-5. http://dx.doi.org/10.1111/jog.12897 PMid:26628290

17. Yefet E, Tovbin D, Nachum Z. Pregnancy outcomes in patients with Alport syndrome.Arch Gynecol Obstet. 2016; 293(4):739-47. http://dx.doi.org/10.1007/s00404-015-3893-9 PMid:26411580

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):443-448.
http://dx.doi.org/10.3889/oamjms.2016.079
eISSN: 1857-9655
Case Report

The Role of Rehabilitation in the Management of Patients with Charcot-Marie-Tooth Disease: Report of Two Cases

Erieta Nikolikj Dimitrova*, Ivana Božinovikj, Simona Ristovska, Aleksandra Hadzieva Pejcikj, Aleksandra Kolevska, Mirjeta Hasani

Institute of Physical Medicine and Rehabilitation, Medical Faculty, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia

Citation: Nikolikj Dimitrova E, Božinovikj I, Ristovska S, Hadzieva Pejcikj A, Kolevska A, Hasani M. The Role of Rehabilitation in the Management of Patients with Charcot-Marie-Tooth Disease: Report of Two Cases. Open Access Maced J Med Sci. 2016 Sep 15; 4(3):443-448. http://dx.doi.org/10.3889/oamjms.2016.079

Keywords: Charcot-Marie-Tooth disease; rehabilitation; exercise; orthoses.

***Correspondence:** Prof. Dr Erieta Nikolikj Dimitrova. Institute of Physical Medicine and Rehabilitation, Medical Faculty, Ss Cyril and the Methodius University of Skopje, Skopje, Republic of Macedonia. E-mail: erietand@yahoo.com

Received: 19-Jun-2016; **Revised:** 05-Jul-2016; **Accepted:** 06-Jul-2016; **Online first:** 12-Jul-2016

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: Charcot-Marie-Tooth (CMT) disease is a hereditary disease with signs of chronic non-progressive motor-sensory neuropathy which is characterised by symmetric muscle atrophy and weakness of the distal portion of lower extremities.

AIM: The aim is to present two cases with peroneal muscular atrophy, applied rehabilitation procedures and rehabilitation outcome.

MATERIAL AND METHODS: Patient DR, aged 51, and patient KH, aged 78. Both patients had weakness and pronounced atrophy of the distal portion of lower extremities, numbness down the legs, contractures in the ankles and walking difficulties. Evaluation of patients included a clinical examination, Barthel Index, Time Up and Go test, measurement of the ankle range of motion, and a manual muscle test. On admission, the Barthel Index score was 60 in the first case, and 80 in the second. The rehabilitation program included exercise therapy with for lower extremity, occupational therapy, stationary bicycle riding, galvanic current, water exercises, and ankle-foot orthoses for both legs.

RESULTS: The therapy applied had no significant changes in the clinical neurological status of the patients, but yet it provided some improvement in ankle contractures, better mobility, and a more stable gait.

CONCLUSION: The application of rehabilitation procedures in patients with Charcot-Marie-Tooth disease can improve their functional status and walking stability.

Introduction

Charcot-Marie-Tooth (CMT) disease is the most common rare inherited neurological disorder with prevalence 1:2500. It represents a genetically heterogeneous group of symmetrical peripheral motor-sensory neuropathies sharing the same clinical phenotype characterised by distal limb muscle wasting and weakness, skeletal deformities, distal sensory loss and reduced deep tendon reflex [1], pes cavus or pes cavovarus, functional ankle instability and higher falls frequency [2]. The most frequent are demyelinating form (CMT 1) that is a result of the chronic progressive axonal degeneration and reinnervation with segmental demyelinisation; and axonal form (CMT 2) has electrodiagnostic findings more consistent with axonal loss involving the anterior horn cell of the spinal cord without evidence of segmental demyelination [3, 4]. They account for over 70% of cases [4].

These disorders are characterised by slowly progressive neuropathic processes involving the distal muscles with a highly variable severity and onset. Foot drop or steppage gate, foot pain secondary to foot deformity is also common [4]. The disease gradually progresses causing smaller or greater disability. Occasionally, there is also a weakness in the distal parts of the upper extremities.

Concerning the therapies, there is no pharmacology treatment [5]. The treatment procedures of such patients include rehabilitation, as well. Since muscle wasting and sensory disturbance are the main features of these syndromes, treatments aim to improve motor impairment and sensory disturbances [3] The rehabilitation processes are directed toward the prevention of contractures, maintenance of circulation, strengthening of the remaining unaffected muscles, improvement of gait, and improvement of quality of life [4, 6].

The aim is to present two patients with Charcot-Marie-Tooth (CMT) disease and the effects of the applied rehabilitation procedures.

Material and Methods

Design architecture: The proposed study was a report of two cases. The report was interventional with subjects acting as their own control. The comparison was made between the pre and post outcome measures. The follow up was made 3 months later.

Outcome measures: The outcome measures in this study included: clinical examination, measurement of active and passive ankle range of motion with goniometer, force capabilities were tested for dorsal and plantar flexor muscles according to the Medical Research Council 0-5 Scale (Manual Muscle Testing) by same clinician, functional parameters with Time Up and Go test (three timed tests of motor function were assessed to stand from the chair and walk 3 meters, turn on back, go back and sit on the chair) (7), 6 meters walking test (three timed tests of motor function were assessed including time taken to walk 6 meters at the participant's fastest and self-selected speed), Barthel Index for assessing disability, Visual Analogue Scale (VAS) for low back pain.

Cases Presentation

Case 1

Patient DR, aged 51. On admission, the patient complained of weakness in his extremities, more pronounced in lower parts, numbness down his arms and legs, occasional pain in lower back and in the back of his right hip. The disease started 4 years ago with weakness in the distal parts of extremities, more pronounced in lower extremities, with pains in his legs, walking difficulties, and fatigue. The following year, the patient was hospitalised at the Department of Neurology, where he was diagnosed with Charcot-Marie-Tooth type II disease.

EMG of chronic neurogenic lesions of moderate to a severe degree, mainly in the distal limb muscles with combined characteristics, mainly axonal damage. Computed tomography of the brain showed normal findings. EEG: on the basis of proper brain activity, groups of high-volt and slow waves were registered bilaterally frontotemporal.

Neuropsychological testing: the behavioural plan is dominated by depressive symptoms; the person is inhibited, hypersensitive, emotionally immature, and registers the phenomenon of fear and insecurity leading to a chronic anxiety condition.

Patient environmental and social history: finished primary school, unemployed, the beneficiary of social assistance, unmarried, lives with his parents, suffers from epilepsy, and receives medical treatment with the table. Carbamazepine a 200 mg 2 x 1, table. Phenobarbital a 100 mg 1 x 1.

Clinical examination on admission

Normal posture of the spine, no tension in paravertebral muscles in the cervical and lumbar area. Neck movements are partly limited with pronounced latero flexion and rotation; movements in the lumbar area are easily restricted in all directions.

Upper Extremities: postural tremor of fingers. Muscles with relatively preserved trophic. Active movements performed in all the joints to its full extent. Reduced rough motion force of both hands was noticed. Tendon reflexes give numerous bad responses, almost extinct. Superficial sensibility shows hand hyperesthesia. He uses his arms in everyday life.

Lower Extremities: both feet have equinovalgus deformity, with thumbs flexed. There is a pronounced weakness of the below knee muscles, more pronounced in the left leg. The muscles of the calves with reduced tone. The patient maintained their posture by balancing. He performed active movements of the hips and knees in quite a limited extent, in the ankles plantar flexion on both sides is 10 degrees, actively runs dorsal flexion in the partial volume due to muscle weakness (MMT 2), and passively it is minus 5 degrees to the left and 0 degrees to the right. Patellar reflex with the bilaterally symmetric response, Achilles tendon reflex is extinguished on both sides. He has somewhat reduced sensitivity to touch of the knees down, more pronounced in his left leg. He stands on his toes, but cannot stand on his heels. His gait is without aids, a typical peroneal gait, on a large scale.

Barthel Index score was 60 on admission. Time Up and Go test 11.7 sec. VAS for lumbal pain was 4. Walking time at a distance of 6 meters was 9.02 sec.

Following procedures were applied during rehabilitation: exercise therapy with active exercises for hip and knee muscles (Fig. 1), passive exercises for foot muscles, stationary bike ride, occupational therapy, manual massage and galvanization of lower extremities. Infrared rays and diadynamic current (DF, CP, LP 6 minutes) were applied for low back pain. Two ankle-foot orthoses for both legs to limit ankle plantar flexion were prescribed.

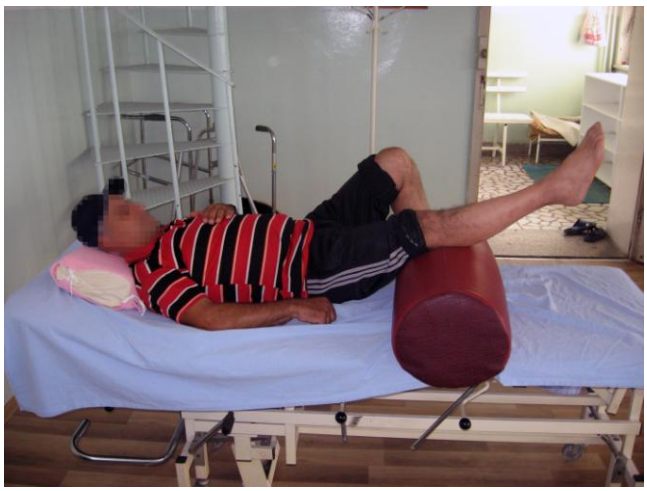

Figure 1: Active exercises for quadriceps

Upon dismissal, the clinical findings of the patient indicated no significant improvement in the lower leg muscle strength, no changes in MMT, a slight improvement of the left ankle contracture, and dorsal flexion passive 0 degrees. The patient's gait was more stable with two ankle-foot orthoses. Barthel Index scores 60, Timed Up and Go test 10.3 sec. Walking time at a distance of 6 m. was 8.64 sec. The low back pain has disappeared - VAS was 0.

Follow-up examinations after 3 months have shown that the clinical findings and the Barthel Index are the same as on discharge. The patient continued to exercise at home.

Case 2

Patient KH, aged 78. On admission, the patient complained of weakness in his lower extremities, numbness down legs, swelling and pain in his left ankle, difficulties when walking. The disease started some 38 years ago showing weakness in the distal portions of his lower extremities. He was examined by a neurologist in the regional hospital and diagnosed with Atrofia neural progressive. The muscle weakness was gradually developing 35 years ago; the patient was hospitalised at the University Department of Neurology, where the tests confirmed the diagnosis of Charcot-Marie-Tooth disease.

EEG in favour of deficit dominant in the muscles of the lower extremities most pronounced in the small toe, and to a lesser extent at the m. tibialis and m. quadriceps femoris. There is a large reduction in motor units, while the remaining motor units show signs of hypertrophy. Findings of the upper extremities are within limited values. There is a reduced conduction n. peroneus and a great loss of motor axons. The upper extremity findings are in favour of segmental demyelination.

The patient has been repeatedly subjected to spa-balneotherapy. He was admitted to our rehabilitation institution for the first time. Patient environmental and social history: completed higher education, retired, worked as a school teacher, suffers from arterial hypertension.

Clinical examination on admission

Upper Extremities: increased contours of the PIP and DIP joints on both hands are present; more pronounced in the DIP joints. Hand muscules have a slight hypertrophy corresponding to his age. The patient fully performs active movements in all of his joints. Superficial sensibility is normal. Tendon reflexes have weak response levels. Rough motion force of both hands is slightly reduced. He uses his hands in everyday life.

Lower Extremities: Both feet are in the equine position. There is a small swelling in the left ankle, painless to palpation. The lower limbs have a pronounced hypotrophy and muscle hypotonia. The patient maintains his lower extremities in posture. He performs active movements in both hips and knees to the full extent, slightly slower, whereas his active movements are possible in ankles in traces only because of the pronounced muscle weakness (MMT 1+), plantar flexion is mutually 10 degrees, active dorsal flexion is possible in traces minus 5 degrees left and minus 10 degrees to the right, and passive dorsal flexion is 0 st. Superficial sensibility is preserved. Tendon reflexes are extinguished. He stands on his toes, but not on his heels. He walks without walking aids, with a typical peroneal gait on both sides.

Barthel Index scores 80 on admission. Time Up and Go Test 13.54 sec. Walking time at a distance of 6 meters was 9.99 sec. VAS for pain in his left ankle was 4.

Following procedures were applied during rehabilitation: exercise therapy with active exercises for hip and knee muscles, passive exercises for foot muscles, stationary bicycle riding, and water exercises in the pool (Fig. 2), occupational therapy, manual massage and galvanization of lower extremities. Dyadinamic currents (DF, CP, LP 6 minutes) were applied for swelling and slight pain in the left ankle. Two ankle-foot orthoses for both legs to limit ankle plantar flexion were prescribed.

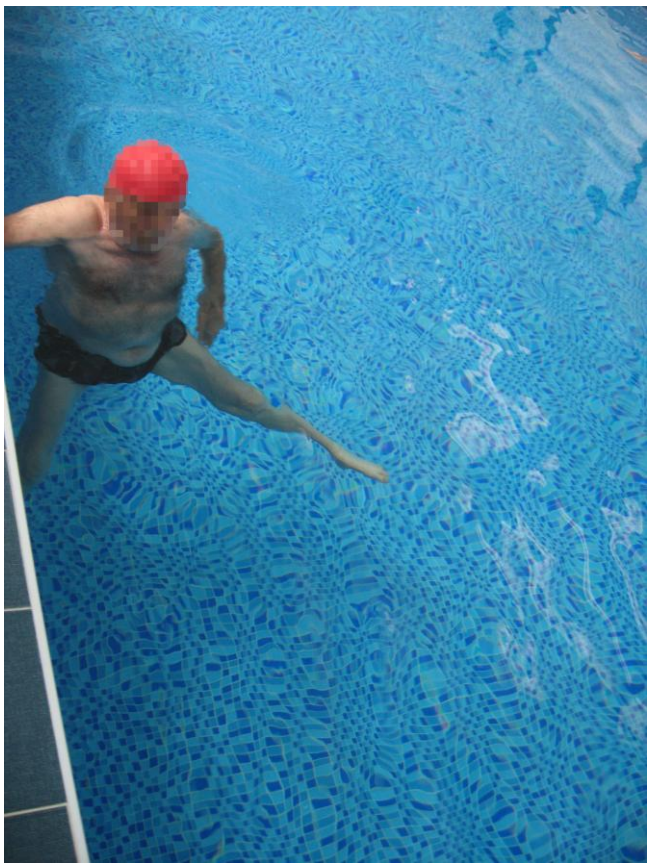

Figure 2: Water exercises for lower extremities in the pool

Upon dismissal, the patient had subjective improvement, but the clinical findings of the patient indicated no significant improvement in the lower leg muscle strength, no changes in MMT, swelling decreased in the left ankle, no pain in the left ankle. The patient's gait was more stable with below-knee plastic orthoses. Barthel Index scores 80, Timed Up and Go test 12.3 sec. Walking time at a distance of 6 m. was 9.14 sec.

Follow-up examinations after 3 months have shown that the clinical findings and the Barthel Index are the same as on discharge. The patient continued to exercise at home.

Discussion

There are no effective drugs for therapeutic approach to Charcot-Marie-Tooth (CMT) disease at present [8]. Six RCTs compares the effect of oral ascorbic acid (1 to 4 grammes) and placebo treatment in CMT 1A. High-quality evidence indicates that ascorbic acid does not improve the course of CMT1A and outcomes in adults, as well as by low-quality evidence in children [5].

Treatments are aimed at functional restoration of gait with an orthosis, exercises and surgery [9], and improvements in sensory disturbances and pain reduction [3, 9]. Rehabilitation is usually suggested by physicians, but only a few studies were performed in this field and some evidence confirmed the benefit deriving from physical therapy. The rehabilitation programs are heterogeneous, differ from therapist to therapist, should be tailored to the patients and may be changed from time to time. Moreover, usually, the patient is not able to define and quantify precisely the type of rehabilitation procedures [8]. It is a rare disease with published studies with case reports, or studies with small numbers of patients and that is why there is no high-quality evidence for suggested methods of rehabilitation.

Carter GT, et al. believe that patients with Charcot-Marie-Tooth disease should be treated in a comprehensive multi-disciplinary ward in which a neurologist, physiatrist, orthopaedic surgeon, physiotherapist, occupational therapist and an orthopedist should be involved. Treatment should be aimed at achieving maximum independence and quality of life [6].

In an observational study of total 26 adult patients with CMT, the results suggested that gait parameters correlated with both dorsal- and plantar – flexors strength, whereas postural parameters correlated only with plantar-flexor strength. They concluded that improved knowledge of postural and gait capacities may constitute a basis to emphasise the corrections that should be enabled by rehabilitation exercises or orthotic devices [10]. Rose et al. in the group of 29 children and adolescents participants with CME reported moderate to severe bilateral functional ankle instability, reporting functional ankle instability significantly associated with cavus foot structure, female gender and impaired balance [2].

Padua L, et al in the observational survey study with 123 patients with CMT, showed that patients have physical and mental benefit from rehabilitation, but also perceived that do not perform the best rehabilitation program for their pathology. They concluded that there was a lack of consensus on rehabilitation tailored to CMT patients need and familiar/caregiver [8].

During rehabilitation, our patients received Achilles tendon stretching exercises, ankle extension exercises, preserved muscles strengthening, occupational exercises, and water exercises in the pool for the second patient. The applied therapy enabled patients a certain reduction in ankle contracture, improved mobility, and more stable gait.

According to a systematic review, exercises may be beneficial to maintain strength and function for people with CMT. Significant effects described included improvements in strength, functional activities, and physiological adaptations the following

exercise. There was few studies available and moderate quality of evidence. The optimal exercise modality and intensity for people with CMT as well as the long-term of exercise remain unclear [11]. Foot extension exercises are recommended to prevent shortening of the Achilles tendon [12].

Both patients received electrotherapy with galvanic current for both lower extremities, longitudinally because of its excitatory effect, as well as dyadinamic currents for the lumbar pain of the first patient and for the left ankle pain of the second patient. After 10 days of dyadinamic current application, the patients had no pain.

Some authors for rehabilitation of CMT patients recommended iontophoresis, electric stimulation (indirect and direct), dyadinamic currents (rhythm syncope), middle-frequency currents with alternative or constant regime with frequency from 100 to 150 Hz, depth of 100%, as well as exercises, exercises in water, four-cell bath, paraffin therapy, healing mud therapy and manual massage [13].

In the previously published case report a patient with CMT after rehabilitation treatment with galvanic currents, exercise therapy, occupational therapy, orthopaedic devices (ankle- foot orthosis and a pair of crutches) achieved some improvement in mobility and gait [14].

On admission our patients walked with a typical peroneal gait, without walking aids, whereas on discharge, their gait was with two ankle-foot orthoses for limiting the ankle plantar flexion without walking aids. The mobility and gait were better, more stable then on admission.

Special shoes with good ankles support may be necessary. Good evaluation of movements, application of ankle foot orthosis and training for its application is indispensable. Patients frequently need AFO orthosis for foot equines correction and gait improvement [12]. In the study of 7 Charcot-Marie-Tooth type 1A patients, the use of anterior elastic ankle-foot orthoses improved walking economy by reducing the energy cost of walking per unit of distance, thus reflected a lower level of metabolic effort and improved mechanical efficiency in comparison with shoes only [15].

Some patients need under-elbow crutches or a stick to improving their gait stability, and only less than 5% of the patients need a wheelchair. It is desirable to educate the patients in relation to weight control because obesity makes difficulties in movement. Exercises should support the capacity of each individual patient. The majority of the Charcot-Marie-Tooth patients remain physically active [16]. Adapted aids may be used occasionally to improve the activities of daily living and self-care. Wrist and hand orthoses are occasionally used. Professional occupational therapy important for employment and career may also be needed [16].

In a clinical study testing sensitivity of various scales in rehabilitation and lungs condition with 8 CMT patients compared to healthy persons, it was determined that all the rehabilitation measures were much worse with CMT patients than was the case with the healthy persons. Treatments with walking belts, extension exercises, respiratory exercises and proprioceptive exercises were carried out twice a week for a period of 8 weeks. After the treatment, there was a significant improvement in the movement range of the ankles and in the walking time at a distance of 6 meters. The authors concluded that patients should be treated at least 2 times a year because regression of outcome measures was determined after a 6-month period without exercises [17].

In the recently published survey of 44 patients with CMT disease, the qualitative content analysis revealed that personal factors such as fatigue, poor balance, muscle weakness, and pain were important barriers to physical activity behaviour. They concluded that understanding of these factors can guide health professionals to facilitate physical activity behaviour in this group of patients [18].

According to the systematic review larger randomised controlled trials are needed for any form of pharmacological intervention as well as for the form of physical intervention [3].

In conclusion, the application of rehabilitation procedures in patients with Charcot-Marie-Tooth disease can improve both their functional status and walking stability. Treatment decisions must be individualised and based upon a clear history, careful examination, and well-defined patient goals.

References

1. Pareyson D, Scaioli V, Laura M. Clinical and electrophysiological aspects of Charcot-Marie-Tooth disease. Neuromolecular Med. 2006; 8:3-22. http://dx.doi.org/10.1385/NMM:8:1-2:3

2. Rose KJ, Hiller CE, Mandarakas M, Raymond J, Refshauge K, Burns J. Correlates of functional ankle instability in children and adolescents with Charcot-Marie-Tooth disease. Journal of Foot and Ankle research. 2015; 8: 61-68. http://dx.doi.org/10.1186/s13047-015-0118-1 PMid:26543504 PMCid:PMC4634800

3. Young P, de Jonghe P, Stogbauer F, Butterfass-Bahloul T. Treatment for Charcot-Marie-Tooth disease. Cochrane Database Syst Rev. 2008; (1):CD006052. http://dx.doi.org/10.1002/14651858.cd006052.pub2

4. Njegovan ME et al. Rehabilitation medicine approach to Charcot-Marie-Tooth disease. Clin Podiat Med Surg. 1997; 14:99-116. PMid:9030448

5. Gess B, Baets J, de Jonghe P, Reilly MM, Pareyson D, Young P. Ascorbic acid for the treatment of Charcot-Marie-Tooth disease. Cochrane Database Syst Rev. 2015;12:CD011952. PMid:26662471

6. Carter GT, Weiss MD, Han JJ, Chance PF, England JD. Charcot-Marie-Tooth disease. Curr Treat Options Neurol. 2008; 10 (2): 94-102. http://dx.doi.org/10.1007/s11940-008-0011-3 PMid:18334132

7. Podsialdo D, Richardson S, The Tume "Up&Go": A test of basic functional mobility for frail elderly persons. Journal of American Geriatrics Society. 1991; 39(2):142-148. http://dx.doi.org/10.1111/j.1532-5415.1991.tb01616.x

8. Padua L, Pazzaglia C, Schenone A, Ferraro F, Biroli A, Esposito C, Pareyson D. Rehabilitation for Charcot-Marie-Tooth: A survey study of patients and familiar/caregiver perspective and perception of efficacy and needs. Eur J Rehabil Med. 2014; 50:25-30.

9. Rankin P. The Bournemouth quaestionnaire as an outcome measure in the rehabilitation of a person suffering with mechanical neck and arm pain and concurrent Charcot-Marie-Tooth disease: a case report. J Can Chiropr Assoc. 2006;50(3):190-194. PMid:17549156 PMCid:PMC1839966

10. Guillebastre B, Calmels P, Rougier P. Effects of muscular deficiency on postural and gait capacities in patients with Charcot-Marie-Tooth disease. J Rehabil Med. 2013; 45:314-317. http://dx.doi.org/10.2340/16501977-1113 PMid:23412436

11. Sman AD, Hackett D, Fiatarone Singh M, Fornusek C, Menezes MP, Burns J. Systematic review of exercise for Charcot-Marie-Tooth disease. J Peripher Nerv Syst. 2015; 20 (4):347-362. http://dx.doi.org/10.1111/jns.12116 PMid:26010435

12. Guillebastre B, Calmels P, Rougier PR. Assessment of appropriate ankle-foot orthoses models for patients with Charcot-Marie-Tooth disease. Am J Phys Med Rehabil. 2011; 90(8):619-27. http://dx.doi.org/10.1097/PHM.0b013e31821f7172 PMid:21681059

13. Kolev Z. Nevralna muskulna atrofija na Charcot-Marie-Tooth. Vo Rakovodstvo po fizikalna terapia, Ur. Gatev S. Medicina i fiskultura: Sofia, 1992: 143.

14. Nikolikj-Dimitrova E. Rehabilitation of patient with peroneal muscular atrophy – case report. Acta Morphologica. 2012; 9(2): 43-47.

15. Menotti F, Laundani I, Damiani A, Mignogna T, Macaluso A. An anterior ankle-foot orthosis improves walking economy in Charcot-Marie-Tooth type 1A patients. Prosthet Orthot Int. 2014; 38 (5): 387-92. http://dx.doi.org/10.1177/0309364613506250 PMid:24100074

16. Kedlaya D, Meier R.H. Physical Medicine and rehabilitation for Charcot-Marie-Tooth disease. E-medicine. Accessed at 10.05.2012.

17. Maggi G, MontiBragadin M, Padua L, Fiorina E, Bellone E, Grandis M, Reni L, Bennicelli A, Grosso M, Saporiti R, Scorsone D, Zuccarino R, Crimi E, Schenone A. Outcome measures and rehabilitation treatment in patients affected by Charcot-Marie-Tooth neuropathy: a pilot study. Am J Phys Med Rehabil. 2011; 90 (8): 628-37. http://dx.doi.org/10.1097/PHM.0b013e31821f6e32 PMid:21681064

18. Anens E, Emtner M, Hellstrom K. Exploratory study of physical activity in persons with Charcot-Marie-Tooth disease. Arch Phys Med Rehabil. 2015; 96(2):260-8. http://dx.doi.org/10.1016/j.apmr.2014.09.013 PMid:25286435

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):449-452.
http://dx.doi.org/10.3889/oamjms.2016.078
eISSN: 1857-9655
Case Report

Cytomegalovirus Infection during Pregnancy and Its Impact on the Intrauterine Fetal Development – Case Report

Mariya Angelova[1*], Emil Kovachev[2], Nikolai Todorov[1]

[1]Department of Obstetrics and Gynecology, Trakia University, Faculty of Medicine, Stara Zagora, Bulgaria; [2]Department of Obstetrics and Gynecology, Paraskev Stoyanov Medical University, Varna, Bulgaria

Citation: Angelova M, Kovachev E, Todorov N. Cytomegalovirus Infection during Pregnancy and Its Impact on the Intrauterine Fetal Development – Case Report. Open Access Maced J Med Sci. 2016 Sep 15; 4(3):449-452. http://dx.doi.org/10.3889/oamjms.2016.078

Keywords: Cytomegalovirus infection; pregnancy; a case report.

***Correspondence:** Mariya Angelova. Department of Obstetrics and Gynecology, Trakia University, Faculty of Medicine, Stara Zagora, Bulgaria. E-mail: angelova70@abv.bg

Received: 27-Apr-2016; **Revised:** 19-Jul-2016; **Accepted:** 25-Jul-2016; **Online first:** 08-Aug-2016

Competing Interests: The authors have declared that no competing interests exist.

Abstract

AIM: The aim of this publication is to present a case of CMV infection during pregnancy, with clinical manifestations of the development of microcephaly and simultaneous dilatation of the 3rd and 4th brain ventricle at 23 weeks gestation. This article discusses the role of ultrasound screening in the second trimester of pregnancy.

CASE PRESENTATION: We present the case of a 25-year-old woman with the initials S.K. in her second pregnancy that came to our antenatal Consulting Centre. The first screening for blood count, blood group, biochemistry and serology showed results within the reference range. The patient came for a second comprehensive biochemical screening at 17 – 18 weeks gestation. The results showed the low genetic risk of congenital anomalies. Fetal morphology of the fetus was normal. S.K. came again for consultation at 22 weeks gestation in connection with the admittance of her first 3-year-old child to the hospital because of pneumonia. Serological tests of the child had shown elevated CMV titer - specific IgM. Then we made new serological tests of the patient and the results have shown that the patient was most likely infected by CMV primarily in the first trimester of pregnancy. After consulting about the risk of transmission of CMV to the fetus, the woman chose monthly ultrasound scans and refused amniocentesis. At 36 weeks gestation, in addition to the microcephaly already established, enlargement of the IV brain ventricle at the expense of underdevelopment of the cerebellum was noticed. Also, 2nd to 3rd stage of placenta maturity and low quantity of amniotic fluid was established. A male fetus of weight 2,890 g and height 50 cm was delivered. The fetus was with skin petechiae and hepatosplenomegaly. Neurological examination showed no abnormalities.

CONCLUSIONS: In the described case the time interval between infection and ultrasonic manifestations is more than 17 weeks. The long interval between infection and occurrence of ultrasound markers can be a good prediction sign, as it may reflect less aggressive viral infection than present in cases where similar ultrasound findings were obtained shortly after infection of the mother.

Introduction

The cytomegalovirus infection is caused by a DNA virus of the viral family known as herpes viruses. Presently, CMV can be encountered in every fourth woman in childbirth age [1]. Once in the body, the virus remains for a lifetime. Usually, the cytomegalovirus “sleeps” in the body and does not cause any damages [2]. However, when the immunity drops, in cases of frequent colds, stress situations and pregnancy, the virus becomes active [3]. It is important to note that infecting the fetus or the newborn infant can happen only with active virus infection. During pregnancy, the virus attacks the nervous system of the fetus which is connected with early miscarriages or with the development of a number of defects in the newborn infants, whereas 20-30% of them die [4].

The low pathogenicity of the virus in the adult human body is sharply contrasting with the impact on the fetus which can cause serious damages. The harm for the fetus is even more serious when the mother has recently had a primary infection and has not developed any specific CMV antibodies. The clinical forms of fetal damages are: congenital cytomegalic infection acquired perinatal infection and acquired postnatal infection [5].

The focus of this publication is a case of CMV

infection during pregnancy which was clinically expressed with the development of microcephaly and simultaneous dilatation of the 3-rd and 4-th cerebral ventricle in the 23 g.w. This paper presents a discussion on the role of the ultrasound testing in the second trimester of pregnancy.

Case Presentation

The following is a description of the case of a 25-year-old woman with the initials S.K. in her second pregnancy who came to our antenatal consulting centre.

The first screening for blood count, blood group, biochemistry (ALAT, ASAT, creatine, urea, blood sugar) showed results within the reference range. Hbs Ag, HIV, Wasserman, IgG, IgM (toxoplasmosis), IgG, IgM (rubella), IgG, IgM (CMV) were negative.

At that time the woman was at 6 – 7 weeks gestation. Biochemical screening generally is done at 12 – 13 weeks gestation showed results within the reference range (low risk of development of a baby with an abnormality, low risk of developing preeclampsia in the second half of pregnancy and low risk of premature birth).

The patient came for a second comprehensive biochemical screening at 17 – 18 weeks gestation. The results showed the low genetic risk of congenital anomalies. Fetal morphology of the fetus was normal.

S.K. came again for consultation at 22 weeks gestation in connection with the admittance of her first 3-year-old child to the hospital because of pneumonia. Serological tests of the child had shown elevated CMV titer - specific IgM.

Then we made new serological tests of the patient and the results were as follows: CMV – specific IgG – 98; CMV – specific IgM – 2.1. It was then understood that the patient was most likely infected by CMV primarily in the first trimester of pregnancy. After consulting about the risk of transmission of CMV to the fetus the woman chose monthly ultrasound scans and refused amniocentesis.

The results of all ultrasound scans were normal until that time. At 24 weeks gestation fetal brain ultrasound scan showed thalamic calcifications bilaterally, hyperechogenic foci in the right side of the ventricular wall, asymmetric ventriculomegaly > 10 mm (Fig. 1).

Similar changes were imaged at 28 weeks gestation and also cerebellar developmental delay was noticed. Also, hepatomegaly was found at otherwise eutrpophically developing a fetus.

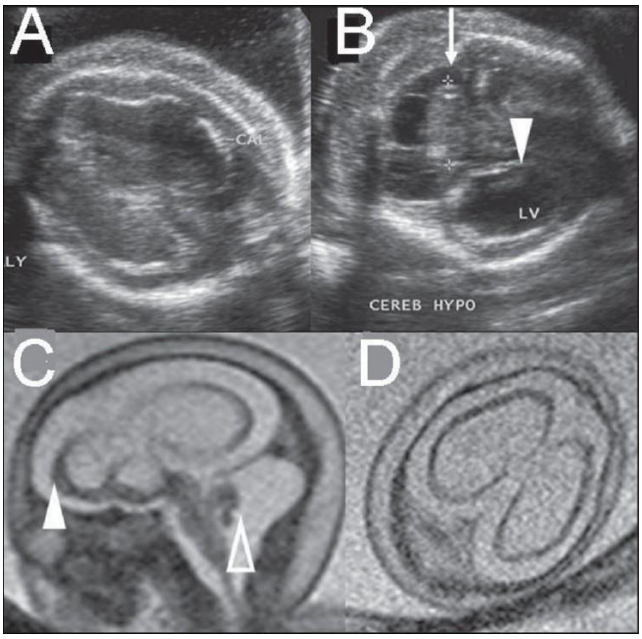

Figure 1: Fetal brain ultrasound scan at 24 weeks gestation

After that patient, S.K. consented to do of amniocentesis as changes were already verified by ultrasound. Amniotic fluid analysis detected that the fetus was positive for CMV (CMV – specific IgM titer was 68), thrombocytopenia – 46 g/l. Levels of fetal liver enzymes were normal. Following consultation on the possible consequences of CMV infection of the fetus, the parents chose to continue with the pregnancy.

In the next ultrasound scanning to follow at 32 weeks gestation not only cerebellar developmental delay was noticed, but also of the head as a whole (microcephaly).

At 36 weeks gestation, in addition to the microcephaly already established, enlargement of the IV brain ventricle at the expense of underdevelopment of the cerebellum was noticed. Also, 2nd to 3rd stage of placenta maturity and low quantity of amniotic fluid was established (Fig. 2).

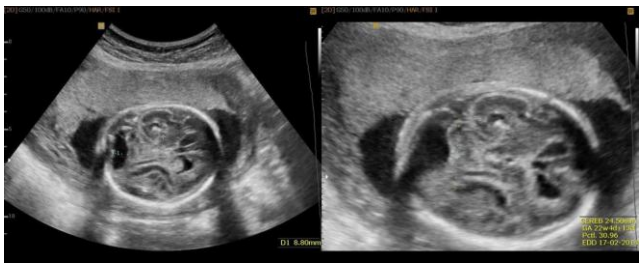

Figure 2: Fetal brain ultrasound scan at 36 weeks gestation

The patient was admitted to an obstetrics and gynaecology clinic for active monitoring. Cardiotocography was done on a daily basis. Upon the occurrence of two consecutive non-reactive NST and presence of late decelerations, a decision for

delivery was taken. Because of the relatively immature fetus and low pelvic score a caesarian section delivery was done. A male fetus of weight 2,890 g and height 50 cm was delivered. The fetus was with skin petechiae and hepatosplenomegaly. Neurological examination showed no abnormalities. Thrombocytopenia was confirmed (Hg 66 g/l). The newborn baby was treated with Acyclovir i.v. – 5 mg / g / 12 h for 6 weeks (Fig 3).

Figure 3: A male fetus of weight 2,890 g and height 50 cm was delivered. The fetus was with skin petechiae and hepatosplenomegaly

Presently, the baby is six-month-old, of normal neurological status and preserved hearing.

Discussion

CMV is the most common congenital viral infection and is the leading infectious cause of sensory deafness and cerebral mental retardation. The estimated risk of vertical transmission in the first trimester of pregnancy is 36-45%. Ten % of infected newborn babies are symptomatic at birth and 15 % of asymptomatic newborn babies display developmental disorders later [6].

In the described case the time interval between infection and ultrasonic manifestations is more than 17 weeks. The long interval between infection and occurrence of ultrasound markers can be a good prediction sign, as it may reflect less aggressive viral infection than present in cases where similar ultrasound findings were obtained shortly after infection of the mother.

This case shows that when there is suspicion of CMV and patients refuse invasive diagnostics (amniocentesis, cordocentesis), monthly ultrasound examinations are of exceptional crucial importance.

According to world statistical data, more than 80% of the population is infected with the virus. Presently, CMV is isolated in every fourth woman of childbearing age. Once this virus enters the body, it remains there for life [7].

Normally, the cytomegalovirus "sleeps" in the body and causes no harm. At fall of immunity, frequent colds, stress and pregnancy, the virus becomes active. It is important to note that infection of the fetus or neonate can take place only in an active viral infection. Only the test by DNA analysis clearly demonstrates active viral infection.

The most serious complication caused by active cytomegalovirus infection during pregnancy is an infection of the fetus or neonate. In pregnancy, the virus affects the nervous system of the fetus which attributes to early miscarriage or development of a number of defects in neonate so that 20-30 % of them die [8].

Unfortunately, in practice congenital cytomegalovirus infection is not susceptible to treatment and therefore taking of preventive measures is required. In pregnant women, it is advisable to monitor and control the activity of the virus by screening in each trimester [9].

In conclusion, the overcoming of the CMV infection is possible through the administration of antiviral drugs. The purpose of such drugs is to suppress viral division (propagation) and to facilitate the passage of the virus from active phase to latent phase of viral rest, in which the virus is not able to infect and harm the body organs.

References

1. Kenneson A, Cannon MJ. Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection. Rev Med Virol. 2007;17:253–76. http://dx.doi.org/10.1002/rmv.535 PMid:17579921

2. Gaytant MA, Rours GI, Steegers EA, Galama JM, Semmekrot BA. Congenital cytomegalovirus infection after recurrent infection: case reports and review of the literature. Eur J Pediatr. 2003;162:248–53. PMid:12647198

3. Boppana SB, Rivera LB, Fowler KB, Mach M, Britt WJ. Intrauterine transmission of cytomegalovirus to infants of women with preconceptional immunity. N Engl J Med. 2001;344:1366–71. http://dx.doi.org/10.1056/NEJM200105033441804 PMid:11333993

4. Raynor BD. Cytomegalovirus infection in pregnancy. Semin Perinatol. 1993;17:394–402. PMid:8160023

5. Nelson CT, Demmler GJ. Cytomegalovirus infection in the pregnant mother, fetus, and newborn infant. Clin Perinatol. 1997;24:151–60. PMid:9099507

6. Revello MG, Gerna G. Diagnosis and management of human cytomegalovirus infection in the mother, fetus, and newborn infant. Clin Microbiol Rev. 2002;15:680–715. http://dx.doi.org/10.1128/CMR.15.4.680-715.2002 PMCid:PMC126858

7. Enders G, Bader U, Lindemann L, Schalasta G, Daiminger A. Prenatal diagnosis of congenital cytomegalovirus infection in 189 pregnancies with known outcome. Prenat Diagn. 2001;21(5):362-77. http://dx.doi.org/10.1002/pd.59 PMid:11360277

8. Revello MG, Zavattoni M, Baldanti F, Sarasini A, Paolucci S, Gerna G. Diagnostic and prognostic value of human cytomegalovirus load and IgM antibody in blood of congenitally infected newborns. J Clin Virol. 1999;14:57–66. http://dx.doi.org/10.1016/S1386-6532(99)00016-5

9. Barbi M, Binda S, Caroppo S, Primache V. Neonatal screening for congen- ital cytomegalovirus infection and hearing loss. J Clin Virol. 2006;35:206–9. http://dx.doi.org/10.1016/j.jcv.2005.08.010 PMid:16384745

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):453-454.
http://dx.doi.org/10.3889/oamjms.2016.083
eISSN: 1857-9655
Case Report

Enduring Personality Changes after Intense Stressful Event: Case Report

Slavica Arsova[1*], Nensi Manusheva[1], Gabriela Kopacheva-Barsova[2], Stojan Bajraktarov[1]

[1]University Psychiatry Clinic, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia; [2]University Clinic for Ear, Nose and Throat, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Vodnjanska 17, Skopje 1109, Republic of Macedonia

Citation: Arsova S, Manusheva N, Kopacheva-Barsova G, Bajraktarov S. Enduring Personality Changes after Intense Stressful Event: Case Report. Open Access Maced J Med Sci. 2016 Sep 15; 4(3):453-454. http://dx.doi.org/10.3889/oamjms.2016.083

Keywords: posttraumatic stress disorder; enduring personality changes; SSRI anti-depressive; anti-psychotic; psycho stabilising therapy; case report; Republic of Macedonia.

***Correspondence:** Slavica Arsova, MD. University Psychiatry Clinic, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia. E-mail: arsova_s@yahoo.com

Received: 14-Jun-2016; **Revised:** 09-Jul-2016; **Accepted:** 28-Jul-2016; **Online first:** 17-Aug-2016

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: World statistical data show that a large number of individuals suffer from posttraumatic stress disorder (PTSD) after exposure to the intense traumatic event. PTSD can have a chronic course with enduring changes in the functioning of the person.

CASE PRESENTATION: Here we report two adult individuals of different gender and education who were exposed to the extremely severe stressful event after which difficulties in psychological functioning developed. The first case we present is a 46-year-old man, with completed high education, divorced, father of two children, who lives with his parents, and is retired. Disorders appeared 20 years ago when he was exposed to extremely severe stressful events in war circumstances that included captivity, torture, and loss of fellow soldiers. The second case is a 50-year-old female patient, with a university degree, professor of art, married, and mother of two children of whom the son died six years ago. She suffered from disorders after the sudden injury of her son that ended with his death.

CONCLUSION: Posttraumatic stress disorder after the intense stress is a risk of development enduring personality changes with serious individual and social consequences.

Introduction

World statistical data show that a large number of individuals suffer from posttraumatic stress disorder (PTSD) after exposure to the intense traumatic event. PTSD can have a chronic course with enduring changes in functioning of the person [1].

It is considered that the majority of healthy individuals will not develop posttraumatic stress disorder; however, if the individual has experienced an extremely traumatizing event associated with violence and conflict situations such as war, concentration camp, rape, domestic violence, bullying during childhood, then the chances for development of this disorder are greater [2-6].

Literature data show that men tend to experience more traumatic events, but women more often develop stress-related disorder if they have been exposed to traumatic events such as family violence, loss of a child, rape, etc [7, 8].

According to ICD 10, if psychological difficulties last for years after the survived traumatic event maladaptive forms of behaviour develop including distinct difficulties in social and personal functioning that lead to enduring personality changes [9].

The aim of this study was to present the influence of intense stressful event on the development of enduring personality changes after survived catastrophic event.

Case Presentation

Here we report two adult individuals of different gender and education who were exposed to the extremely severe stressful event after which difficulties in psychological functioning developed. The diagnosis was made in line with the ICD 10, and the following diagnostic criteria were used: HAMA, HAMD, and clinical interview, psychological and social findings.

The first case we present is a 46-year-old man, with completed high education, divorced, father of two children, who lives with his parents, and is retired. Disorders appeared 20 years ago when he was exposed to extremely severe stressful events in war circumstances that included captivity, torture, and loss of fellow soldiers. Within the course of the first years he developed posttraumatic stress disorder diagnosed and managed on an outpatient basis, and later several hospital stays in day hospital care have been recorded. Variable psychological difficulties with the gradual development of enduring personality changes have been observed along with a persistent feeling of insecurity, jeopardy, social inequality, withdrawal and marked work-related dysfunction.

The second case is a 50-year-old female patient, with a university degree, professor of art, married, and mother of two children of whom the son died six years ago. She suffered from disorders after the sudden injury of her son that ended with his death. Within the course of the first month's acute stress reaction was noticed, which was later transformed into a posttraumatic stress disorder of chronic course and development of enduring personality changes.

During the day hospital stay patients were treated with pharmacologic agents (SSRI anti-depressive, anti-psychotic and psycho stabilising therapy), which showed a modest success, that is, reduction in the impulsive behaviour; sleep improvement and reduction in self-harmful behaviour. In addition to pharmacology therapy patients were treated with individual and group psychosocial therapy procedures but without significant success in their normal reintegration in their families, social and professional environment.

Discussion

Literature shows that PTSD most commonly develops in individuals who have been exposed to severe stress associated with violence when the lives of the victims or their close relatives or friends have been in jeopardy, or they have been threatened, tortured, molested, or suffered a sudden loss of a close family member or friend. Long-lasting difficulties might result in permanent maladaptive forms of behaviour [10]. These individuals have paranoid attitude towards the environment, a feeling of uncertainty, social withdrawal, work-related dysfunction, irritability, and they are prone to interpersonal conflicts, with a low threshold of tolerance for frustrating situations [1].

In conclusion, posttraumatic stress disorder after the intense stress is a risk of development enduring personality changes with serious individual and social consequences.

References

1. Kaplan HI, Sadock BJ, Grebb JA, editors. Kaplan and Sadock's synopsis of psychiatry: Behavioural sciences, clinical psychiatry, 7th ed., 1994.

2. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, 5th ed. Arlington, VA: American Psychiatric Publishing, 2013.

3. Crawford C, Dawn B. A Systematic Review of Biopsychosocial Training Programs for the Self-Management of Emotional Stress: Potential Applications for the Military. Evidence-Based Complementary and Alternative Medicine. 2013:1–23. http://dx.doi.org/10.1155/2013/747694 PMid:24174982 PMCid:PMC3794660

4. Dobry Y, Braquehais MD, Sher L. Bullying, psychiatric pathology and suicidal behavior. International Journal of Adolescent Medicine and Health. 2013;25(3):295–9. http://dx.doi.org/10.1515/ijamh-2013-0065 PMid:24006324

5. Fullerton CS, Ursano RJ, Wang L. Acute Stress Disorder, Posttraumatic Stress Disorder, and Depression in Disaster or Rescue Workers. Am J Psychiatry. 2004;161(8):1370–6. http://dx.doi.org/10.1176/appi.ajp.161.8.1370 PMid:15285961

6. Dobry Y, Braquehais MD, Sher L. Bullying, psychiatric pathology and suicidal behavior. International Journal of Adolescent Medicine and Health. 2013;25(3):295–9. http://dx.doi.org/10.1515/ijamh-2013-0065 PMid:24006324

7. Pereira A. Combat trauma and the diagnosis of post-traumatic stress disorder in female and male veterans. Military Medicine. 2002;167(1):23–27. PMid:11799808

8. Suris A, Lind L, Kashner M, Borman P, Petty F. Sexual Assault in Women Veterans: An Examination of PTSD Risk, Health Care Utilization, and Cost of Care. Psychosomatic Medicine. 2004;69:749–756. http://dx.doi.org/10.1097/01.psy.0000138117.58559.7b PMid:15385701

9. National Collaborating Centre for Mental Health (UK). Post-Traumatic Stress Disorder: The Management of PTSD in Adults and Children in Primary and Secondary Care". NICE Clinical Guidelines, No. 26. Gaskell, 2005.

10. Ozer EJ, Best SR, Lipsey TL, Weiss DS. Predictors of posttraumatic stress disorder and symptoms in adults: a meta-analysis. Psychol Bull. 2003;129(1): 52–73. http://dx.doi.org/10.1037/0033-2909.129.1.52 PMid:12555794

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):455-457.
http://dx.doi.org/10.3889/oamjms.2016.098
eISSN: 1857-9655
Case Report

Aortic Annular Enlargement during Aortic Valve Replacement

Selman Dumani*, Ermal Likaj, Laureta Dibra, Stavri Llazo, Ali Refatllari

University Hospital Center "Mother Theresa", Tirana, Albania

Citation: Dumani S, Likaj E, Dibra L, Llazo S, Refatllari A. Aortic Annular Enlargement during Aortic Valve Replacement. Open Access Maced J Med Sci. 2016 Sep 15; 4(3):455-457. http://dx.doi.org/10.3889/oamjms.2016.098

Keywords: AAE-aortic annulus enlargement; Manouguian technique; PPM-patient - prosthesis mismatch; AVR-aortic valve replacement; iEOA-indexed effective orifice area.

***Correspondence:** Selman Dumani. University Hospital Center "Mother Theresa", Tirana, Albania. E-mail: selmandumani@yahoo.co.uk

Received: 04-Aug-2016; **Revised:** 20-Aug-2016; **Accepted:** 24-Aug-2016; **Online first:** 02-Sep-2016

Competing Interests: The authors have declared that no competing interests exist.

Abstract

In the surgery of aortic valve replacement is always attempted, as much as possible, to implant the larger prosthesis with the mains goals to enhance the potential benefits, to minimise transvalvular gradient, decrease left ventricular size and avoid the phenomenon of patient-prosthesis mismatch. Implantation of an ideal prosthesis often it is not possible, due to a small aortic annulus. A variety of aortic annulus enlargement techniques is reported to avoid patient-prosthesis mismatch. We present the case that has submitted four three times open heart surgery. We used Manouguian technique to enlarge aortic anulus with excellent results during the fourth time of surgery.

Introduction

In the surgery of aortic valve replacement is always attempted, as much as possible, to implant the larger prosthesis with the mains goals to enhance the potential benefits, to minimise transvalvular gradient, decrease left ventricular size and avoid the phenomenon of patient-prosthesis mismatch. Implantation of an ideal prosthesis often it is not possible, due to a small aortic annulus. A variety of aortic annulus enlargement techniques is reported to avoid patient-prosthesis mismatch. We present the case that has submitted four three times open heart surgery. We used Manouguian technique to enlarge aortic annulus with excellent results during the fourth time of surgery.

Case Presentation

Patient Z.M. 52 years old was admitted to our service with the diagnosis: Status post replacement of mitral and aortic valve, dysfunction of the aortic prosthetic valve, heart failure NYHA III-IV.

The patient was operated three times: 1990- open mitral commissurotomy for rheumatic valvular disease, 1997 mitral and aortic valve replacement with a mechanical prosthesis, 2004 replacement of aortic prosthesis for prosthetic dysfunction.

The patient at admission had the prosthesis Sorin Nr.19 in the aortal position. The echocardiographic data present normal function and diameters of left ventricle, while mean aortic trans-prosthetic gradient was 70 mmHg. The mobility of prosthetic leaflets was normal. There were no data for prosthetic panus or thrombus. There was a normal function of the mitral prosthesis. The patient was in NYHA III clinical status. It was clear that the main problem was the patient-prosthesis mismatch. The patient was in severe patient – prosthesis mismatch. The calculated indexed effective orifice area was 0.64 cm^2/m^2.

In these circumstances, it was established the indication for redo surgery to resolve the problem of patient - prosthesis mismatch. The patient underwent routine preoperative examinations to be prepared for intervention.

Intervention was performed through median sternotomy with standard cardiopulmonary bypass and systemic hypothermia to 32^0C. An oblique aortotomy was performed and myocardial protection was provided by intermittent antegrade cristaloidcardioplegia delivered directly into the coronary ostia. We have inspected carefully the prosthesis and there was no panus or thrombus near the prosthesis. In these conditions, we decided that the best solution was the replacement of the prosthesis with a new one and in the same time doing enlargement of the aortic annulus. After removing the old prosthesis, aortic annulus enlargement was done using Manouguian technique [1]. Aortotomy was extended through annulus into the fibrous trigone between the noncoronary cusp and the left coronary cusp to the subaortic curtain and anterior mitral valve leaflet. This defect was closed using the synthetic Teflon patch.

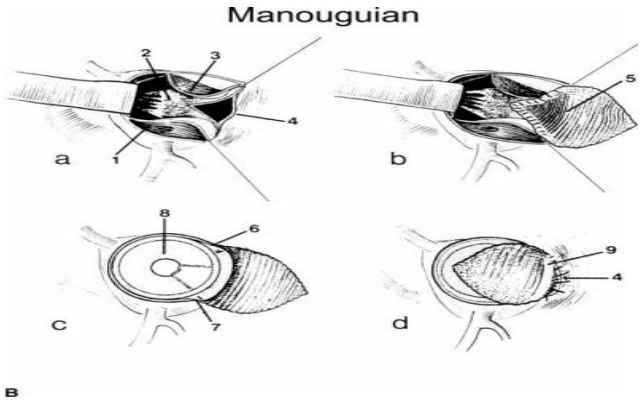

Figure 1: Schematic Manouguian Technique

We implanted SJM prosthesis Nr 21. Aortic cross-clamping time was 110 minutes. Cardiopulmonary bypass time was 130 minutes. The patient did the usual postoperative course as standard aortic valve replacement.

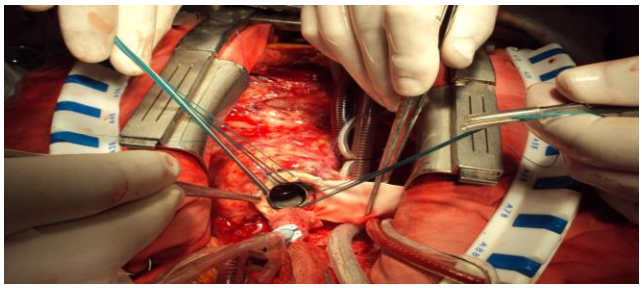

Figure 2: Photo during intervention

Discussion

Rahimtola et al [2] presented for the first time since 1978 the issue of prosthesis - patient mismatch which is defined as a condition in which the effective surface of the prosthesis is less than that of the normal patient valve.

The most accurate and used a parameter to define patient - prosthesis mismatch (PPM) actually is the indexed prosthetic effective orifice area (iEOA) that is the ratio of the orifice area of the prosthesis (EOA) with the patient's body surface area (BSA). Based on these values EOAi ≤ 0.85 cm^2/m^2 is regarded as the threshold for the occurrence of PPM to continue with moderate PPM when iEOA value is between 0.65 cm^2/m^2-0.85 cm^2/m^2 and severe when iEOA < 0.65 cm^2/m^2. The patient-prosthesis mismatch is common phenomenon during aortic valve replacement. The reported incidence varies 2-11 % [3].

There are four ways to resolve the problem of mismatch: implantation of the stentless prosthesis, homograft, autograft and aortic annulus enlargement (AAE) [1, 4-6].

The first three are associated with an increased operative mortality and morbidity [3]. Aortic annulus enlargement remains the more simple and reproducible surgical procedure to avoid this phenomenon.

Aortic annulus enlargement is an additional surgical procedure and it is performed in an anatomical area with high risk of bleeding. These facts have provoked the debate about the impact of this procedure on the early results of aortic valve surgery.

Aortic annulus enlargement procedure [7, 8] does not affect negatively early results of aortic valve surgery in terms of hospital mortality and morbidity even while, due to the complexity of the procedure, cross-clamping and cardiopulmonary bypass times are relatively longer than in standard aortic valve replacement. In this context Coutinho et al [7] recommend strongly the necessity to involve the aortic annulus enlargement procedure as part of operating strategy whenever is necessary during aortic valve replacement in patients with small aortic annulus. These suggestions are supported by other authors with a smaller contingent of patients operated that have realised aortic annulus enlargement [9, 10].

There are authors that analysing their results report higher mortality and morbidity in the group with AAE. They criticise the routine use of the aortic annulus enlargement and recommend being careful in the management of patient –prosthesis mismatch [11].

Mayo Clinic presented one of the largest studies where are involved 2366 patients in which 10.5 % of patients have been the subject of aortic annulus enlargement during aortic valve replacement. This study shows that the small number of a prosthesis implanted is an independent important risk factor in the early operative results while the aortic annulus enlargement procedure does not influence perioperative mortality and morbidity [12].

The accurate indications for AAE in the contingent of patients who are at risk to show to have postoperative patient-prosthesis mismatch improved significantly results of aortic valve surgery. Peterson et al report that in the modern era AAE is importantly improved. They present a comparison between two large groups of patients operated different periods find out a significant decline in hospital mortality from 7.5 to 3 % respectively for the periods 1995-2000 and 2001-2005 [13]. Aortic annulus enlargement, in a multifactorial analysis, is not a risk factor in aortic valve surgery and is recommended to be performed specifically in separate contingents of patients as in young patients and in those with reduced function of the left ventricle.

In our case, the early and late results of aortic annulus enlargement demonstrate effective solution of patient - prosthesis mismatch. The iEOA is 0.84 cm^2/m^2. The aortic cross-clamping and cardiopulmonary bypass time were longer in comparison with standard aortic valve replacement but the patient did very good postoperative period. We had no excessive bleeding and usual respiratory assistance, intensive care unit and postoperative hospital stay in comparison with standard aortic valve replacement. The early postoperative period was very good. The patient is in very good health after hospital discharge. She is in NYHA class I and does normal life for her age.

Manouguian technique is used successfully in a significant number of patients operated in our service, but this is not the topic of this presentation. This fact encourages us to involve the aortic annulus enlargement procedure in patient with high risk of patient - prosthesis mismatch, during aortic valve replacement.

In conclusion, **a**ortic annulus enlargement during aortic valve replacement according to Manouguian is a safe technique that solves the problem of patient- prosthesis mismatch.

References

1. Manouguian S, Seybold-Epting W. Patch enlargement of the aortic valve ring by extending the aortic incision to the anterior mitral leaflet. J Thorac Cardiovasc Surg. 1979;78:402—12. PMid:470420

2. Rahimtoola SH. The problem of valve prosthesis—patient mismatch. Circulation. 1978;58:20—4. http://dx.doi.org/10.1161/01.CIR.58.1.20 PMid:348341

3. Pibarot P, Dumesnil JG. Prosthesis-patient mismatch: definition, clinical impact, and prevention. Heart. 2006;92:1022–1029. http://dx.doi.org/10.1136/hrt.2005.067363 PMid:16251232 PMCid:PMC1861088

4. Nu-ez L, Gil Aguado M, Pinto GA, Larrea JL. Enlargement of the Aortic Annulus by Resecting the Commissure Between the Left and Noncoronary Cusps. Tex Heart Inst J. 1983; 10(3): 301–303.

5. Nicks R, Cartmill T, Bernstein L. Hypoplasia of the aortic root: the problem of aortic valve replacement. Thorax. 1970;25:339—46. http://dx.doi.org/10.1136/thx.25.3.339 PMid:5452289 PMCid:PMC472719

6. Kinsley RH, Antunes MJ. Enlargement of the narrow aortic root and oblique insertion of a St. Jude prosthesis. Br Heart J. 1983;50:330—2. http://dx.doi.org/10.1136/hrt.50.4.330 PMid:6626394 PMCid:PMC481419

7. Coutinho GF, Correia PM, Paupe´rio G, De Oliveira F, Antunes MJ. Aortic root enlargement does not increase the surgical risk and short-term patient outcome? European Journal of Cardio-thoracic Surgery. 2011;40: 441—447. http://dx.doi.org/10.1016/j.ejcts.2010.11.064

8. Castro LJ, Arcidi MJ, Fisher AL, Gaudiani VA. Routine enlargement of the small aortic root: a preventive strategy to minimize mismatch. Ann Thorac Surg. 2002;74:31—6. http://dx.doi.org/10.1016/S0003-4975(02)03680-9

9. St. Rammos K, Ketikoglou DG, Koullias GJ, Tsomkopoulos SG, Rammos CK, Argyrakis NP. The Nicks–Nunez posterior enlargement in the small aortic annulus:immediate–intermediate results. Interactive CardioVascular and Thoracic Surgery. 2006; 5:749–754. http://dx.doi.org/10.1510/icvts.2006.136457 PMid:17670704

10. Srivastava DK, Sanki P, Bhattacharya S, Siddique JV. Strategy to avoid patient-prosthesis mismatch: aortic root enlargement. Asian Cardiovasc Thorac Ann. 2014;22(2):137-41. http://dx.doi.org/10.1177/0218492312469117 PMid:24585782

11. LaPar DJ, Ailawadi G, Bhamidipati CM, Stukenborg G, Crosby IK, Kern JA, Kron IL. Small Prosthesis Size in Aortic Valve Replacement Does Not Affect Mortality. Ann Thorac Surg. 2011;92:880-888. http://dx.doi.org/10.1016/j.athoracsur.2011.04.105 PMid:21871273 PMCid:PMC3260881

12. Dhareshwar J, Sundt TM 3rd, Dearani JA, Schaff HV, Cook DJ, Orszulak TA. Aortic root enlargement: what are the operative risks? J Thorac Cardiovasc Surg. 2007;134(4):916-24. http://dx.doi.org/10.1016/j.jtcvs.2007.01.097 PMid:17903507

13. Peterson MD, Borger MA, Feindel CM, David TE. Aortic Annular Enlargement During Aortic Valve Replacement: Improving Results with Time. Ann Thorac Surg. 2007;83:2044 –9. http://dx.doi.org/10.1016/j.athoracsur.2007.01.059 PMid:17532393

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):458-460.
http://dx.doi.org/10.3889/oamjms.2016.097
eISSN: 1857-9655
Brief Communication

Mental Health Legislation and Involuntary Hospitalization in the Republic of Macedonia

Nensi Manuševa[1*], Slavica Arsova[1], Silvana Markovska-Simoska[2], Antoni Novotni[1], Branislav Stefanovski[1], Marija Raleva[1]

[1]University Psychiatry Clinic, Psychophysiology, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia; [2]Division of Neuroinformatics, Research Center for Energy, Informatics and Materials, Macedonian Academy of Sciences and Arts, Skopje, Republic of Macedonia

Citation: Manuševa M, Arsova S, Markovska-Simoska S, Novotni A, Stefanovski B, Raleva M. Mental Health Legislation and Involuntary Hospitalization in the Republic of Macedonia. Open Access Maced J Med Sci. 2016 Sep 15; 4(3):458-460. http://dx.doi.org/10.3889/oamjms.2016.097

Keywords: mental health; legislation; psychiatry; involuntary hospitalisation; patient rights.

***Correspondence:** Nensi Manuševa, MD. University Psychiatry Clinic, Psychophysiology, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia. E-mail: nensi.man@gmail.com

Received: 11-Aug-2016; **Revised:** 15-Aug-2016; **Accepted:** 16-Aug-2016; **Online first:** 01-Sep-2016

Competing Interests: The authors have declared that no competing interests exist.

Abstract

As psychiatrists, we are often obliged to provide non-consensual treatment. This institute comprises the rights of the patients with mental health disorders. The aim of this paper is to explain the contemporary mental health legislation in our country the Republic of Macedonia and the problems with the implementation of involuntary hospitalisation. This could be overcome with close cooperation between the judicial and health care system.

Introduction

Psychiatrists are often confronted with the problem of non-consensual treatment when patients with severe mental illnesses have to be hospitalised against their will. There is always a controversy as to whether compulsory treatment reduces their right for equality and freedom to decide. We would like to emphasise that if something is compulsory you must do it or accept it because it is the law or because someone in a position of authority says you must. This is important especially in the last several decades with the processes of deinstitutionalization and downsizing of large psychiatric hospitals and establishment of alternative community services [1].

Involuntary treatment is, therefore, the 'last resort'. The second question that arises does this treatment improves the clinical outcome and social functioning of that patient. Given the widespread use of such powers, it is important to assess the effects of this type of legislation [2]. That is why in several European countries these dilemmas are well understood and analysed [3, 4].

Besides the meaning of this institute, taking into practice the involuntary hospital treatment has several disadvantages that are the result of actual law regulations in our county.

Mental Health Reforms in the Republic of Macedonia

After declaring the independence in 1991 the Republic of Macedonia (RM) has adopted an

extensive set of legal reforms and many of them were concerned with and address distinct aspects of providing appropriate health care for the population. Also signed and ratified were several international instruments like Convention against torture and other cruel, inhuman or degrading treatments or punishment [5]. The Parliament of RM enacted National Mental Health Policy on October 13th, 2005 [6]. It includes several components such as developing community mental health services and downsizing large mental health hospitals and also developing a mental health component in primary health care. This document also addresses issues as providing access to mental health care including the least restrictive care and rights of mental health service consumers, family members and caregivers. It covers competency, capacity and guardianship issues for people with mental illness, especially voluntary and involuntary treatment with mechanisms to oversee involuntary admission and treatment practices, also Law enforcement and other judicial system issues for people with mental illness. Regular inspections and complaints processes are reviewed by a national human right review body. There are some disparities in practising some of them mainly because there is an unjustified delay in legislating compulsory hospitalisation in our country although 4% of all admissions to community-based inpatient psychiatric units and 4% of all admissions to mental hospitals were involuntary [6].

We need to mention the National Program for the treatment of people with mental disorders with Law on Mental Health [7] and Strategy for Mental Health promotion in the RM 2005-2012 that offers improvement with decentralisation of mental health care in community mental care centres distributed in various parts of the country. Currently, there are eight Centers that work on re-socialization and re-integration into society of the mentally ill persons [8].

The manner of hospitalisation can be voluntary or forced

Pursuant to the Law on non-litigation procedure when a person is admitted with their consent they should submit a statement in written in front of two adult witnesses who are not employed in the public health institution and are not relatives of the person that is being hospitalised [9]. In cases where imminent and very likely danger is present when the patient with mental disorder is to harm him/herself or others and/or the surroundings (aggressive or suicidal behaviour etc.) and the patient is not willing to accept the treatment or is not in a position to comprehend the need for treatment, involuntary/or non-consensual treatment could be performed. The compulsory treatment is permitted only when the clear benefit from the treatment is obvious and solely alternative for treatment. Also, immobilisation in psychiatric hospitals is with special protocols that are applied, which has elaborated the policy and the rules for restriction measures (immobilisation) of patients and the means which may be used [10].

In our country, there is a clear legal process in accordance with the international standards that regulates detention and medical treatment without their consent. It is regulated by the provisions of the articles 58 and 59 from the Non-litigation law. Criteria for detention and treatment are the presence of mental illness and aggressive or suicidal behaviour and the Court decides when the mentally ill person should be deprived the right for freedom of movement and contacting the surrounding environment.

When mental health organisation is to treat mentally ill patient without their consent or without a Court order the public health organisation is obliged to inform the regional Court in 48 hours. The Court is obliged to appoint two independent doctors one of them specialised in psychiatry, and the examination is performed in the stationary institution in which the treatment is provided. Then after the medical examination and expertise the Court is obliged to examine the circumstances and to provide the decision in the next 72 hours.

Discussion

Post-independence law reforms in R. Macedonia provide substantial and procedural protection of rights of the patients [11, 12] with mental disorders and they are generally in line with international best practices. The Republic of Macedonia has had a mental health policy and mental health legislation since 2005. There is a national human right review body which performs regular inspections and reviews complaints processes. But, there are some disparities in practicing these laws mainly because there is unjustified delay in legislating compulsory hospitalization in our country, especially the provisions from the article 59 par.2 of the Non-litigation law that are not fully implemented, mainly due to the difficulties in its implementation (or non-implementation) of this provision, because there are no (or only in rare cases) two adult witnesses that fulfill the legally binding pre-conditions. Still, there is a need for standardisation of the rules and regulations for involuntary admission [13], implementation of guidelines [14] and research in this field [15] and also close cooperation between the judicial and health care system.

Reference

1. Chow WS, Priebe S. Understanding psychiatric institutionalization: a conceptual review. BMC Psychiatry. 2013:13:169-83. http://dx.doi.org/10.1186/1471-244X-13-169 PMid:23773398 PMCid:PMC3702490

2. Kisely SR, Campbell LA, Preston NJ. Compulsory community and involuntary outpatient treatment for people with severe mental disorders. Cochrane Database Syst Rev. 2011: Feb 16 (2): CD004408.

3. Maître E, Debien C, Nicaise P, et al. Advanced directives in psychiatry: A review of the qualitative literature, a state-of-the-art and viewpoints. Encephale. 2013: 39(4):244-51. http://dx.doi.org/10.1016/j.encep.2012.10.012 PMid:23537636

4. Thorneycroft G, Farrelly S, Birchwood M, et al. CRIMSON [CRisis plan IMpact: Subjective and Objective coercion and eNgagement] protocol: a randomized controlled trial of joint crisis plans to reduce compulsory treatment of people with psychosis. Trials. 2010 Nov 5;11:102. http://dx.doi.org/10.1186/1745-6215-11-102 PMid:21054847 PMCid:PMC2992058

5. Alcheva G, Gerovski F, Beletsky L. Implementation of patients' rights legislation in the Republic of Macedonia: gaps and disparities. Health Hum Rights. 2013:15(2):20-31. PMid:24421164

6. WHO-AIMS Report on Mental Health System in The Former Yugoslav Republic of Macedonia. WHO and Ministry of Health, Skopje, the Former Yugoslav Republic of Macedonia (2009). http://www.who.int/mental_health/macedonia_who_aims_report.pdf . Accessed May 22, 2016.

7. Law on Mental Health. Official Gazette of the Republic of Macedonia. 2006: 71:43-51.

8. Law on amendment and addenda to the Law on Health Care. Official Gazette of Republic of Macedonia. 2005:84:44-5.

9. Non-litigation law. Official Gazette of the Republic of Macedonia. 2008:9:40-1.

10. Memeti I, Tomsik Stojkovska A, Boskovski D, Aliu Alili M. Annual report 2012-National preventive mechanism. Ombudsman of the Republic of Macedonia, 2012:213-308.

11. Law on the Protection of Patients' rights. Official Gazette of the Republic of Macedonia. 2008:82:47-67.

12. Law on amendment and addenda to the law on the protection of patients' rights. Official Gazette of the Republic of Macedonia. 2011:53:59-61.

13. Zhang S, Mellsop G, Brink J, Wang X. Involuntary admission and treatment of patients with mental disorder. Neurosci Bull. 2015:31(1):99-112. http://dx.doi.org/10.1007/s12264-014-1493-5 PMid:25595369

14. Bislimovska-Karadzinska J, Dabovic-Anastasovska J, Chichevalieva S, et al. Human rights in mental care: A practitioner guide- Macedonia. Skopje, Republic of Macedonia, Medical Faculty, Sts. Cyril and Methodius University and Centre for Regional Policy research and Cooperation Studiorum, 2010.

15. Burris S, Wagenaar AC, Swanson J, Ibrahim JK, Wood J, Mello MM. Making the case for laws that improve health: a framework for public health law research. Milbank Q. 2010:88(2):169-210. http://dx.doi.org/10.1111/j.1468-0009.2010.00595.x PMid:20579282 PMCid:PMC2980343

ID Design 2012/DOOEL Skopje
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):461-467.
http://dx.doi.org/10.3889/oamjms.2016.052
eISSN: 1857-9655
Stomatology

Characterization and Cytotoxicity Analysis of a Ciprofloxacin Loaded Chitosan/Bioglass Scaffold on Cultured Human Periodontal Ligament Stem Cells: a Preliminary Report

Maha I. Abdelfattah[1*], Sherine A. Nasry[2], Amani A. Mostafa[3]

[1]Oro-dental Genetics Department, Human Genetics and Genome Research Division, National Research Centre, Cairo, Egypt; [2]Surgery and Oral Medicine Department, Oro-dental Research Division, National Research Centre, Cairo, Egypt; [3]Refractories, Ceramic & Building Materials Department & Nanomedicine & Tissue Engineering Laboratory, MRCE, National Research Centre, Cairo, Egypt

Citation: Abdelfattah MI, Nasry SA, Mostafa AA. Characterization and Cytotoxicity Analysis of a Ciprofloxacin Loaded Chitosan/Bioglass Scaffold on Cultured Human Periodontal Ligament Stem Cells: a Preliminary Report. Open Access Maced J Med Sci. 2016 Sep 15; 4(3):461-467. http://dx.doi.org/10.3889/oamjms.2016.052

Keywords: stem cells; periodontal; cytotoxicity; ciprofloxacin; chitosan.

***Correspondence:** Dr. Maha Ibrahim Abdelfattah. National Research Centre, Oro-dental Genetics, El Behoos street-Dokki-Giza, Cairo, Egypt. Phone: 01224509450. E-Mail: dr_maha80@yahoo.com

Received: 19-Mar-2016; **Revised:** 12-Apr-2016; **Accepted:** 13-Apr-2016; **Online first:** 22-May-2016

Competing Interests: The authors have declared that no competing interests exist.

Abstract

AIM: The aim of this study was to analyze the cytotoxicity of ciprofloxacin (CIP) loaded on chitosan bioactive glass scaffold on human periodontal ligament stem cells (PLSCs) in vitro.

MATERIALS AND METHODS: PLSCs obtained from human third molars, cultures treated with medium containing 15 x 15 mm chitosan/bioactive glass scaffolds without/with different concentration 0, 5, 10, and 20 % of CIP. A total of 15 x 10^3 cells were plated in 6 well plates. The attached cells of each group were harvested from the plates after 1, 4 and 8 days of culture to detect the viability of cells. The cell number was determined using a hemocytometer and the trypan blue dye-exclusion assay. Data was analyzed using normality using Shapiro-Wilk test. Comparisons between groups were made using One-way ANOVA complemented by Tukey's test.

RESULTS: When comparing the proliferation rate of cells in the four groups, no statistically significant difference was found (P = 0.633). With regards to cell viability, no statistical difference was found between the 0, 5, and 10 % CIP concentrations, while the 20 % CIP concentration demonstrated the least viability with a high statistically significant difference (P = 0.003).

CONCLUSION: Twenty percentages CIP demonstrated the least proliferation rate and viability.

Introduction

Periodontitis is a complex disease which occurs when environmental and bacterial factors with allelic variants of multiple genes act synergistically to increase or decrease the likelihood of developing a disease [1]. Dental pathogens are not planktonic but form complex communities called biofilms that cause chronic infections by resisting antibiotic treatments and killing by the host immune system [2].

Aggressive periodontitis (AgP) is a type of periodontal diseases that causes rapid destruction of the periodontal attachment apparatus and the supporting alveolar bone. The familial nature of AgP has led to speculation that a major gene defect is responsible for its transmission and many genetic disorders are associated with AgP such as Papillon Lefevre syndrome and Down syndrome [1]. Patients with AgP often present with limited microbial deposits that seem inconsistent with the severity of tissue destruction but often have elevated levels of Aggregatibacter actinomycetemcomitans (Aa), that possess variety of virulence factors that can impair PMNL's function and potentiate the disease process

[3] and does not respond to mechanical therapy alone as these pathogens have been found to be invasive and re-infect the pocket. Thus, augmenting mechanical therapy with antibiotics should be used to eliminate the pathogens left in the tissues [4].

Many clinical studies and systematic reviews have attested to the beneficial effects of using systemic as well as local antibiotics as an adjunctive therapy to mechanical scaling and root planning in terms of pocket reduction and attachment gain, and these effects are more pronounced in AgP patients. However, it has been reported that the benefits of systemic antibiotics administrated in periodontal therapy should be balanced against the possible side effects of the repeated use of these antibiotics in treating periodontal diseases, and that in comparison to systemic antibiotics, the application of antimicrobials by sustained-delivery devices may offer a better pocket depth reduction and gain in attachment level without the side effects seen in systemic antibiotics [5].

It has been found that both systemic antibiotics and topical chlorhexidine in patients with AgP did not reduce the percentage of invaded epithelial cells, which points out the fact that intracellular reservoirs of bacteria exist and may lead to disease recurrence and/or refractory treatment in these patients. This invasion might cause the bacteria to withstand systemic antibiotic treatment [6].

Porous three-dimensional scaffolds have the ability to attach to cells and allow their proliferation [7]. Chitosan (C), a deacetylation derivative of chitin has gained much attention as a functional material for biomedical applications due to its non-antigenicity, biocompatibility and the ability to support cell attachment and proliferation [7]. It is a potential candidate for targeting antibiotic resistant microorganism due to a broad spectrum of antimicrobial activity [8]. Bioactive glasses (G) are ideal candidates for regeneration and the incorporation of these glasses into a chitosan scaffold have been shown to provide a backbone for the scaffold [9]. Ciprofloxacin (CIP), a fluoroquinone is more effective against gram-negative bacteria, particularly Aa [10]. It is the only antibiotic used in periodontal therapy to which all strains of Aa are susceptible [11]. CIP level in the gingival crevicular fluid was also demonstrated to be significantly higher than in serum [12]. Antibiotics loaded on scaffolds and released into the periodontal pocket should not be toxic to the living cells [13]. Cells from the pulp and apical papilla were shown to be susceptible to CIP toxic effects. These effects also depend on the dosage and time of exposure of these cells to CIP [12, 13].

The purpose of this study was to evaluate the cytotoxic effect of different concentration of CIP loaded on a chitosan/glass scaffold on the proliferation and viability of PLSCs.

Materials and Methods

Fabrication of scaffold

Tetraethyl orthosilicate (TEOS: Fluka, wt = 208.33), calcium nitrate hydrate (Fluka, M.wt = 236), sodium hydroxide (Prolab, M.wt = 40) and ammonium dihydrogenphosphate (MERK, M. wt = 115.03) in addition with chitosan of medium molecular weight were used in this study. The 46S6 bioactive glass with the composition (46% SiO_2, 24% CaO, 24% Na_2O, 6% P_2O_5wt%) was prepared by sol-gel technique and named (S) [16]. A mixture of chitosan (C) in a concentration of 3% w/vand the previously prepared bioactive glass (G) of the ratio 1:2 was used in this study. CIP in different ratios: 0, 5, 10 and 20 wt% were added to the above formula during preparation and named; CIP- 0%, CIP -5%, CIP -10% and CIP -20%, respectively. The freeze drying technique was used to get porous scaffolds. The composition of all the prepared scaffold formulations was cast in a mould with dimensions1.2 x 0.3 cm, kept at -80^{o}C for overnight, and freeze dried at the same temperature for 24 h before further cell culture analysis [15,16].

Characterization

Scaffold morphology by SEM

Scanning electron microscope (SEM) coupled with energy dispersive spectroscopy (EDS) was used for morphological evaluation and elemental analysis. SEM analyses were performed on the structure of the drug and the surface of scaffolds without/with CIP.

Ciprofloxacin releases from the investigated scaffolds

Phosphate buffer saline (PBS) with pH 7.4 at 37^{o}C was used to investigate the CIP release from the scaffolds. The scaffolds were immersed in 10 ml PBS and shacked by an incubator shaker at 100 struck per minute. Samples were removed at different time intervals and the drug concentration was determined using spectrophotometer at 277 nm [19].

Sampling and Cell Culture

Healthy two lower right impacted molars, (as it is the latest erupted teeth and has the youngest cells), were extracted a traumatically, (to avoid loss of periodontal ligament during the extraction procedure), from two different healthy female patients, aged from 18-25 years. The teeth were extracted for patients attending the Clinic of the Oral and Maxillofacial Surgery Department, National Research Centre, Egypt and informed consent was obtained from the patients before extraction. The extracted teeth were immediately put in a sterile 50 ml polypropylene tube

supplemented with culture media (DMEM) (Lonza Bioproducts, Belgium). Then, the teeth were transported to the cell culture laboratory in about 20 minutes.

With totally aseptic conditions, the periodontal ligament tissue was minced into pieces and digested in a solution of 2 mg/ml Collagenase NB 4 (SERVA, crescent chemical company, USA) for 30 minutes at 37°C in a water bath. Single cell suspension was seeded into a T-25 flask (Costar, Cambridge, USA) with DMEM supplemented with 10% fetal bovine serum (FBS) (Equitech-Bio Inc., Kerrville, USA), 100 U/ml penicillin, 100 µg/ml streptomycin and 1% Fungizone **(**Lonza Bioproducts, Belgium), then incubated in 5% carbon dioxide incubator at 37°C. The samples were sub-cultured after reaching 80-85% confluence by utilizing 0.25% trypsin and 0.02% EDTA to get the next passage of cells. The third and fourth passages were used in all the coming procedures.

Detection of the self-renewal capability of PLSCs

For this assay, 100,000 cells counted by hemocytometer were plated on a T-25 flask and incubated for 10 days. Subsequently, the cultures were fixed with 4% paraformaldehyde and afterward stained with 0.1% toluidine blue. Aggregates superior to or equivalent to 50 cells were scored as colonies [20].

Identification of PLSCs by immunocytofluorescence

PLSCs were sub-cultured into 24- well cell culture plates, just as cells attained semi-confluency they were fixed with 4% paraformaldehyde for 30 min, afterward blocked with PBS containing 10% goat serum at room temperature for 30 min, and then incubated with primary antibody (Anti-STRO-1, mouse monoclonal IgM anti-human STRO- 1) (Millipore, Darmstadt, Germany) at dilution 1 µl: 200 µl of PBS for overnight at 4°C. Following washing, the samples were incubated with fluorescein- conjugated secondary antibody (goat anti-mouse IgM) for 60 minutes in the dark. Regarding nuclei staining, DAPI (4, 6-diamino-2-phenylindol) was used for five minutes. Following washing with PBS, the samples were examined by fluorescence microscopy with 40 X original magnification [21].

Multilineage differentiation

For osteogenic differentiation in vitro, PLSCs cultures were supplemented with 0.01 µmol/L dexamethasone, and 1.8 mmol/L inorganic phosphates (Sigma-Aldrich, United States). The medium was changed twice weekly. After that, the samples were stained after 14 days by 2% alizarin red stain to detect calcium accumulation in vitro [22]. For adipogenic differentiation in vitro, PLSCs cultures were supplemented with 0·5 µmol/L isobutylmethylxanthine, 0·5 µmol/L hydrocortisone, 60 µmol/L indomethacin and 10 µg ml insulin (Sigma-Aldrich, United States) for 21 days to induce adipogenic differentiation, then Oil red O staining was applied to identify lipid-laden fat cells [21].

Cytotoxic analysis of scaffold-CIP on cultured human PLSCs.

The scaffolds (15x15 mm) were first sterilized by UV radiation of the laminar flow (30 min for each side), followed by 30 min immersion in 70 % ethanol [23]. A total of 15 x 10^3 cells were plated in 6 well plates, after 24 hours of culture, the culture medium was changed. Afterward, chitosan bioglass loaded with different ciprofloxacin concentrations (5, 10, and 20%) and 0 % (positive control) was added. After 1, 4 and 8 days of culture, the cells were harvested and the cell number was determined by counting the viable cells by a hemocytometer using the trypan blue dye-exclusion assay, where 100 micro liter of trypan blue was mixed to 100 micro liter of treated cellular suspension and left for 10 minutes, then 10 µl of the mixture was spread into both chambers of the hemocytometer. Subsequently, the hemacytometer was observed under an inverted light microscope using the 20 x objective lens. The number of viable cells harvested was obtained by the following equation: UC×D×104/nSQ, where UC is the unstained cell count (viable cells), D is the dilution of the cell suspension, and nSQ is the number of counted squares in the hemocytometer. Viable cells appear colorless and bright under phase contrast microscopy. Nonviable cells appear blue-stained and are non-refractile. The viable percentage of the cell population was obtained using the equation: UC/TC× 100, where UC is the unstained cell count (viable cells) and TC is the total cell count (stained plus unstained cells) [24].

Statistical Analysis

Analysis of data was performed utilizing SPSS 18 (Statistical Package for Scientific Studies) for Windows. Data were explored for normality using Shapiro-Wilk test. Comparisons between groups were made utilizing One-way ANOVA complemented by Tukey's test. The level of significance was $p < 0.05$.

Results

Representative SEM micrographs of the synthesized scaffolds (CIP-0%) are presented in (Fig. 1), where it can be noticed that the addition of (G) to

the (C) scaffold gave a homogeneous structure of the scaffold with a rough texture compared to the smooth structure of a previously prepared chitosan scaffold alone. The mean pore diameter of the prepared scaffolds ranged between 40-60 μm, pointing to the wide range of interconnected pores of the scaffolds which can facilitate cell migration, adhesion, and proliferation.

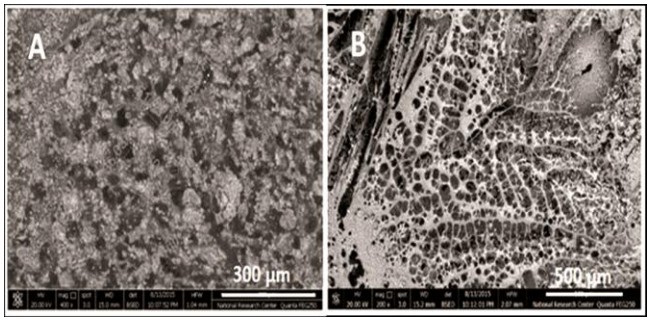

Figure 1: Scanning Electron Micrograph of scaffold without ciprofloxacin (CIP-0%), the glass particles homogeneously distributed as in (A) and the porosity are in the range of 40-60 μm (B), magnification 300 μm and 500 μm, respectively

The SEM micrographs and the elemental analyses of the CIP drug denoted in Fig. 2A,B, while the prepared scaffold loaded with (CIP-20%) presented in Fig. 2 C, D. The rod shape of the drug could be seen, where the elemental analysis proved the presence of carbon (C) and oxygen (O) of the drug.

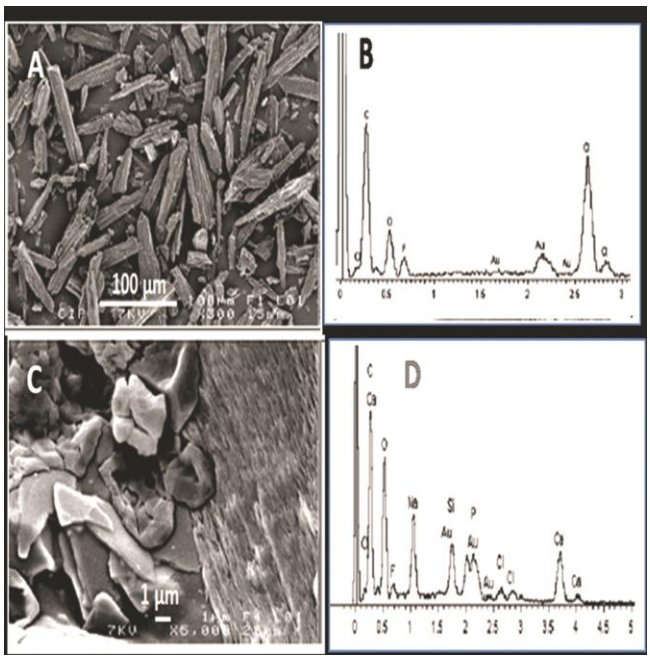

Figure 2: SEM micrographs and EDS of: the drug ciprofloxacin, CIP (A, B) with magnification 100μm, rod shape of the drug (A) elements of C and O appears in (B) and for chitosan/ bioactive glass scaffold loaded with CIP-20% (C,D) with magnification 1 μm, the glass crystals appear and the rod shape of the drug embedded in the chitosan matrix (C) and the elements of Ca, Na, Si and P of the glass beside that of the drug C and O (D)

However, addition of the glass in the scaffold was confirmed by the presence of silicon (Si), phosphorous (P), sodium (Na) and calcium (Ca) in addition to (C) and (O) of the drug, which indicates the homogenous incorporation of the glass in the structure of the scaffold. CIP concentrations above 5 wt% led to the formation of fibers with significantly smaller diameters than PDS ($p < 0.001$).

In our assay, it is obvious that the drug release increased with increasing the drug concentration (from 5, 10 and 20 %). This is attributed to the difference between the concentration of the drug in the scaffold and that in the surrounding media, which could be explained by the large driving force of the drug release.

Cell culture

PLSCs were successfully isolated from extracted impacted teeth. By observation of PLSCs under inverted microscopy, they appeared as fibroblast-like shaped cells after 3 days of culturing, which are characteristic to PLSCs. The cultured cells were rapidly proliferated till became confluent about 80%-90% after 7 days of culturing.

Colony forming unit-fibroblast (CFU-F) assay

PDLSCs possess the potential to form clonogenic cell clusters, detected by the development of about 120 colonies, engendered from 100,000 single cells after culturing at low density, suggesting the large proliferative capacity for PLSCs. The cells within each colony were defined with a fibroblast-like morphology as revealed in (Fig. 3A).

Immunocytofluorescence

Indirect immunocytofluorescence technique was utilized to identify STRO 1 antigen. In the current research, PDLSCs that was isolated were discovered to express the mesenchymal stem-cell marker STRO - 1 as demonstrated in (Fig. 3B).

Multilineage differentiation

On behalf of the osteogenic potential of PLSCs, our findings revealed that isolated PDLSCs revealed positive staining for calcium accumulation in vitro by utilizing Alizarin red stain after 14 days of culturing in osteogenic media (Fig. 3C). In addition, for the adipogenic potential of PLSCs, our results presented that PLSCs expressed oil red O-positive lipid clusters after 21 days of induction (Fig. 3D).

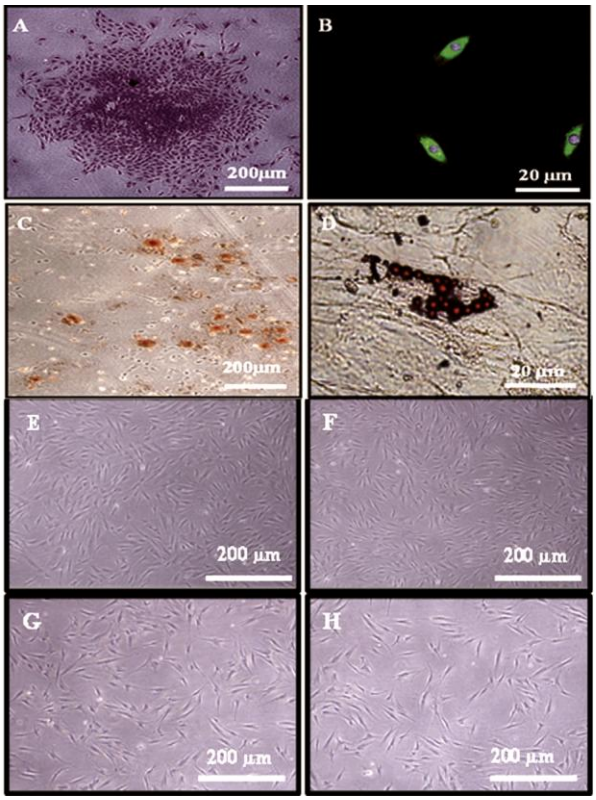

Figure 3: (A) Phase contrast micrograph showing a colony formed by PDLSCs after staining by toluidine blue stain. (4X). (B) Phase contrast micrograph showing PDLSCs express the MSC marker STRO-1 (Green colour). Notice counter stain of the nuclei by DAPI (Blue colour). (Indirect immunocytofluorescence, 40X). (C) Phase contrast micrograph showing extracellular calcium accumulation (Orange - red nodules) by differentiated PLSCs after 14 days of osteogenic induction (Alizarin red stain, 4X), and (D) Fat droplets formed by PLSCs after 21 days of adipogenic induction (oil red O stain, 40X). Phase contrast micrograph showing PLSCs after 8 days of culturing with scaffold loaded with (E) 0% Cipro, (F) 5% Cipro, (G) 10% Cipro and (H) 20% Cipro, maintained their PLSCs morphological characteristics

Cytotoxic analysis of scaffold-CIP on cultured human PLSCs

Results are summarized in Tables 1 & 2. PLSCs after 8 days of culturing with CIP with different concentrations retained their PLSCs morphological characteristics compared to control untreated cells as shown in Fig. 3 E,F,G,H. After 24-hour time period, CIP 0%, CIP 5%, CIP 10% resulted in cell proliferation rates of 85×10^3, 65×10^3, 55×10^3, respectively and viabilities of 100 % in all three groups, while the CIP group of 20 % concentration resulted in cell proliferation rate of 50 $\times10^3$ and viability of 98 %. After 4 days, the cell proliferation rates of the CIP 0%, CIP 5%, CIP 10% and CIP 20% ciprofloxacin groups resulted in 180×10^3, 120×10^3, 90×10^3 and 80 $\times10^3$ respectively, while the cell viabilities were 100% in the CIP 0% and CIP 5% concentration and 99 and 98 % in the CIP 10 % and CIP 20 % concentration groups, respectively. At the 8th day, the CIP 0%, CIP 5%, CIP 10% and CIP %20 concentration groups showed variable cell proliferation rates of 295×10^3, 245×10^3, 180×10^3, and 165×10^3, respectively. The cell viability remained 100% in the CIP 0% and CIP 5% concentration, and also remained at 99 % for the third group CIP 10% at 99%, while it showed the least viability in the CIP 20 % group at 96%.

Table 1: The cell proliferation rate for tested scaffolds at different time intervals

Group	Day 1	Day 4	Day 8	P-value
CIP- 0%	85 x 10^3	180 x 10^3	295 x 10^3	0.633
CIP- 5%	65 x 10^3	120 x 10^3	245x 10^3	
CIP- 10%	55 x 10^3	90 x 10^3	180 x 10^3	
CIP- 20%	50 x 10^3	80 x 10^3	165x 10^3	

When comparing the proliferation rate of cells between the four concentrations groups, no statistical significance difference was found (P = 0.633). With regards to cell viability, no statistical difference was found between the 0, 5, and 10 % CIP concentrations, while the 20 % CIP concentration demonstrated the least viability with a high statistical difference (P = 0.003).

Table 2: Cell viability for tested scaffolds at different time intervals

	Cell viability at:			
Group	Day 1	Day 4	Day 8	P-value
CIP- 0%[a]	100%	100%	100%	0.633
CIP- 5%[a]	100%	100%	100%	
CIP- 10%[a]	100%	99%	99%	
CIP- 20%[b]	98%	98%	96%	
The same superscript letters indicate statistically non-significant values (p > 0.05).				

Discussion

Aggressive periodontal diseases are caused by particular groups of microorganisms as A.a which is not eliminated by mechanical means alone due to the bacterial invasion, accordingly, adjunct anti-infective therapy is recommended. Periodontal disease treatment necessitates an anti-infective agent to infection sites and sustaining its localized concentration at effective levels for a sufficient time whereas at the same time evoking minimal or no side effects [25].

Unfortunately, the regular systemic antibiotic protocol for the treatment of AgP was found to be not effective in eradicating bacteria that reside within the epithelial cells. Although such treatment resulted in a reduction of bacteria and improved the clinical parameters, the number of invaded epithelial cells was not reduced [6]. Thus, local antibiotics are proposed compared to systemic antibiotics in terms of providing adequate dose and time to eradicate the infection and, also, prevent relapse without the side effects [26].

The use of drug delivery systems containing antimicrobial agents is advocated as it aims for releasing a sufficient level of the drug directly inside the periodontal pocket. Moreover, increasing the exposure of target microorganisms to higher concentrations could be realizing, thus minimizing the

side effects associated with systemic drug administration. This, also, helps in patients where there is an intolerance to systemic administration and serves as a treatment option for localized areas of exudation and deep pockets not responding well to mechanical and systemic antibiotics [23, 24].

The use of antibiotic-loaded scaffold combined with bioactive glass as targeting drug-delivery systems for treatment of AP may allow the release of the drug directly into the periodontal pocket thus minimizes undesirable side effects caused by systemic drug administration and may also enhance patient compliance. However, these drugs should not exert any toxic effect on the underlying tissue. Therefore, this study aimed at evaluating the cytotoxic effects of different concentration of CIP on the viability and rate of proliferation of PDL stem cells. CIP was selected in the present study because, at present, it is the only antibiotic in periodontal therapy to which all strains of A.a. are susceptible. Previous results suggested that exposure of periodontal surfaces to CIP reduced the micro-colony size and cell surface density of A.a in the biofilm. Moreover, A.a resistance to CIP is rare or non-existent. It was also suggested that CIP retained bactericidal activity inside PMNs ultimately contributed to the enhanced intracellular killing of susceptible bacteria [10].

The appropriate scaffold fabrication technique is a critical choice as it can significantly influence the scaffold properties and its degradation rate [29]. In a former study fibroblasts attached successfully to chitosan proving that it is not only a biocompatible but also a successful tissue engineering scaffold [30]. In the present study, a chitosan/glass scaffold loaded with different concentration of CIP was formulated using the freeze-drying technique. This technique provides a convenient and easy mean for formulating highly porous scaffold, with interconnected pores, that has the ability to enhance regeneration. The bioactive glass was incorporated into the chitosan scaffold to adjust the quality of pores, the mechanical strength and degradation rate of chitosan scaffolds through [31].

In vitro testing using primary cells, as stem cells, to test the cytotoxicity of dental materials is more preferable than using established cell lines as these cells have been cultured for the first time, and are therefore similar to their original tissue. They are characterized by their largely unchanged metabolic status, and a high degree of differentiation [24]. PLSCs were used as target cells in the present study because they are easily accessible and are able to differentiate into other types of dental cells [32]. In the present study, CFU-F assay realized the successful self-renewal capacity of PLSCs from the primary cell culture, as well as indirect immunocytofluorescence, revealed that PLSCs are stained positive for the early MSC marker (STRO-1). Additionally, multilineage differentiation (osteogenic and adipogenic) of PLSCs was emphasized. The osteogenic potentiality was detected by orange – red nodules of calcium accumulation in vitro using alizarin red satin after 14 days of induction. The adipogenic potentiality of PLSCs was shown through their positive lipid clusters after 21 days of induction. These findings demonstrated that isolated periodontal ligament cells fulfill the criteria of mesenchymal stem cells. Our results are in agreement with other investigators [17, 18]. The trypan blue exclusion staining technique was chosen to differentiate nonviable from viable cells as it is a preferred assay due to its quickness and ease of performance [24].

It was found that the process of drug release increased when the concentration of CIP increased from 5 to 20 % which was related to the increase in the concentration gradient between the drug in the scaffold and the surrounding medium. Dental cells were found to be susceptible to the toxic effects of CIP in terms of time and dosage, where decreasing drug concentrations and time of exposure may significantly improve stem cell viability [14]. In the present study high concentration of CIP (20%) decreased growth rate and viability of PLSCs. In a former study, 10 and 20 mg/ml concentration of CIP significantly reduced the rate of growth of human embryonic stem cells but did not affect their viability and differentiation characteristics. The cytotoxic effect of CIP was however reversed when the antibiotic was withdrawn and the cells regained their normal growth rate [13]. Also, previous data demonstrated that high concentrations of antibiotics are harmful to the survival of stem cells of the apical papilla and that it is important that when using bactericidal medications they should not have a detrimental effect on the stem cell viability [15].

In conclusion, based on the results of this study we suggest that 5% CIP concentration might be a suitable concentration because it was less toxic to the cells than other concentrations. Our results, however, are preliminary and require further preclinical and in vivo research, to confirm the safety and effectiveness of this drug and ensure that its biological activity is retained in the delivery system.

Acknowledgment

The authors wish to thank Dr. Phoebe Magdy Abd El-Massieh, Assistant Researcher at the Oro-Dental Genetics Department, National Research Centre, Cairo, Egypt for her technical support.

References

1. Bali D, Pandit N, Kathuria R, Bali A. Genetics and Aggressive Periodontal Disease : An Update Review. J Oral Heal Community Dent. 2012;6:97–101.

2. Leid JG. Bacterial Biofilms Resist Key Host Defenses. Microbe.

2009;4(2):66–70.

3. Mukherjee S(1) KD. Study of neutrophils isolated from peripheral blood of patients suffering from aggressive periodontitis at the cellular level: Receptors and cytoskeletal reorganization. J Indian Soc Periodontol. 2012;16(1):59–64. http://dx.doi.org/10.4103/0972-124X.94606 PMid:22628965 PMCid:PMC3357037

4. Thanakun S, Pornprasertsuk-Damrongsri S, Gokyu M, Kobayashi H, Izumi Y. Inverse Association of Plasma IgG Antibody to Aggregatibacter actinomycetemcomitans and High C-Reactive Protein Levels in Patients with Metabolic Syndrome and Periodontitis. PLoS One. 2016;11(2):e0148638. http://dx.doi.org/10.1371/journal.pone.0148638 PMid:26871443 PMCid:PMC4752452

5. Jepsen K, Jepsen S. Antibiotics / antimicrobials : systemic and local administration in the therapy of mild to moderately advanced periodontitis. Periodontol 2000. 2016;71:82–112. http://dx.doi.org/10.1111/prd.12121 PMid:27045432

6. Johnson JD, Chen R, Lenton PA, Zhang G, Hinrichs JE, Rudney JD. Persistence of extracrevicular bacterial reservoirs after treatment of aggressive periodontitis. J Periodontol. 2008;79(12):2305–12. http://dx.doi.org/10.1902/jop.2008.080254 PMid:19053921 PMCid:PMC2597013

7. Semwal A, Singh R, Dutta PK. Chitosan: A Promising Substrate for Pharmaceuticals. J Chitin Chitosan Sci. 2013;1(2):87–102. http://dx.doi.org/10.1166/jcc.2013.1012

8. Pichayakorn W, Boonme P. Evaluation of cross-linked chitosan microparticles containing metronidazole for periodontitis treatment. Mater Sci Eng C Mater Biol Appl. 2013 1;33(3):1197–202.

9. Yao Q, Li W, Yu S, Ma L, Jin D, Boccaccini AR, et al. Multifunctional chitosan/polyvinyl pyrrolidone/45S5 Bioglass® scaffolds for MC3T3-E1 cell stimulation and drug release. Mater Sci Eng C Mater Biol Appl. 2015 1;56:473–80. http://dx.doi.org/10.1016/j.msec.2015.06.046 PMid:26249617

10. Suci P, Young M. Selective killing of Aggregatibacter actinomycetemcomitans by ciprofloxacin during development of a dual species biofilm with Streptococcus sanguinis. Arch Oral Biol. 2011;56(10):1055–63. http://dx.doi.org/10.1016/j.archoralbio.2011.03.013 PMid:21507381

11. Prakasam A, Elavarasu SS, Natarajan RK. Antibiotics in the management of aggressive periodontitis. Journal of pharmacy & bioallied sciences. 2012; 4:252–5. http://dx.doi.org/10.4103/0975-7406.100226 PMid:23066264 PMCid:PMC3467876

12. Tezel A, Yucel O, Orbak R, Kara C, Kavrut F, Yagiz H, et al. The gingival crevicular fluid ciprofloxacin level in subjects with gingivitis and periodontitis, and its effects on clinical parameters. J Periodontal Res. 2005;40(5):395–400. http://dx.doi.org/10.1111/j.1600-0765.2005.00820.x PMid:16105092

13. Romorini L, Riva DA, Blüguermann C, Videla Richardson GA, Scassa ME, Sevlever GE, et al. Effect of antibiotics against Mycoplasma sp. on human embryonic stem cells undifferentiated status, pluripotency, cell viability and growth. PLoS One. 2013;8(7):e70267. http://dx.doi.org/10.1371/journal.pone.0070267 PMid:23936178 PMCid:PMC3728093

14. Chuensombat S, Khemaleelakul S, Chattipakorn S, Srisuwan T. Cytotoxic effects and antibacterial efficacy of a 3-antibiotic combination: an in vitro study. J Endod. 2013;39(6):813–9. http://dx.doi.org/10.1016/j.joen.2012.11.041 PMid:23683284

15. Ruparel NB, Teixeira FB, Ferraz CCR, Diogenes A. Direct effect of intracanal medicaments on survival of stem cells of the apical papilla. J Endod. 2012;38(10):1372–5. http://dx.doi.org/10.1016/j.joen.2012.06.018 PMid:22980180

16. Mabrouk M, Mostafa A, Oudadesse H, Wers E, Lucas-Girot MIE-G A. Study of Nanobioactive Glass Quaternary System 46S6. Bioceram Dev Appl. 2014;4:1000072.

17. Mabrouk M, Mostafa AA, Oudadesse H, Mahmoud AA, Gaafar AM, El-Gohary MI. Fabrication, Characterization and Drug Release of Ciprofloxacin Loaded Porous Polyvinyl Alcohol / Bioactive Glass Scaffold for Controlled Drug Delivery. Bioceram Dev Appl. 2013;S1(1):2–5.

18. Mabrouk M, Mostafa AA, Oudadesse H, Mahmoud AA, El-Gohary MI. Bioactivity and drug delivering ability of a chitosan/46S6 melted bioactive glass biocomposite scaffold. Int Ceram Rev. 2013;62(6):444–52.

19. Mostafa AA, Zaazou MH, Chow LC, Mahmoud AA, Zaki DY, Basha M, et al. Injectable nanoamorphous calcium phosphate based in situ gel systems for the treatment of periapical lesions. Biomed Mater. 2015;10(6):065006. http://dx.doi.org/10.1088/1748-6041/10/6/065006 PMid:26541174

20. Liu J, Wang L, Liu W, Li Q, Jin Z, Jin Y. Dental follicle cells rescue the regenerative capacity of periodontal ligament stem cells in an inflammatory microenvironment. PLoS One. 2014;9(9):e108752. http://dx.doi.org/10.1371/journal.pone.0108752 PMid:25275580 PMCid:PMC4183515

21. Lee S-Y, Chiang P-C, Tsai Y-H, Tsai S-Y, Jeng J-H, Kawata T, et al. Effects of cryopreservation of intact teeth on the isolated dental pulp stem cells. Journal of Endodontics. 2010; 36:1336–40. http://dx.doi.org/10.1016/j.joen.2010.04.015 PMid:20647092

22. Perry BC, Zhou D, Wu X, Yang F-C, Byers M a, Chu T-MG et al. Collection, cryopreservation, and characterization of human dental pulp-derived mesenchymal stem cells for banking and clinical use. Tissue Eng Part C Methods. 2008;14(2):149–56. http://dx.doi.org/10.1089/ten.tec.2008.0031 PMid:18489245 PMCid:PMC2963629

23. Kamocki K, Nör JE, Bottino MC. Effects of ciprofloxacin-containing antimicrobial scaffolds on dental pulp stem cell viability-In vitro studies. Arch Oral Biol. 2015;60(8):1131–7. http://dx.doi.org/10.1016/j.archoralbio.2015.05.002 PMid:26042622

24. Er K, Polat ZA, Özan F, Taşdemir T, Sezer U, Siso SH. Cytotoxicity analysis of strontium ranelate on cultured human periodontal ligament fibroblasts: A preliminary report. J Formos Med Assoc. 2008;107(8):609–15. http://dx.doi.org/10.1016/S0929-6646(08)60178-3

25. Kaplish V, Walia MK, Kumar SLH. Pharmacophore. Pharmacophore. 2013;4(2):39–49.

26. Koort JK, Makinen TJ, Suokas E, Veiranto M, Jalava J, Tormala P, et al. Sustained release of ciprofloxacin from an osteoconductive poly(DL)-lactide implant. Acta Orthop. 2008;79(2):295–301. http://dx.doi.org/10.1080/17453670710015111 PMid:18484258

27. Joshi D, Garg T, Goyal AK, Rath G. Advanced drug delivery approaches against periodontitis. Drug Deliv. 2016;23(2):363–77. http://dx.doi.org/10.3109/10717544.2014.935531 PMid:25005586

28. Roshna T, Nandakumar K. Generalized Aggressive Periodontitis and Its Treatment Options: Case Reports and Review of the Literature. Case Rep Med. 2012;2012:1–17. http://dx.doi.org/10.1155/2012/535321 PMid:22291715 PMCid:PMC3265097

29. Yang S, Leong KF, Du Z, Chua CK. The design of scaffolds for use in tissue engineering. Part I. Traditional factors. Tissue Eng. 2001;7(6):679–89. http://dx.doi.org/10.1089/107632701753337645 PMid:11749726

30. Mochizuki M, Kadoya Y, Wakabayashi Y, Kato K, Okazaki I, Yamada M, et al. Laminin-1 peptide-conjugated chitosan membranes as a novel approach for cell engineering. FASEB J. 2003;17(8):875–7. http://dx.doi.org/10.1096/fj.02-0564fje

31. Catauro M, Renella RA, Papale F, Vecchio Ciprioti S. Investigation of bioactivity, biocompatibility and thermal behavior of sol-gel silica glass containing a high PEG percentage. Mater Sci Eng C Mater Biol Appl. 2016 1;61:51–5. http://dx.doi.org/10.1016/j.msec.2015.11.077 PMid:26838823

32. Rodríguez-Lozano FJ, García-Bernal D, Aznar-Cervantes S, Ros-Roca MA, Algueró MC, Atucha NM, et al. Effects of composite films of silk fibroin and graphene oxide on the proliferation, cell viability and mesenchymal phenotype of periodontal ligament stem cells. J Mater Sci Mater Med. 2014;25(12):2731–41. http://dx.doi.org/10.1007/s10856-014-5293-2 PMid:25081645

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):468-475.
http://dx.doi.org/10.3889/oamjms.2016.067
eISSN: 1857-9655
Stomatology

Midfacial Reconstruction – A Systematic Review

Aala Emara[1], Adel Abou ElFetouh[1], Maha Hakam[1], Basma Mostafa[2*]

[1]Oral Maxillofacial Department, Faculty of Dentistry, Cairo University, Cairo, Egypt; [2]Surgery and Oral Medicine Department, Oral and Dental Research Division, National Research Centre, Cairo, Egypt

Citation: Emara A, Abou ElFetouh A, Hakam M, Mostafa B. Midfacial Reconstruction – A Systematic Review. Open Access Maced J Med Sci. 2016 Sep 15; 4(3):468-475. http://dx.doi.org/10.3889/oamjms.2016.067

Keywords: Midface; reconstruction; oncologic defect; maxillectomy; Maxilla.

***Correspondence:** Basma Mostafa, Assistant Professor of Oral Medicine and Periodontology, Surgery and Oral Medicine Dep., Oral and Dental Research Division, National Research Centre. E-mail: boshta@hotmail.com

Received: 15-Apr-2016; **Revised:** 31-May-2016; **Accepted:** 01-Jun-2016; **Online first:** 17-Jun-2016

Competing Interests: The authors have declared that no competing interests exist.

Abstract

AIM: Different lesions affecting the midfacial regions require surgical reconstruction. The aim of this study was to assess the different methods used in midfacial reconstruction after maxillectomy procedures. The various reported surgical reconstructive techniques focusing on the esthetic and functional outcomes are to be reviewed in this article.

MATERIAL AND METHODS: A thorough PUBMED and hand-search of journals of relevance was performed on related terms and yielded 772 titles of which 45 abstracts were selected and obtained as full articles for further evaluation while the rest were excluded by title/abstract. According to the inclusion criteria; 14 of these studies were used to complete this article.

RESULTS: In this review we showed that fibular and radial vascularized grafts were the most commonly reported methods in literature with a few other options. Computer aided design and surgical planning has been also reviewed and seems to be a rapidly evolving option for maxillofacial reconstruction. Lack of RCTs (randomized controlled trials) and large scale case series was noticed in this review making the evidence of poor quality.

CONCLUSION: Methods of evaluation of reconstruction options mainly qualitative and subjective made the evaluation of the techniques in this review difficult.

Introduction

Benign and malignant lesions commonly affecting the maxillary/midface regions require surgical ablation. The surgical removal of such lesions leaves behind a defect of esthetic and functional components. The esthetic component of the defect is caused by the loss of facial support of the soft tissues related to the region especially the cheek prominence resulting in facial asymmetry. In cases where the defect extended to the orbital region causing orbital exenteration the esthetic disfigurement is paramount. The functional problems are caused by the loss of the maxillary alveolar process with subsequent loss of the masticatory function. Moreover, the speech and cosmetic problems they cause; make their reconstruction very difficult [1].

Another functional issue encountered is the oro-nasal/antral communication which occurs in cases of lesions perforating the antral / nasal floor. Palatal defects may be covered by acrylic obturators and sparing the patient the sequelae of oro-antral communication [2]. Obturators have historically been the sole reconstructive option till the introduction of microvascular surgical techniques [3]. Microvascular surgery is widely used to reconstruct maxillary-midface defects overcoming the patient's dissatisfaction with obturators' drawbacks and donor site selection [4]. Microvascular reconstruction provides better esthetics, mastication and speech than obturators.

On the other hand, microvascular procedures are complex procedures requiring special training. The donor site needed to harvest the graft adds to the surgical morbidity, increases the intraoperative time and may require more than one surgical reconstructive procedure [5, 6]. The microsurgical

techniques introduced are still being updated and researched greatly; there still remains its complex nature and the complications accompanied with it [3].

The various surgical reconstructive techniques used focusing on the esthetic and functional outcomes are to be reviewed in this systematic review.

Materials and Methods

This review aimed to study the different reported techniques of midfacial reconstruction.

Search strategy

A search in MEDLINE (Pub Med) was performed using the following search query:

- #1 midface
- #2 rehabilitation OR reconstruction.

A hand search of international journals in the scope of maxillofacial surgery was also performed to identify any skipped relevant articles (British journal of oral and maxillofacial surgery (OMFS), International journal of OMFS, Journal of OMFS, Journal of craniofacial surgery, Journal of craniomaxillofacial surgery, Journal of plastic and reconstructive surgery, Journal of aesthetic plastic surgery, Journal of clinical oral investigation, Journal of clinical otorhinolaryngeology, Journal of craniomaxillofacial trauma and reconstruction, Head & neck journal, Annals of plastic surgery, Journal of prosthetic dentistry, Journal of oral surgery-pathology-radiology-endodontics).

Study selection

The results of this search yielded 772 titles that were screened by the authors. Forty five of the resulting titles were chosen for abstract evaluation. Clinical trials reporting different techniques of maxillary reconstruction of pathologic defects were selected and after screening 29 articles were obtained as full articles and studied thoroughly and those not fulfilling the inclusion criteria were excluded.

The 14 publications fulfilling the inclusion criteria were selected to perform this review. Author disagreements were negotiated till satisfactory results reached (Figure 1).

Clinical trials reporting different techniques of maxillary reconstruction of pathologic defects were selected and after screening at each level studies were excluded according to the following exclusion criteria.

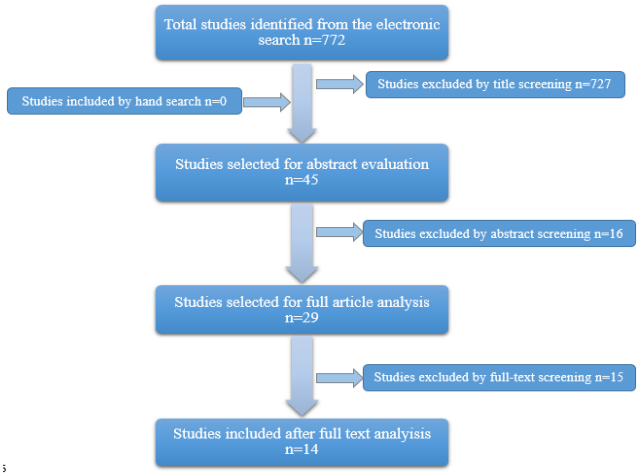

Figure 1: Study selection process

Exclusion criteria

- Traumatic fractures of the face (non-pathologic defects)
- Cosmetic / plastic surgeries
- Animal studies
- Studies evaluating surgical approach rather than reconstruction technique
- Soft tissue defects without a bony component
- In languages other than English
- Non clinical trials / review articles

Data Collection

Data collection forms were custom designed for the selected articles including the following items:

- Authors & study date
- Study type
- Number of patients
- Age of patients
- Treatment provided
- Follow up duration
- Patient satisfaction
- Histopathological assessment

The selected articles were studied according to the method of reconstruction used and outcome accomplished. The different methods of reconstruction reported included; iliac grafts , fibular grafts, radial forearm free flap, scapular, calvarial, computer guided procedures, distraction ostoegenesis or a combination of two of these techniques together.

Critical appraisal

Risk of bias was assessed according to study design, randomized selection, specification of the inclusion/exclusion criteria, reporting of lost follow-up and complications, objective evaluation and statistical analysis of the results.

Results

Of the 14 articles selected none were randomized controlled trial; the study design was either retrospective (6 studies), case report (5 studies) or case series (3 studies) and so no meta- analysis was possible and the results will be presented in a descriptive manner. Data was collected from the selected articles in customized forms and tabulated (Table 1) and the risk of bias assessment presented in Table 2.

The articles were published in the period from 1998 to 2015. The outcome in all of them was a subjective assessment of esthetic and functional patient satisfaction and so no numerical evaluation of the different reconstruction techniques was possible. Histopathologic assessment of the lesions in most cases reported a malignant pathology; and in some cases adjunctive chemotherapy and/or radiotherapy was necessary. Risk of bias in all selected articles was substantially high due to the study design which was either retrospective studies or prospective non-controlled case series/reports. This literature review will therefore only present the studies narrative without any meta-analysis.

Fibular graft

A total of 55 cases of reconstruction using fibular grafts were found in the search of which 3 were associated with an anterolateral thigh soft tissue flap. Moreno et al. reported the use of an osteocutaneous fibular graft in 11 patients with total loss of the graft in one patient; and partial flap loss of less than 10 % in 2 cases A combination of an ALT and an osteocutaneous fibular graft was reported in 3 of these cases with reported uneventful healing and successful grafting [7]. An osteomyocutaneous fibular graft was used to reconstruct a midface defect using a 3D simulation technique reported as a case report. The patient's preoperative computed tomography (CT) scans were imported and a virtual osteotomies and graft harvesting performed. The fibular graft to be harvested was adapted virtually as well to reach the best esthetic result preoperatively and to reduce intraoperative time. A 3D resin model of the final reconstructed midface was printed to aid in intraoperative orientation. The authors reported patient's satisfaction with the function and esthetics; dental rehabilitation was provided 6 months postoperatively [8].

Table 1: list of selected articles

Study – publication, yr	Study design	No. of pts	Mean age (Year)	TTT provided	Followup mean (months)	Histopathologic report	Results (patient satisfaction)
Mueller et al. 2014	Retro.	10	65	-RFFF 3 -Fibular 1 -RFFF + ALT 1 -Iliac +ALT 1	43	SCC Adenoid CC BCC AB	5/10 wore prosthesis 3/10 back to social life
Moreno et al. 2010	Retro.	18	50.8	-Fibular 11 -Fibula +ALT 3 -RFFF 2 -Lateral arm 1 -Serratus composite 1	27.3	N	Unrestricted diet 55% Excellent speech 47.5%
Mertens et al. 2013	Case report	1	50	-Scapular +pt. specific implant	48	Undiff. Pl. S.	Good esthetics after corrective surgery
Echo et al. 2013	Case report	1	52	-MEDPOR implant	9	Cellular myoma	Acceptable esthetics
Dediol et al. 2013	Case series	7	57	-Titanium mesh + LD 1 + ALT 4 + Fibular 1	15	SCC SAC Periosteal sarcoma	Average facial appearance grade = Excellent
Shaw et al. 2009	Case report	1	62	-Ost.my.c. DCIAP flap	1	Antral SCC	Esthetics = Good
Andrades et al. 2008	Retro.	23	67	-Ost.c. RFFF	11	SCC Sarcoma Melanoma	Esthetics = Good
Chepeha et al. 2005	Case series	7	50	-Ost.c. RFFF	27.9	SCC Spindle CC Hemangio.p.c Sarcoma AB Ad. CC	Esthetics = Very good
Bidros et al. 2005	Case report	2	27.5	-TDAPSOC flap	8	Epitheliod s. Adenoid CC	N
Parhiscar et al. 2002	Retro.	2	50	-TOF flap	27	SCC Haemangioma	N
Futran et al. 2002	Retro.	27	59	-Ost.C. Fibular	26	N	Diet:14 normal, 13 soft only; Speech: all good Cosmetics: 14 excellent, 8 good, 4 fair, 1 poor with obturator
Pollice et al. 1988	Case series	6	59.5	-Calvarial +TPF	6.5	AB Ad. CC SCC Met.Thy.C.	Good 1 with moderate deformity
Schmelzem et al. 1998	Retro.	8	47.75	-Scapular	N	Osteosarcoma SCC Oss fibroma	2nd correctional surgery needed in 5 cases
He et al. 2009	Case report	1	21	-3D fibula	6	Osteosarcoma	Good esthetics and function

AB: Ameloblastoma, Ad. CC: Adenoid cystic carcinoma, ALT: anterolateral thigh, BCC: Basal Cell Carcinoma, Met.Thy.C.: Metastatic thyroid carcinoma RFFF: radial forearm flap, LD: Latissmus Dorsi, DCIAP: Deep circumflex iliac artery perforator, OSS: osseus, Ost.my.c: osteomyocutaneous flap, pt: patient, SAC: Sinoantral carcinoma, SCCC: Squamous Cell Carcinoma TOF: Temporoparietal osteofascial, TDAPSOC: Thoracodorsal Artery Perforator-Scapular Osteocutaneous, TPF: Temporoparietal fascial, Retro: retrospective, Undiff Pl. S.: undifferentiated pleomorphic sarcoma.

The patients reported excellent (14/27), good (8/27), fair (4/27) and poor (1/27) esthetics. Regarding function, the study reported regaining of normal diet in 14 patients while 13 others only followed a soft diet regimen. The speech of all patients in this retrospective study was reported to be intelligible over the phone. The feasibility of elongating the vascular pedicle when necessary as applied in several cases in this study is another advantage of the fibular graft [9]. A single case report published in 2013 showed uneventful healing and good esthetic outcome of the use of fibular grafts along with a patient specific titanium mesh [10].

Another study used fibular osteocutaneous reconstruction for 27 cases with midfacial defects. Dental implants were placed 3-6 months after the initial surgery and 6 months later the maxillary prosthesis was placed. The study reported a single case of flap failure, while 5 other required intervention to salvage the flap.

Iliac grafts

Three cases of reconstruction using iliac grafting were found in the selected articles. The use of iliac bone accompanied by the insertion of implants into simultaneously harvested anterolateral thigh flaps was reported in Mueller et al' s study. The final prosthesis was installed after an average of 13 months postoperatively. Patients wore the prosthesis and were satisfied to an extent although only 3 of the patients wore the prosthesis publicly [11]. Osteomyocutaneous deep circumflex iliac artery perforator was reported to reconstruct the defect caused by midfacial squamous cell carcinoma. The patient showed excellent healing and regain of function and esthetics [12].

Radial forearm free flap

In reviewing the literature, 40 cases of reconstruction using RFFF as the primary reconstruction graft were found; of which 3 cases were accompanied by ALT soft tissue flap. A case series reported the reconstruction of 23 midfacial defects using osteocutaneous radial forearm free flap (OCRFFF). Recipient site complications ranging from hematoma formation to wound dehiscence was noted in 10 cases. Oronasal fistulas occurred in 3 cases and were locally treated by advancement flaps. While donor site complications occurred in 7 cases with radial bone fracture in 1 case and skin graft loss in 6 others. Although none of the patients received dental rehabilitation, a normal diet was regained except for 2 patients. Smaller defect size understandably showed a lower rate of recipient site complications and showed better esthetic results than larger defects [13]. Another study reported the use of OCRFFF for midfacial reconstruction with /without orbital exenteration. Follow-up lasted for a mean of about 23 months with postoperative moderate deformity reported. Functionally, a normal diet was attained by all patients and understandable speech reported; moreover esthetically all patients returned to their social life postoperatively [14]. Moreno et al reported 2 cases of reconstruction using a RFFF enabling further prosthetic rehabilitation and with satisfactory functional and esthetic outcomes [7]. Another study reported the use of RFFF alone (3 cases) or in conjunction with ALT (1 case) to reconstruct midfacial reconstruction with the insertion of extraoral implants for final prosthetic integration. None of the implants failed and the final prosthesis was integrated after 7-12 months of the microvascular reconstruction [11].

Scapular flap

Scapular flaps were used in 13 maxillary reconstruction cases in the included articles. A case series published in 1998 reported the use of the scapular grafts to reconstruct midface defects. This was reported in 8 cases; 7 carcinomas and 1 hemangioma. 4 out of the 8 cases required a second procedure due to flap loss or volume correction [15]. The use of a scapular flap with a different vascular pedicle (thoracodorsal artery perforator) has been reported to reconstruct midfacial defects with healing and radiographic evidence of graft consolidation 8 months later. The second case showed fat necrosis at the anterior portion of the graft requiring a secondary surgery to correct the defect but later healing was uneventful [16]. A single case reconstructed with a scapular tip with a composite serratus anterior flap was reported with the graft maintaining viability at the end of the follow-up period and patient reporting satisfactory results [7].

Calvarial bone graft

A retrospective study of 6 patients reported the reconstruction of the midface using calvarial bone grafts along with a temporoparietal local vascularized graft. The follow-up period extended to a range of 3-36 months with 1 patient complaining of a moderate facial deformity postoperatively due TPF loss and requiring a revision surgery. The authors concluded that the use of calvarial bone grafts in this manner should only be confined to small defects not necessitating further soft tissue grafting [17]. Another retrospective study reported the use of calvarial bone grafts along with a rectus abdominus flap for midfacial reconstruction. Although not all the treated patients received prosthetic rehabilitation that did not affect the functional final reported outcome. It was reported as satisfactory and mastication was reported to be unaffected [18].

Table 2: Risk of bias assessment for the selected articles

Study	Type of study	Inclusion /exclusion criteria	Selection randomization	Reported loss to follow-up	Reported complications	Objective evaluation	Statistical analysis	Risk of bias
Mueller et al. 2014	Retro.	No	No	No	Yes	No	No	High
Moreno et al. 2010	Retro.	Yes	No	N.I.	Yes	Yes	Yes	Moderate
Mertens et al. 2013	Case report	No	No	No	Yes	No	No	High
Echo et al. 2013	Case report	No	No	No	No	No	No	High
Dediol et al. 2013	Case series	No	No	Yes	Yes	No	No	Moderate
Shaw et al. 2009	Case report	No	No	No	No	No	No	High
Andrades et al. 2008	Retro.	No	No	Yes	Yes	No	No	High
Chepeha et al. 2005	Case series	Yes	No	No	Yes	Yes	Yes	Moderate
Bidros et al. 2005	Case report	No	No	No	Yes	No	No	High
Parhiscar et al. 2002	Retro.	No	No	No	Yes	No	No	High
Futran et al. 2002	Retro.	No	No	No	Yes	No	No	High
Pollice et al. 1988	Case series	Yes	No	No	No	No	No	High
Schmelzem et al. 1998	Retro.	Yes	No	No	Yes	No	No	High
He et al. 2009	Case report	No	No	No	No	No	No	High

Temporoparietal osteofascial flap

A case series studied the use of temporoparietal osteofascial flap in maxillofacial reconstruction including 2 cases of midface reconstruction. Both cases were reported to show good uneventful healing and successful results along the follow-up periods which lasted 24 and 30 months. The authors concluded that the TOF has the obvious advantage of the absence of a second surgical donor site and so less morbidity. On the other hand, alopecia and dural tears have been reported so extreme care intraoperatively is essential [19].

Computer guided procedures

The rapidly evolving advancements in the scope of computer software has allowed for the introduction of virtual surgical planning techniques and the fabrication of patient specific implants and/or stereolithographic models.

A single case of the use of a specifically fabricated titanium mesh in conjunction with a latissimus dorsi flap was reported. Mesh exposure was reported requiring secondary surgical intervention with another soft tissue graft. The latissimus dorsi flap provides an abundance of soft tissue which was advantageous in this case to cover the titanium prosthesis. The same amount of soft tissue from another donor site would be accompanied by significant donor-site morbidity [10].

Another case report of a single case with a secondary midfacial depression was treated using a computer guided prefabricated MEDPOR implant. The preoperative CT scans of the patient were used to make a model of the patient's facial skeleton. The model was then used intraoperatively to adapt the MEDPOR implant. After 9 months of follow-up, both the patient and clinician reported good esthetics and function. The use of MEDPOR in this case avoided a secondary surgical site with its morbidity. On the other hand, the main disadvantage of MEDPOR is the risk of infection and the added cost of the implant [20].

In 2013 a published case report introduced the use of a patient specific implant along with a scapular osteomyocutaneous flap. The patient had an ablative surgery earlier and the CTs were imported into the computer software where a virtual surgery was performed by designing the components of the defect to be reconstructed with the scapular graft (palate and alveolus). A patient specific titanium implant was also designed by mirror imaging the normal side to obtain a structure of the midface as close to normal as possible. The zygomatic and orbital components of the defect were reconstructed using the PSI. Intraoperatively the scapular flap was harvested and microvascular anastomosis achieved by using the facial artery and vein as recipient vessels. Proper insertion and fixation of the device were detected intraoperatively using intraoperative navigation systems. The myocutaneous component of the flap was used to better support the soft tissue of the cheek. Healing was uneventful with good epithelialization of the recipient mucosa and good contour and symmetry. Later on due to the poor vascularity of the radiated recipient bed, as reported by the authors, part of the titanium implant was exposed at the lower eyelid necessitating a secondary rotation cheek flap to cover it. Three months postoperatively complete healing and epithelialization was reported. Although this is a costy technique; it provides superior esthetics and requiring less graft harvesting [21].

Discussion

Maxillectomy defects have been reported to be treated by prosthetic appliances; obturators; as early as the 1950s aiming to provide adequate esthetics and function after surgical ablation. The advantages of the obturator at that time was to restore functions, reduce bleeding and maintain a clean wound. Obturators have been reported to restore the drinking ability of the patient by closing the oro-antral/nasal caused by the resection [22, 23]. On the other hand, reports of poor masticatory function and improper drinking with an obturator which was attributed to the large initial maxillectomy defect must be taken into consideration [1]. Drawbacks of

obturators include leakage, constant need for proper cleaning to maintain hygiene and constant modification of the prosthesis [2]. A higher tendency of improper nasalance; whether hyponasality or hypernasality; in patients receiving obturators post-maxillectomy is also an issue to be considered [24]. Poorer swallowing ability was also reported in patients rehabilitated with an obturator; especially in cases of a larger horizontal defect such as extensive palatal defects (Okay Class III) [7].

Complications of obturators were overcomed with the introduction of microvascular techniques which has become the internationally accepted method of maxillary/midface reconstruction. Donor sites have varied according to the size and type of the defect with pros and cons to each of the reported donor sites in literature.

Fibular grafts are a common option in cases requiring a bony component in the graft. The use of osteocutaneous flaps in midface defects has been reported to be highly reliable and providing acceptable esthetics in cases other than those requiring restoration of bony parts of the orbit. Authors commented that the use of an osteocutaneous fibular graft for midfacial reconstruction is beneficial especially in the freely movable soft tissue component which is not the case in other composite grafts [9]. It has also been reported to successfully provide a platform for the future insertion of dental implants and prosthesis placement although not following the normal anatomy [25]. The use of an osteomyocutaneous fibular flap was also applied in several studies to reconstruct oncologic defects with esthetically and functionally acceptable results to both patients and surgeons [8, 24]. On the other hand, the inadequate alveolar height in cases of mandibular reconstruction has been overcomed by applying the “double-barrel” technique to enable future prosthetic rehabilitation [26, 27]; although this caused a deficiency in bone length [28]. No reports of the application of this technique in maxillary/midface reconstruction were encountered in the literature search.

Radial forearm free flap is another commonly used option. It may supply a wide variety of tissue components; skin, muscle and/or bone according to the defect characteristics and clinician’s preference. Flap harvesting is quite a simple technique with a thin, long reliable pedicle. Osseocutaneous radial forearm grafts have been used to reconstruct midface defects and restoring the infraorbital rim using the bony component of the graft. High success rates in terms of graft incorporation; function restoration (speech and oronasal separation) and esthetic outcomes (patient accepting social interaction) have been reported [14]. Moreover; the presence of a second donor site to harvest the split thickness skin graft increases morbidity. The use of a local full thickness skin graft from the incision already needed to dissect the vascular pedicle of the graft was reported to overcome the necessity of a 2nd donor site. Authors of this trial reported uneventful healing in all 29 cases included with only a single case of seroma formation which was treated locally and eventually healed as well [29]. The use of a narrower and longer skin paddle was introduced allowing easy primary closure of the defect site with satisfactory results [30].

Scapular flaps have also been discussed vastly in the literature with several different applications with/without other tissue grafts. Combination with the latissimus dorsi muscle and harvesting a part of the scapular bone has been reported to provide sufficient bone and soft tissue components to reconstruct maxillectomy defects. Some authors considered it to be the first reconstructive option in cases of maxillectomy with orbital exenteration defects [31] while others noted that the use of the TDA provides a more reliable vascular pedicle with a lower risk of kinking and thrombus formation and the bony component when needed may be sufficient for dental implant placement [16]. Moreover primary closure of the donor site and the longer pedicle are the main advantages of this technique. The anatomical similarity of the scapular tip to that of the palate makes its use for palatal defect reconstruction preferable [32]. The advantages of the scapular flap are; the presence of bony, muscle and skin tissue with proportionate amounts, the long large-caliber donor vessel and the morphological similarity with the maxillary structures [33].

Iliac grafts: the iliac region may provide bone with or without accompanying soft tissue components for maxillofacial reconstruction. The outstanding advantage of iliac grafts is the abundant bone stock available with its vascular pedicle. The cutaneous tissue of the groin has the advantage of being hairless and so mimicking the recipient area but the skin tone unfortunately does not match that of the face. The hairless nature of the groin skin is advantageous although the non-matching skin tone may cause an esthetic concern [12].

Despite the obvious advantage of autogenous tissue grafting, reconstruction of the maxilla/midface has higher morbidity rates and is a complex procedure. Prolonged surgical time complications, donor site morbidity, technique sensitivity and expensive equipment complicate the use of microvascular surgery. With the introduction of computer-guided surgeries, preoperative surgical planning and fabrication of patient specific implants were reported and aimed at overcoming such drawbacks [8, 21].

The technique of computer-assisted planning and mirror-imaging of the same patient’s normal side was used by Mertens et al to fabricate a patient specific titanium implant (PSI) to act as a scaffold for an osteomyocutaneous scapular flap to reconstruct an oncologic midface defect. Postoperative CT was ordered at the end of the 4 month followup period to

enable the fabrication of implant-retained rehabilitation was used. The authors considered it to be a promising technique combining advantages of several reconstructive techniques yet no further details on the postoperative period have been provided [26]. The main obstacle to generalize the use of computer guided and navigation modalities is their cost [34]. Preoperative planning of both donor and recipient sites is also done to attain precise bony harvests to fit the preplanned bed. This may be performed by printing models of the recipient and donor sites and shaping the donor graft accordingly preoperatively [8]. Virtual planning may greatly reduce human error by mirror-duplication of the contralateral side in cases of hemifacial defects and so attaining better facial symmetry than that planned arbitrarily [35]. Although several computer applications have been introduced; the use of these applications remains quite complicated and requires extensive training. These techniques have undoubtedly made the intraoperative surgical steps easier and their outcome expectable. Intraoperative surgical time previously wasted in planning, adaptation and even evaluating surgical steps has been saved by finishing these steps preoperatively. The reduced intraoperative time reduce surgical cost and complications.

In conclusion, the articles included in this review were chosen to target methods used to overcome facial asymmetry/deformity caused by pathologic defects. The lack of controlled/ RCTs and a fixed postoperative evaluation scale made the comparison of the results impossible. It was noted however that outcomes were directly affected by the extent of the defect with larger defects usually accompanied by poorer outcomes. The computer-guided novel techniques are yet to be researched to allow for further assessment but the show great potential for esthetically satisfactory simple midface reconstruction. The risk of bias in all assessed articles was substantially high making the proper evaluation of the reported techniques impossible. Outcomes in the selected articles were vague reports of patients' satisfaction as regards function and esthetics. Properly arranged RCTs with fixed postoperative assessment measures are highly recommended to enable further analysis of the results to come up with guidelines for midface/maxillary reconstruction.

References

1. Vero N, Mishra N, Singh BP, et al. Assessment of swallowing and masticatory performance in obturator wearers: a clinical study. J Adv Prosthodont. 2015;7:8–14. http://dx.doi.org/10.4047/jap.2015.7.1.8 PMid:25722831 PMCid:PMC4341192

2. Davison SP, Sherris D, Meland NB. An algorithm for maxillectomy defect reconstruction. Laryngoscope. 1998;108:215–9. http://dx.doi.org/10.1097/00005537-199802000-00010 PMid:9473070

3. Brown JS, Shaw RJ. Reconstruction of the maxilla and midface: introducing a new classification. Lancet Oncol. 2010;11:1001–8. http://dx.doi.org/10.1016/S1470-2045(10)70113-3

4. Mücke T, Hölzle F, Loeffelbein DJ, , et al. Maxillary reconstruction using microvascular free flaps. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2011;111:51–7. http://dx.doi.org/10.1016/j.tripleo.2010.03.042 PMid:20591701

5. Frederick JW, Sweeny L, Carroll WR, et al. Outcomes in head and neck reconstruction by surgical site and donor site. Laryngoscope. 2013;123:1612–7. http://dx.doi.org/10.1002/lary.23775 PMid:23686870 PMCid:PMC3695001

6. Sekido M, Yamamoto Y, Makino S. Maxillary reconstruction using a free deep inferior epigastric perforator (DIEP) flap combined with vascularised costal cartilages. J Plast Reconstr Aesthet Surg. 2006;59:1350–4. http://dx.doi.org/10.1016/j.bjps.2006.01.048 PMid:17113517

7. Moreno MA, Skoracki RJ, Hanna EY, Hanasono MM. Microvascular free flap reconstruction versus palatal obturation for maxillectomy defects. Head Neck. 2010;32(7):860-8. PMid:19902543

8. He Y, Zhu HG, Zhang ZY, He J, et al. Three-dimensional model simulation and reconstruction of composite total maxillectomy defects with fibula osteomyocutaneous flap flow-through from radial forearm flap. Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontology. 2009;108:e6–12. http://dx.doi.org/10.1016/j.tripleo.2009.07.027 PMid:19913721

9. Futran N. Midface Reconstruction With the Fibula Free Flap. Arch otolaryngolo head neck Surg. 2002;128:161–6. http://dx.doi.org/10.1001/archotol.128.2.161

10. Dediol E, Uglešić V, Zubčić V, Knežević P. Brown class III maxillectomy defects reconstruction with prefabricated titanium mesh and soft tissue free flap. Ann Plast Surg. 2013;71:63–7. http://dx.doi.org/10.1097/SAP.0b013e318246e895 PMid:23241764

11. Mueller S, Hohlweg-Majert B, Buergers R, Steiner T, Reichert TE, Wolff KD, et al. The functional and aesthetic reconstruction of midfacial and orbital defects by combining free flap transfer and craniofacial prosthesis. Clin Oral Investig. 2014;26: 413-9.

12. Shaw RJ, Brown JS. Osteomyocutaneous deep circumflex iliac artery perforator flap in the reconstruction of midface defect with facial skin loss: a case report. Microsurgery. 2009;29:299–302. http://dx.doi.org/10.1002/micr.20623 PMid:19274653

13. Andrades P, Rosenthal EL, Carroll WR, et al. Zygomaticomaxillary Buttress Reconstruction of Midface Defects with the Osteocutanous Radial Fforearm Free Flap. Head Neck. 2008; 30:1295–302. http://dx.doi.org/10.1002/hed.20874 PMid:18642322

14. Chepeha DB1, Moyer JS, Bradford CR, et al. Osseocutaneous Radial Forearm Free Tissue Transfer for Repair of Complex Midfacial Defects. Arch otolaryngolo head neck Surg. 2005;131:513–7. http://dx.doi.org/10.1001/archotol.131.6.513 PMid:15967885

15. Schmelzeisen R, Schliephake H. Interdisciplinary microvascular reconstruction of maxillary, midfacial and skull base defects. J Cranio-Maxillo-Facial Surg. 1998;26:1–10. http://dx.doi.org/10.1016/S1010-5182(98)80028-0

16. Bidros RS, Metzinger SE, Guerra AB. The Thoracodorsal Artery Perforator-Scapular Osteocutaneous (TDAP-SOC) Flap for Reconstruction of Palatal and Maxillary Defects. Ann Plast Surg. 2005; 54:59–65. http://dx.doi.org/10.1097/01.sap.0000139561.64564.d7 PMid:15613885

17. Pollice PA1 FJJ. Secondary reconstruction of upper midface and orbit after total maxillectomy. Arch Otolaryngol Head Neck Surg. 1998;124:802–8. http://dx.doi.org/10.1001/archotol.124.7.802

18. Futran N. Retrospective case series of primary and secondary microvascular free tissue transfer reconstruction of midfacial defects. J Prosthet Dent. 2001;86:369–76. http://dx.doi.org/10.1067/mpr.2001.118875 PMid:11677530

19. Parhiscar A, Har-El G, Turk JB, Abramson DL. Temporoparietal osteofascial flap for head and neck reconstruction. J Oral Maxillofac Surg. 2002;60:619–22. http://dx.doi.org/10.1053/joms.2002.33104 PMid:12022094

20. Echo A, Wolfswinkel EM, Weathers W, McKnight A, Izaddoost S. Use of a Three-Dimensional Model to Optimize a MEDPOR Implant for Delayed Reconstruction of a Suprastructure Maxillectomy Defect. Craniomaxillofac Trauma Reconstr. 2013;6(4):275-80. http://dx.doi.org/10.1055/s-0033-1356762 PMid:24436774 PMCid:PMC3820743

21. Mertens C, Löwenheim H, Hoffmann J. Image data based reconstruction of the midface using a patient-specific implant in combination with a vascularized osteomyocutaneous scapular flap. J Cranio-Maxillofacial. 2013;41:219–25. http://dx.doi.org/10.1016/j.jcms.2012.09.003 PMid:23073039

22. Aramany M. Basic principles of obturator design for partially edentulous patients. Part II: Design principles. J Prosth Dent; 1978;5: 656-62. http://dx.doi.org/10.1016/0022-3913(78)90065-3

23. Shah D, Meenakshi A. The obturator prostheses for maxillectomy. J Res Dent Sci. 2012;3:193–7. http://dx.doi.org/10.4103/0976-433x.107402

24. Eckardt A, Teltzrow T, Schulze A, et al. Nasalance in patients with maxillary defects - Reconstruction versus obturation. J Craniomaxillofac Surg. 2007; 35:241–5. http://dx.doi.org/10.1016/j.jcms.2007.07.001 PMid:17870609

25. Laure B, Sury F, Martin T, et al. Reconstruction of bony mandibular and maxillary defects with one single transfer of a free fibula osteocutaneous flap. J Plast Reconstr Aesthet Surg. 2008; 61:200–3. http://dx.doi.org/10.1016/j.bjps.2007.06.007 PMid:17658304

26. Yu H, Shen SG, Wang X, et al. The indication and application of computer-assisted navigation in oral and maxillofacial surgery-Shanghai's experience based on 104 cases. J Craniomaxillofac Surg. 2013;41:770–4. http://dx.doi.org/10.1016/j.jcms.2013.01.016 PMid:23462802

27. Zhang C, Sun J, Zhu H, et al. Microsurgical free flap reconstructions of the head and neck region: Shanghai experience of 34 years and 4640 flaps. Oncology Reports. 2015; 26: 813-23. http://dx.doi.org/10.1016/j.ijom.2015.02.017

28. Shen Y1, Guo XH, Sun J, Li J, et al. Double-barrel vascularised fibula graft in mandibular reconstruction: a 10-year experience with an algorithm. J Plast Reconstr Aesthet Surg. 2012; 66: 364–71. PMid:23122692

29. Riecke B, Assaf a T, Heiland M, et al. Local full-thickness skin graft of the donor arm-a novel technique for the reduction of donor site morbidity in radial forearm free flap. Int J Oral Maxillofac Surg. 2015; 21:1–5. http://dx.doi.org/10.1016/j.ijom.2015.02.021

30. Zhang YX, Xi W, Lazzeri D, et al. Bipaddle radial forearm flap for head and neck reconstruction. J Craniofac Surg. 2015;26:350–3. http://dx.doi.org/10.1097/SCS.0000000000001468 PMid:25668117

31. Kosutic D, Uglesic V, Knezevic P, et al. Latissimus dorsi-scapula free flap for reconstruction of defects following radical maxillectomy with orbital exenteration. J Plast Reconstr Aesthet Surg. 2008;61:620–7. http://dx.doi.org/10.1016/j.bjps.2007.11.004 PMid:18226591

32. Pagedar N, Gilbert RW, Chan H, et al. Maxillary reconstruction using the scapular tip free flap: a radiologic comparison of 3D morphology. Head Neck. 2012;34:1377–82. http://dx.doi.org/10.1002/hed.21946 PMid:22287228

33. Piazza C, Paderno A, Taglietti V, et al. Evolution of complex palatomaxillary reconstructions: the scapular angle osteomuscular free flap. Curr Opin Otolaryngol Head Neck Surg. 2013;21: 95–103. http://dx.doi.org/10.1097/MOO.0b013e32835e8445 PMid:23385785

34. Rohner D, Guijarro-Martínez R, Bucher P, et al. Importance of patient-specific intraoperative guides in complex maxillofacial reconstruction. J Craniomaxillofac Surg. 2013;41:382–90. http://dx.doi.org/10.1016/j.jcms.2012.10.021 PMid:23228282

35. Acero J, García E. Reoperative Midface Reconstruction. Oral Maxillofac Surg Clin North Am. 2011;23:133–51. http://dx.doi.org/10.1016/j.coms.2010.10.004 PMid:21126882

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):476-481.
http://dx.doi.org/10.3889/oamjms.2016.089
eISSN: 1857-9655
Stomatology

A Comparative Clinical Study of the Effect of Denture Cleansing on the Surface Roughness and Hardness of Two Denture Base Materials

Amani Ramadan Moussa, Wessam Mohamed Dehis, Asmaa Nabil Elboraey, Hisham Samir ElGabry*

Prosthodontics Department, National Research Centre, Giza, Egypt

Citation: Moussa AR, Dehis WM, Elboraey AN, ElGabry HS. A Comparative Clinical Study of the Effect of Denture Cleansing on the Surface Roughness and Hardness of Two Denture Base Materials. Open Access Maced J Med Sci. 2016 Sep 15; 4(3):476-481. http://dx.doi.org/10.3889/oamjms.2016.089

Keywords: Clinical study; complete denture; denture cleansing; denture base roughness; denture base hardness.

***Correspondence:** Hisham Samir ElGabry. Researcher, Prosthodontics Dept, National Research Centre, 33 El Bohouth St., Dokki, Giza, P.O. 12622, Egypt. E-mail: HishamElgabry@live.com

Received: 30-Jun-2016; **Revised:** 29-Jul-2016; **Accepted:** 30-Jul-2016; **Online first:** 22-Aug-2016

Competing Interests: The authors have declared that no competing interests exist.

Abstract

AIM: This study aimed to verify the influence of oral environment and denture cleansers on the surface roughness and hardness of two different denture base materials.

METHODS: A total of sixteen identical removable disc specimens (RDS) were processed. Eight RDS were made from heat-cured acrylic resin (AR) and the other eight were fabricated from thermoplastic injection moulded resin (TR). Surface roughness and hardness of DRS were measured using ultrasonic profilometry and Universal testing machine respectively. Then the four RDS (two AR and two of TR) were fixed to each maxillary denture, after three months RDS were retrieved. Surface roughness and hardness of RDS have measured again.

RESULTS: The surface roughness measurements revealed no significant difference ($p > 0.05$) for both disc groups at baseline. However, both groups showed a significant increase in the surface roughness after three months with higher mean value for (TR) group. On the other hand, the (AR) group showed higher hardness mean value than (TR) group at baseline with no significant decrease in the hardness values ($p > 0.05$) following three months follow-up period.

CONCLUSIONS: Denture cleansers have an effect on the denture's surface roughness and hardness concurrently with an oral condition which will consequently influence the complete dentures' lifetime and patients' satisfaction.

Introduction

Polymethyl methacrylate (PMMA) resin has a long, clinically established history for being utilised as denture base material, owing to its excellent aesthetic, adequate physical properties, reasonable coast and easy processing technique [1-3]. However, dimensional inaccuracies, microbial adhesion, inadequate mechanical properties and allergic side effects are the greatest disadvantages that affect the clinical performance of PMMA prosthetics [4]. Continuous research focusing on PMMA properties improvement has led to the emergence of new processing techniques and alternative polymeric materials known as thermoplastic resins. These materials exhibit high creep and solvent resistance, excellent wear characteristics and high fatigue endurance. In addition, they have very little or almost no free monomer; therefore, they offer another option for allergic patients. Among thermoplastic resins is PMMA based resin which is used as denture base for both removable and complete dentures [5, 6].

Clinically, dentures are exposed to temperature variations during smoothening and polishing procedures at the time of construction. In the oral environment, dentures are also subjected to thermal alterations through food intake, besides the unavoidable biofilm development and bacterial colonisation on denture surfaces [7]. This colonisation is an important stage in the pathogenesis of denture stomatitis and other diseases not only for elderly and immune-compromised patients but also for healthy individuals [8].

While the surface roughness of the denture base is a contributing factor for bacterial colonisation, the adhesion of microorganisms to a surface is a prerequisite for its colonisation [9-11]. Furthermore, hardness is another property that influences the surface characteristics of denture base material as it facilitates the prosthesis finishing and maximises its resistance to abrasion and scratching during service and cleansing [12, 13]. Nevertheless, the maintenance of denture hygiene and effective microbial film removal represent an essential demand for denture wearers' health. Currently, a number of mechanical and chemical denture cleansers are available. The mechanical method involves brushing with a dentifrice or neutral soap [14, 15]. While in the chemical method, dentures are immersed in products containing chemical agents as alkaline hypochlorite solution and alkaline peroxides (oxygenated cleansers). The latter is safe, easier and frequently utilised procedure, particularly in old aged patients. Beside their chemical efficiency against biofilm, they also eliminate stains mechanically by liberating oxygen [16, 17]. In literature, both the surface roughness and hardness have been widely studied in-vitro; however, no in-vivo reports are available about the effect of oral environment together with denture cleanser. Accordingly, it was interesting to verify the influence of proceeding factors on the surface roughness and hardness of two denture base materials in-vivo.

Materials and Methods

Construction of removal discs' specimens (RDS)

The two different denture base materials used in this study are listed in Table 1. A total of sixteen identical disc specimens (5 mm. in diameter and 2mm. in thickness) were processed (Fig. 1). Eight were made from heat cured acrylic resin (AR) and the other eight were fabricated from thermoplastic injection moulded resin (TR). All specimens were produced in moulds prepared by insertion of stainless steel rings into the metal dental flask filled with type III dental stone [18, 19]. After complete stone setting, each RDS denture base material was proportioned, mixed and processed according to each manufacturer's instructions shown in Table 1. Then the flasks were allowed to bench cool and the specimens were removed.

For TR specimens, spruces were carefully removed with tungsten carbide burs (Bre-dent, GmbH & Co.KG Germany).

AR and TR disc specimens were finished and polished using medium and fine grit acrylic polishers

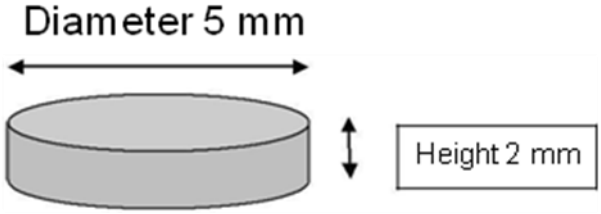

Figure 1: Diagrammatic representation for the circular disc

(Bre-dent, GmbH & Co.KG Germany). Finally, all RDS were cleaned and disinfected utilising denture cleansing tablets (Protefix, Queisser, Germany) and stored in distilled water to measure both surface roughness and hardness.

Table 1: Denture base resins and their processing techniques

Denture resin	Processing type	Polymerization procedure	Powder/Liquid Ratio-
Acrostone (WHW plastic, England Packed by Anglo-Egyptian Lab)	Heat activation fast heat	Pack and press curing at boiling water 100 for 20 min	21/6 ml
Bre-Crystal (bredent- Germany)	Heat activation	Injection-molding 260°C for 26 min. Pressure: 5 bar	Single Component

Dentures construction and specimens' fixation

Four edentulous male volunteers, aged 50-60 year, willing to have a new set of complete dentures, participated in the current clinical study. Patients were selected from National Research Center (NRC) dental clinic fulfilling the following criteria:

i - Healthy firm mucoperiosteum without any signs of inflammation or flabby tissues.

ii - Patients were free from any systemic and neurological diseases that might affect their ability to co-operate, follow the recommendations and instructions of the clinician.

iii - Smokers were not included in the study.

Each participant signed a written informed consent before sharing in this study. The study protocol was approved by the ethics committee at NRC.

Complete Dentures were constructed and processed using conventional heat cured acrylic resin (Acrostone, WHW plastic, England Packed by Anglo Egyptian Lab) following the manufacturer's instructions. The maxillary dentures were prepared to receive the four RDS specimens by creating two circular holes on either side of the midline at the denture's flat palatal portion using tissue bunch with 6.1 mm diameter (Leader, Italy). Then, dentures were replaced with a replica of the master cast to facilitate fixation of the specimens, where two RDS of each denture base material were fixed on each side using self-cured acrylic resin (Acrostone, WHW plastic,

England Packed by Anglo Egyptian Lab).

A fine acrylic polisher (Bre-dent, GmbH & Co. KG ·Germany) was used to eliminate any irregularities or excess of self-cured resin (Fig. 3). Finally, the dentures were disinfected by means of cleansing tablets (Protefix, Queisser, Germany) and stored in room temperature tap water until delivery time.

Dentures were delivered to patients and they were instructed to maintain strict denture hygiene measures using cleansing tablets (Protefix, Queisser, Germany) once a day for 3 months.

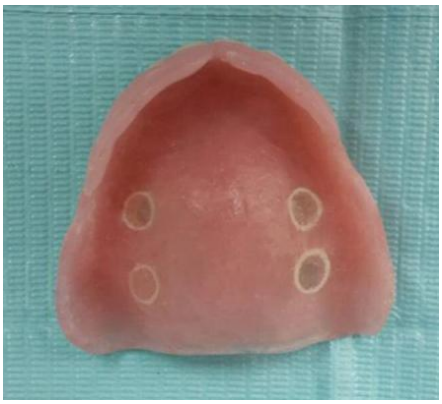

Figure 2: Maxillary complete denture with fixed RDS

Retrieval of Removable Acrylic Resin Specimens

Patients were recalled after 3 months where the maxillary dentures were removed and gently cleansed with soft denture brush to remove any gross soft deposits. The dentures were disinfected with the same given denture-cleansing agent before retrieving of RDS. Then RDS were retrieved with tissue bunch 6.1 mm diameter (Leader, Italy). The holes in maxillary dentures were restored with heat-cured discs using self-cured acrylic resin. The retrieved disc specimens were disinfected once more and stored in tap water for one day before surface roughness and hardness were measured.

Measurements of Surface Roughness and Hardness

Measuring Surface Roughness (μm)

Surface roughness in terms of roughness average (Ra) was estimated by the National Institute for the Standards-Egypt using ultra-sonic profilometer (form Talysurf i200, Taylor Hobson-AMETEK's, USA). The first surface roughness readings were measured immediately after specimens' preparation as a baseline record and the mean of three readings was enrolled. The final roughness measurements were done after 3 months of RDS retrieval.

Measuring Surface Hardness (kg/mm)

The hardness was measured using the Universal testing machine (Nexus 4503, INNOVATEST, Netherlands, Europe) in the National Research Centre, with Vickers diamond indentator. A 100 g load was applied for 10 seconds with 20 x magnification. Every specimen was subjected to three indentations (one on the centre, two on the border) and the average value was recorded for each RDS material. Similarly to roughness, the first hardness readings were achieved immediately after specimens' preparation and the final hardness measurements were carried out after 3 months of RDS retrieval.

Statistics

Data were analysed using IBM® SPSS® (SPSS Inc., IBM Corporation, NY, USA) Statistics Version 23 for Windows. Independent t-test was performed to compare the influence of denture cleansing tablets and oral environment on both the surface roughness and hardness of two different denture base materials utilising removable discs' specimens (RDS) fixed to maxillary complete dentures. The significance level was set at $p \leq 0.05$.

Results

Table 2 and 3 represents the mean and standard deviation (SD) values for the two denture base materials RDS; heat cured acrylic resin (AR) and the thermoplastic resin (TR) prior to fixing it to dentures and 3 months following insertion and utilising using of cleansing tablets.

Despite the lower mean roughness value of heat cured resin compared to thermoplastic resin (0.20 *μ*m & 0.35 *μ*m respectively) as shown in Table 2; the surface roughness measurements revealed no significant difference ($p > 0.05$) both before and after fixation of RDS and the use of cleansing tablets for both RDS materials. Moreover, each RDS material showed a significant increase ($p < 0.05$) in the surface roughness after three months (Table 2) with higher mean value for TR than AR. (0.37 *μ*m & 0.35 *μ*m respectively).

Table 2: Roughness measurement (*μ*m) for heat cured (AR) and thermoplastic resin (TR) DRS before and 3 months following the use denture cleansing tablets

		Denture Base				P value
		Heat Cure AR		Thermoplastic resin		
		Mean	SD	Mean	SD	
Roughness	Before	0.20	0.08	0.26	0.08	0.06 NS
	After	0.35	0.11	0.37	0.11	0.723 NS
p-value		0.001*		0.023*		

*Note: Means with the same letter within each row are not significantly different at p ≥ 0.05. *= Significant, NS= Non-significant.*

Comparison of surface hardness of the two denture base RDS is shown in Table 3. The hardness measurement before fixing RDS to dentures demonstrated the statistically significant difference ($P < 0.05$); with heat cure AR recorded higher hardness mean value than TR (18.46 and 13.65 respectively). With denture insertion and utilising cleansing tablets for three months a slight decrease in mean values were recorded (14.90 & 11.81) for AR and TR respectively, however, it was not statistically significant ($p > 0.05$).

Table 3: Measurement of surface micro hardness (Kg/mm^2*)* for heat cured (AR) and thermoplastic resin (TR) before and 3 months following the use denture cleansing tablets

		Denture Base				
		Heat Cure AR		Thermoplastic PMMA		p-value
		Mean	SD	Mean	SD	
Micro hardness	Before	18.46	2.08	13.65	2.01	0.006*
	After	14.90	4.20	11.81	.53	0.192 NS
p-value		0.151 NS		0.123 NS		

Means with the same letter within each row are not significantly different at p≥0.05.
**= Significant, NS= Non-significant.*

Discussion

Denture base surface properties are of peculiar importance as they affect the denture longevity during the function. Surface roughness and hardness have been investigated utilising different in-vitro methods. All these techniques provide valuable information regarding the mechanical properties of the materials tested, however, none of in vitro techniques can expose the tested materials to conditions similar to that of the oral environment (*in vivo*) such as pH and temperature variations [20].

Hence, the association between the results of in vitro methods and clinical studies are expected to show some discrepancies [21]. In the current study, therefore, a sampling technique developed by Avon et al, [22] was modified and utilised to provide reliable information about the influence of denture cleanser coupled with that of oral cavity on both surface roughness and hardness of two denture base materials [23].

Denture hygiene and disinfection have been recommended as an essential practice for preventing cross-contamination and the maintenance of a healthy oral mucosa. It has been pointed out that some disinfection methods may have unfavourable effects on denture base resins [24, 25]. The surface roughness of any denture base material influences microbial colonisation and biofilm formation [26, 27]. Furthermore, roughness causes denture discoloration and it may predispose patient discomfort. The surface roughness (Ra) of 0.2 µm was reported to be a clinically acceptable value, where no further decrease in plaque accumulation is anticipated indenture prostheses as reported in the literature [28-31]. In this study, maxillary dentures were designated with retrievable removable discs (RDS) from two materials: conventional acrylic resin and thermoplastic one. Both RDS showed roughness within the acceptable value (Ra 0.2 µm) prior to fixing RDS to dentures. Despite the difference in the chemical composition and curing method, the accurate laboratory procedures and following manufacturers' instructions aiming to achieve the smooth surface quality may account for this nearly similar roughness values.

Conversely, after three months of using cleansing tablets, retrieval of RDS revealed an apparent matching increase in roughness (Ra) above the acceptable value. This increase in roughness might be attributed to possible changes occurring in RDS polymer materials as a consequence of the coupled effects of oral environment and the use of denture cleanser. These findings are in agreement with a previous study which reported that effervescent cleansing tablets increase the surface roughness [32].

Furthermore, the results of this study demonstrated that the conventional acrylic resin RDS presented a higher hardness than thermoplastic resin before fixing RDS to dentures. This probably due to the difference in chemical composition were; high monomer-polymer content, presence of methyl-methacrylate monomer and cross-linking agents are influencing factors for the better surface hardness of the conventional acrylic resin [33].

Interestingly, a comparable decrease in the hardness for both RDS materials after retrieval of specimens was evident. This result is in accordance with previous studies demonstrated reduction of hardness after using different disinfection methods on different denture base materials [32, 34, 35]. Several reasons may explain the previous results as water absorption during disinfection may act as a plasticizing agent, which permits relaxation of stresses occurred during processing and consequently, hardness is lowered [36, 37]. It was reported that repeated exposure of the dentures to disinfectant solutions may alter their physical properties. Moreover, some chemical constituents of the disinfectants may result in softening and degradation of the surface layer of denture resin. Another explanation is that denture disinfectant liberates oxygen resulting in hydrolysis and disintegration of the polymerised resin [24, 38, 39].

In conclusion, within the limitations of this in vivo study, it could be concluded that denture cleansers affect the surface roughness and hardness concurrently with oral condition variations, which will consequently influence the durability and satisfaction of complete denture wearers.

References

1. Yunus N, Rasid AA, Azmil L, Abu-Hassan MI. Some flexural properties of a nylon denture base polymer. Journal of Oral Rehabilitation. 2005; 32(1):65-71. http://dx.doi.org/10.1111/j.1365-2842.2004.01370.x PMid:15634304

2. Craig RG. Restorative Dental Materials. 11th ed. St Louis, MO: Mosby, 2003: 87-88. PMid:12781382

3. Pires-de-Souza FC, Panzeri H, Vieira MA, Garcia L-FR, Consani S. Impact and fracture resistance of an experimental acrylic polymer with elastomer in different proportions. Mater Res. 2009; 12(4):415-418. http://dx.doi.org/10.1590/S1516-14392009000400007

4. Dhiman RR, Chowdhury SK. Midline fractures in single maxillary complete acrylic versus flexible dentures. Med J Armed Forces India. 2009; 65:141-145. http://dx.doi.org/10.1016/S0377-1237(09)80128-7

5. Kutsch VK, Whitehouse JW, Schermerhorn K, et al. The evolution and advancement of dental thermoplastics. Dental Town. 2003; 4(2):52-56.

6. Ardelean L, Bortun C, Podariu AC, Rusu LC. Some Alternatives for Classic Thermopolymerisable Acrylic Dentures. Materiale Plastice. 2012; 49(1):30-33.

7. Soygun K, Bolayir G, Boztug A. Mechanical and thermal properties of polyamide versus reinforced PMMA denture base materials. J Adv Prosthodont. 2013; 5(2):153-60. http://dx.doi.org/10.4047/jap.2013.5.2.153 PMid:23755341 PMCid:PMC3675288

8. Orsi IA, Junior AG, Villabona CA, Fernandes FH, Ito IY. Evaluation of the efficacy of chemical disinfectants for disinfection of heat-polymerised acrylic resin. Gerodontology. 2010; 28(4):253-257. http://dx.doi.org/10.1111/j.1741-2358.2010.00400.x PMid:20609007

9. Nikawa H, Jin C, Makihira S, Egusa H, Hamada T, Yamashiro H. Biofilm formation of Candida albicans on the surfaces of deteriorated soft denture lining materials caused by denture cleansers in vitro. J Oral Rehabil. 2003; 30(3):243-250. http://dx.doi.org/10.1046/j.1365-2842.2003.01024.x PMid:12588495

10. Glass RT, Bullard JW, Conrad RS, Blewett EL. Evaluation of the sanitization effectiveness of a denture-cleaning product on dentures contaminated with known microbial flora. An in vitro study. Quintessence Int. 2004; 35(3):194-199. PMid:15119677

11. Berger JC, Driscoll CF, Romberg E, Luo Q, Thompson G. Surface roughness of denture base acrylic resins after processing and after polishing. J Prosthodont. 2006; 15(3):180-186. http://dx.doi.org/10.1111/j.1532-849X.2006.00098.x PMid:16681500

12. Pavarina AC, Vergani CE, Machado AL, Giampaolo ET, Teraoka MT. The effect of disinfectant solutions on the hardness of acrylic resin denture teeth. J Oral Rehabil. 2003; 30(7):749-752. http://dx.doi.org/10.1046/j.1365-2842.2003.01145.x PMid:12791163

13. Pinto Lde R, Acosta EJ, Távora FF, da Silva PM, Porto VC. Effect of repeated cycles of chemical disinfection on the roughness and hardness of hard reline acrylic resins. Gerodontology. 2010; 27(2):147-153. http://dx.doi.org/10.1111/j.1741-2358.2009.00282.x PMid:19508322

14. Basson NJ, Quick AN, Thomas CJ. Household products as sanitizing agents in denture cleansing. J Dent Assoc S Afr. 1992; 47(10):437-439. PMid:9511605

15. Bernabé W, Mendonça-Neto T, Pimenta FC, Pegoraro LF, Scolaro JM. Efficacy of sodium hypoclorite and coconut soap used as disinfecting agents in the reduction of denture stomatitis, Streptococcus mutans and Candida albicans. J Oral Rehabil. 2004; 31(5):453-459. http://dx.doi.org/10.1111/j.1365-2842.2004.01254.x PMid:15140171

16. Paranhos HF, Panzeri H, Lara EH, Candido RC, Ito IY. Capacity of denture laque/biofilm removal and antimicrobial action of a new denture paste. Braz Dent J. 2000; 11(2):97-104.

17. Paranhos HF, Silva-Lovato CH, de Souza RF, Cruz PC, de Freitas-Pontes, E. Watanabe, Ito IY. Effect of three methods for cleaning dentures on biofilms formed in vitro on acrylic resin. J Prosthodont. 2009; 18(5):427-431. http://dx.doi.org/10.1111/j.1532-849X.2009.00450.x PMid:19486455

18. Winkler S. Essential of complete denture prosthodontics, 2nd ed, 1988: 307.

19. Zarb GA, Bolender CL, Carlsson GE. Boucher's Prosthodontic treatment for edentulous patients, 7th ed, 1997: 338.

20. Avon SL, Goulet J-P, Deslauriers N. Removable acrylic resin disk as a sampling system for the study of denture biofilms in vivo. J Prosthet Dent. 2007; 97(1):32-38. http://dx.doi.org/10.1016/j.prosdent.2006.12.001 PMid:17280889

21. Gemalmaz D, Pameijer CH, Latta M, Kuybulu F, Alcan T. In vivo disintegration of four different luting agents. Int J Dent. 2012;2012:831508. http://dx.doi.org/10.1155/2012/831508 PMid:22007219 PMCid:PMC3189560

22. Avon SL, Goulet JP, Deslauriers N. Removable acrylic resin disk as a sampling system for the study of denture biofilms in vivo. 2007; 97(1):32-38.

23. Rashed E, Ibrahim S, Swelem A, Ramadan A. The Effect of Different Cleansing methods on The Surface Roughness of Conventional and Flexible Acrylic Resin denture Base Materials. The Egyptian Dental J. 2012; 58(4):3407-3416.

24. Machado AL, Breeding LC, Vergani CE, da Cruz Perez LE. Hardness and surface roughness of reline and denture base acrylic resins after repeated disinfection procedures. J Prosthet Dent. 2009; 102(2):115-122. http://dx.doi.org/10.1016/S0022-3913(09)60120-7

25. Azevedo A, Machado AL, Vergani CE, et al. Effect of disinfectants on the hardness and roughnessof reline acrylic resins. J Prosthodont. 2006; 15(4):235-242. http://dx.doi.org/10.1111/j.1532-849X.2006.00112.x PMid:16827736

26. Radford DR, Sweet SP, Challacombe SJ, Walter JD. Adherence of Candida albicans to denture- base materials with different surface finishes. J Dent. 1998; 26(7):577-583. http://dx.doi.org/10.1016/S0300-5712(97)00034-1

27. Quirynen M, Marechal M, Busscher HJ, Weerkamp AH, Darius PL, van Steenberghe D. The influence of surface free energy and surface roughness on early plaque formation: an in vivo study in man. J Clin Periodontol. 1990; 17(3):138-144. http://dx.doi.org/10.1111/j.1600-051X.1990.tb01077.x PMid:2319000

28. Yamauchi M, Yamamoto K, Wakabayashi M, Kawano J. In vitro adherence of microorganisms to denture base resin with different surface texture. Dent Mater J. 1990; 9(1):19-24. http://dx.doi.org/10.4012/dmj.9.19 PMid:2098207

29. Bollen CM, Lambrechts P, Quirynen M. Comparison of surface roughness of oral hard materials to the threshold surface roughness for bacterial plaque retention: A review of the literature. Dent Mater. 1997; 13(4):258-69. http://dx.doi.org/10.1016/S0109-5641(97)80038-3

30. Harrison Z, Johnson A, Douglas CW. An in vitro study into the effect of a limited range of denture cleaners on surface roughness and removal of Candida albicans from conventional heat-cured acrylic resin denture base material. J Oral Rehabil. 2004; 31(5):460-67. http://dx.doi.org/10.1111/j.1365-2842.2004.01250.x PMid:15140172

31. Hermann C, Mesquita MF, Consani RL, Henriques GE. The effect of aging by thermal cycling and mechanical brushing on resilient denture liner hardness and roughness. J Prosthodont. 2008; 17(4):318-22. http://dx.doi.org/10.1111/j.1532-849X.2007.00293.x PMid:18266656

32. Durkan Durkan R, Ayaz EA, Bagis B, Gurbuz A, Ozturk N, Korkmaz FM. Comparative effects of denture cleansers on physical

properties of polyamide and polymethyl methacrylate base polymers. Dent Mater J. 2013; 32(3):367-375. http://dx.doi.org/10.4012/dmj.2012-110 PMid:23718995

33. Shah J, Bulbule N, Kulkarni S, Shah R, Kakade D. Comparative Evaluation of Sorption, Solubility and Microhardness of Heat Cure Polymethylmethacrylate Denture Base Resin & Flexible Denture Base Resin. Journal of Clinical and Diagnostic Research. 2014; 8(8)1-4. http://dx.doi.org/10.7860/jcdr/2014/8707.4770

34. Neppelenbroek KH, Pavarina AC, Vergani CE, Giampaolo ET. Hardness of heat-polymerizedacrylic resins after disinfection and long-term water immersion. J Prosthet Dent. 2005; 93(2):171-176. http://dx.doi.org/10.1016/j.prosdent.2004.10.020 PMid:15674229

35. Devlin H, Kaushik P. The Effect of Water Absorption on Acrylic Surface Properties. J Prosthodontics. 2005; 14(4):233-238. http://dx.doi.org/10.1111/j.1532-849X.2005.00050.x PMid:16359479

36. Braun KO, Mello JA, Rached RN, et al. Surface texture and some properties of acrylic resins submitted to chemical polishing. J Oral Rehabil. 2003; 30(1):91-98. http://dx.doi.org/10.1046/j.1365-2842.2003.00997.x PMid:12485391

37. Phoenix RD. Denture base resins. In: Anusavice KJ ed. Phillips' Science of Dental Materials. St Louis: Elsevier, 2003:721-757.

38. Marra J, de Souza RF, Barbosa DB, Pero AC, Compagnoni MA. Evaluation of the bond strength of denture base resins to acrylic resin teeth: Effect of thermocycling. Journal of Prosthodontics. 2009; 18(5):438-443. http://dx.doi.org/10.1111/j.1532-849X.2009.00478.x PMid:19515169

39. Goiato MC, Santos DM, Moreno A, Iyda MG, Rezende MC, Haddad MF. Effect of disinfection and storage on the flexural strength of ocular prosthesis acrylic resins. Gerodontology. 2012; 29(2):838-844. http://dx.doi.org/10.1111/j.1741-2358.2011.00570.x PMid:22004090

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):482-488.
http://dx.doi.org/10.3889/oamjms.2016.096
eISSN: 1857-9655
Stomatology – Review Article

A Comparative Study of Transbuccal and Extraoral Approaches in the Management of Mandibular Angle Fractures: A Systematic Review

Sabah Ali Beza[1*], Sayed Attia[1], Edward Ellis III[2], Layla Omara[1]

[1]*Department of Oral and Maxillofacial Surgery, Faculty of Oral and Dental Medicine, Cairo University, Cairo, Egypt;* [2]*Department of Oral and Maxillofacial Surgery University of Texas Health Science Center at San Antonio, TX, USA*

Citation: Ali Beza S, Attia S, Ellis III E, Omara L. A Comparative Study of Transbuccal and Extraoral Approaches in the Management of Mandibular Angle Fractures: A Systematic Review. Open Access Maced J Med Sci. 2016 Sep 15; 4(3):482-488. http://dx.doi.org/10.3889/oamjms.2016.096

Keywords: extraoral approach; transbuccal approach; mandibular angle fracture; trocar canula; internal fixation; postoperative complications.

***Correspondence:** Dr Sabah Abd El latif Hussein Ali Beza. Cairo University, Faculty of Oral and Dental Medicine, Department of Oral and Maxillofacial Surgery, 11 Saraya Street, Manial, Cairo 11553, Egypt. E-Mail: saba7dentist@gmail.com

Received: 13-Jul-2016; **Revised:** 15-Aug-2016; **Accepted:** 16-Aug-2016; **Online first:** 02-Sep-2016

Competing Interests: The authors have declared that no competing interests exist.

Abstract

AIM: The aim of the present study was to compare the extraoral and transbuccal approaches for the treatment of mandibular angle fractures with regard to postoperative complications.

PATIENTS AND METHODS: An electronic search for relevant articles without language and date restrictions was performed in July 2016. Inclusion criteria were studies in humans including randomised controlled trials (RCTs), controlled clinical trials (CCTs), prospective studies (PS), and retrospective studies (RS). In total, 107 patients were included from four studies (transbuccal = 48, extraoral = 59). The follow-up period varied from 3 months to 24 months.

RESULTS: In extraoral group the average of unsightly scar, facial nerve weakness, infection, malocclusion, plate removal were found to be 55% (range,10% -100%), 26.5% (range, 0%-53%), 11.7% (range, 0% - 20%), 22.5% (range, 0% -50%), 6.7% (range, 3.3% - 10%) respectively while these parameters in the transbuccal approach were found to be no obvious unsightly scar, 6.6 % (range, 0%-13.3%), 8.1% (range, 0% - 20%), 4.8% (range, 0% - 12.5%), 0%. The incidence of postoperative trismus and nonunion/malunion were 0% in both groups.

CONCLUSION: The results of this study suggest that transbuccal approach shows fewer complications than extraoral approach when used for the treatment of mandibular angle fractures.

Introduction

The objective of mandibular fracture treatment is the restoration of anatomical form and function, with particular care to establishing the pre-trauma occlusion. Traditionally, this has been achieved by immobilising the jaws using various dental wiring techniques. In the previous two decades, interest has increased for different methods of open reduction and internal fixation [1]. Methods of open reduction and internal fixation have continued to evolve and have changed enormously in the last few years with the advent of plate and screw fixation hardware. Fixation devices became smaller, simpler to handle, and extraoral incisions have been minimised. However, there still is debate regarding the optimal treatment [2, 3].

Mandibular angle fractures (MAFS) have a high frequency of complications particularly in relation to the insufficient stability of the fixation systems [4-6]. Despite the advances in internal fixation used for the treatment of fractures of the mandibular angle, these fractures still present unpredictable results and difficulties in treatment compared to other mandibular fractures. A large number of studies testifies to the fact that no single approach has been shown to be ideal [7].

Extraoral approaches were traditionally used for open reduction and internal fixation of mandibular

angle fractures. It has the potential disadvantage of leaving an unaesthetic scar and risks damage to the facial nerve, though the advantages are better exposure and direct application of plate fixation [8-10]. The transbuccal approach has the advantages of no external scarring and direct visualisation of the occlusion during placement of the bone plates injury to branches of the facial and other anatomic structures were reduced [9-12].

In the previous decades, increased availability of high quality and easy-to- use trocar instrumentation has made the transbuccal approach prevalent, but research into its complication rate is greatly lacking. Presently, the choice of the approach relies on the surgeon's personal preference [13].

The aim of this study is to focus on the question: "Is there a significant difference in the clinical outcomes between the transbuccal versus extraoral approaches in the management of mandibular angle fractures?

Patients and Methods

Data sources and keywords

An electronic search was performed without language and date restrictions in July 2016 in the following data databases: Pub Med, Cochrane Database of Systematic Reviews, Cochrane Central Register of Controlled Trials (CENTRAL), Alt Health Watch, Health Source: Consumer Edition, Health Source: Nursing/Academic Edition, Scopus, Wily Online Library, and Electronic Journal Centre.

The keywords and their combinations used in this search included:

1. In PubMed: ((extraoral[All Fields] AND approach[All Fields]) OR(extroral[All Fields] AND technique[All Fields])) AND (transbuccal[All Fields] AND approach[All Fields]) OR (("mandible"[MeSH Terms] OR"mandible"[All Fields] OR"mandibular"[All Fields]) AND angle[All Fields] AND ("fractures, bone"[MeSH Terms] OR ("fractures"[All Fields] AND "bone"[All Fields]) OR "bone fractures" [All Fields] OR "fracture" [All Fields]))(700 articles) were collected from this database.

2. In Scopus:"extraoral approach" or" extraoral technique"or" transbuccal approach" and "mandibular angle fracture"(174 articles) in all years.

3. In Wily Online Library: extraoral approach or extraoral technique (in Full Text) OR transbuccal approach in Full Text AND mandibular angle fracture (in Full Text) (195 articles). A manual search of oral and maxillofacial surgery related journals including British Journal of Oral and Maxillofacial Surgery, the International Journal of Oral and Maxillofacial Surgery, Journal of Maxillofacial and Oral Surgery, Journal of Craniofacial Surgery, Journal of Oral and Maxillofacial Surgery, Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Journal of Cranio-Maxillofacial Surgery was performed. Relevant reviews on the subject and the reference lists of the studies identified were scanned for possible additional studies.

Inclusion and exclusion criteria

Inclusion criteria were studies in humans including randomized controlled trials (RCTs), controlled clinical trials (CCTs), prospective studies (RS), retrospective studies (RS), unilateral or bilateral fractures of mandibular angle fracture with the aim of a comparative study between the extraoral approach and transbuccal approach with the use of transbuccal instrumentation for treatment of mandibular angle fractures with regard to postoperative complications and other factors. Exclusion criteria were: combined symphyseal and condylar fractures, comminuted fractures, edentulous patients, technical reports, case reports, in vitro studies, animal studies, and review papers.

Selection of relevant studies

The following data were extracted from the studies included in the final analysis: authors, year of publication, study design, number of participants , patient age range and/or mean age, follow-up period, site of MFs, MAF, fixation methods, surgical approach, duration of operation, postoperative maxillomandibular fixation (MMF), use of antibiotics and/or chlorhexidine, teeth retained and removed in MFs, and postoperative complications including evaluation of the resulting scar from an aesthetic point of view, facial nerve damage evaluation, treatment of tooth in the fracture line and its implication on malunion and non-union, infection, postoperative malocclusion, need for plate removal and mouth opening .

Assessment of Quality

A methodological quality analysis was performed by merging the proposed criteria of the Strobe statement [14], Moose statement [15], and Prisma statement [16], to verify the force of scientific evidence in making clinical decisions. The classification of the risk of potential bias for every article was based on the following criteria: random selection in the participants, the definition of inclusion and exclusion criteria, report of losses to follow-up, the validity of assessments, and statistical analysis. A study that comprised all the criteria mentioned above was categorised as having a low risk of bias, a study that did not comprise one of these criteria was categorised as having a moderate risk of bias. If two

or more criteria were missed, the study was classified to have a high risk of bias.

Results

Summary of the study selection process is shown in Figure 1. The electronic search resulted in 1069 studies; seven additional articles were added from hand- searching and other sources. After the initial screening of articles, 52 articles were excluded because of duplication. Of the remaining 1024 articles assessed, 929 were excluded by title and abstract because they were not related to the topic. Ninety-five studies were selected for full- text analysis leading to the exclusion of 91 articles because they did not meet the inclusion and exclusion criteria. Thus, a total of 4 articles were included in the systematic review.

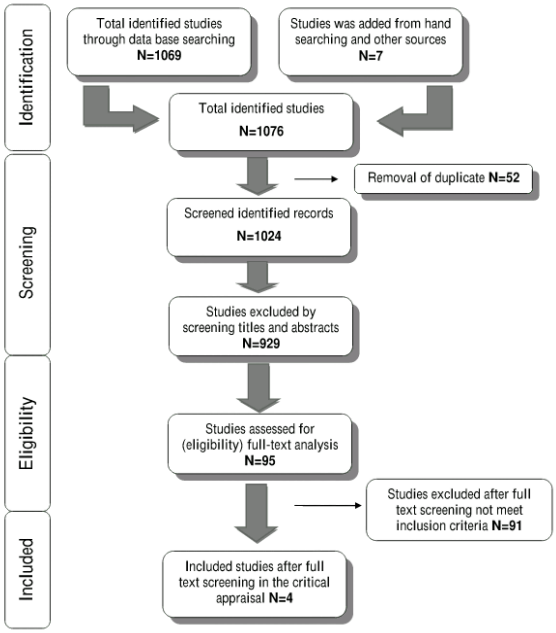

Figure 1: Flow diagram of study selection process

Description of included studies

Extracted data of the included 4 studies are listed in Table 1. Three prospective studies [9, 10, 12], and one retrospective studies [17] were included in this study. A total of 170 patients were enrolled in the four studies, but 63 were excluded because they had other surgical approaches. This left a total of 107 patients with 48 patients in the transbuccal approach group and 59 patients in the extraoral approach group. The ages ranged from 16-62 years. The follow-up period varied from 3 months to 24 months. Additional MFs were reported in three studies [9, 10, 12]. Regarding the transbuccal approach group, three studies performed fixation using a single miniplate, in one of these articles performed the fixation on the lateral aspect of the mandible using 2 mm miniplate, a four-hole centrally spaced standard mini plate and 6-8 mm screws [17]. In the second article a single 2.5 mm, four whole stainless steel mini plate with a gap and 2.5 mm × 8 mm screws was placed along the lateral aspect of the mandible [9]. In a third article [10] used the same fixation method as the second study a single 2.5 mm non-compression, 4-holed with gap stainless steel mini plate and 6 or 8 mm monocortical screws was used. One of the four studies performed the fixation using 2 mini plates in that article [12], 2 mini plates were placed with approximately1 cm distance between them on the lateral cortex. The plate superiorly was a two-hole miniplate fixed superiorly on the external oblique ridge and the inferior plate was a four whole miniplate fixed along the lateral aspect of the mandible.

Regarding the extra-oral group, there was one article [17] that used two 4-hole centrally spaced mini plates; one study [12] used two mini plates with approximately1 cm distance between them and the fixation performed on the lateral cortex; In one article a single 2.5 mm, four hole stainless steel mini plate with gap and 2.5 mm × 8 mm screws was placed on the lateral cortex [9]. In one article [10] a single non-compression 2.5 mm, 4-holed with gap stainless steel mini plate and 6 or 8 mm monocortical screws was used. Two of four studies [9, 12] provided information on the mean operation time.

Assessment of Quality

The risk of bias outcomes is summarised in Table 2. Two [12, 17] were considered to be a high risk of bias and two were considered to be a low risk of bias [9, 10].

Effect of intervention

A summary of the results is presented in Table 3.

Scar from the aesthetic point of view

Three studies with 62 fractures evaluated scarring. In two of these studies with an extraoral approach the incidence of the scar was 55% (range 10 % to 100% while the transbuccal approach showed no obvious scar. The third study with 30 fractures divided into 15 patients in each group evaluated the scar using the Vancouver scar rating scale. The scar rating scale showed a value of 3.6 with the transbuccal approach and 6.73 in the extraoral approach patients.

Table 1: Studies comparing management of mandibular angle fractures via transbuccal and extraoral approaches

Authors, Publication year	Study design	P (n)	Patient age range (mean), years	Follow up	Site of MFs	Methods of fixation MAF	Surgical approach	Duration of surgery, min, mean	Post operative MMF (n)	Antibiotics/ chlorhexidine, days	Teeth retained/ removed (in MAF)
Kale et al., 2010	PS	15	26.9y	7 day 2 weeks 3 months	Angle (n = 14), Body (n = 1), Para-symphysis (n = 8),	(G1) 2 mm two miniplates with around 1 cm distance on lateral cortex (n = 4) (G2) 2 mmt wo miniplates with around 1 cm distance on lateral cortex (n = 10)	Extraoral Intraoral + transbuccal	(G1) 63 (G2) 49.5	NP	NM	2/12
Kumar et al.,2011	RA	80	16-62 (26.6y)	weekly 3 months	Angle N = 80	(G1) four hole centrally spaced 2 mm, two miniplates (n = 30) (G2) four hole centrally spaced 2 mm, one miniplates (n =15) (G3) four hole centrally spaced 2 mm, one miniplates (n = 35)	Extraoral Intraoral + transbuccal Intraoral	NM	NP	NM	Retained teeth in line of fracture 73/NM
Patter et al., 2014	PS	30	NM	1 week 2 weeks 3 weeks 4 weeks 6 weeks 8 weeks 10 weeks 12 weeks 6-24 months	Angle fractures (N = 45) patients associated with other mandibular fractures	(G1) single non-compression 2.5 mm, 4 holed with gap stainless steel mini plate and 6/8 mm monocortical screws (n = 12). (G2) single non-compression 2.5 mm, 4 holed with gap stainless steel mini plate and 6/8 mm monocortical screws (n = 8). (G3) single non-compression 2.5 mm, 4 holed with gap stainless steel mini plate and 6/8 mm monocortical (screws (n = 10)	Intraoral approach Transbuccal approach Extraoral approach	NM	(G1)12 (G2)8 G3)10	NM	NM/1
Sudhakar et al., 2015	PS	45	16-51 (29.6y)	1 week 2 weeks 4 weeks 6 weeks 3 months 6 months	Angle fractures (N = 45) out of 45, 24 patients associated with other facial fractures	(G1) A single 2.5 mm, four hole stainless steel mini plate with gap and 2.5 mm × 8 mm screws, (n = 15) (G2) A single 2.5 mm, four hole stainless steel mini plate with gap and 2.5 mm × 8 mm Screws (n = 15) (G3) A single 2.5 mm, four hole stainless steel mini plate with gap and 2.5 mm × 8 mm screws	Intraoral transbuccal +intraoral approach extraoral approach	(G1) 65 ± 3.27 (G2) 93.5 ± 4.36 (G3) 85.5 ± 4.90	G1)15 (G2)15 (G3)15	Patients were admitted for IV antibiotics/NM	NM

P, participants; MAF, mandibular angle fracture; MMF, maxillomandibular fixation. MF, mandibular fracture; RA, retrospective analysis; PS ,Prospective study; NM, not mentioned; NP, not performed.

Facial nerve damage evaluation

Two studies with 44 fractures divided into 19 fractures in the extraoral approach and 25 fractures in the transbuccal approach evaluated facial nerve function. The incidence of facial nerve weakness in the transoral group 6.6 % (range, 0 % to 13.3%) and in the extraoral approach the incidence was 26.5% (range, 0 % to 53%).

Table 2: Results of the quality assessment

Authors and year of Publication	Random selection of participants	Definition inclusion/ exclusion criteria	Loss of follow-up	Validity of assessment	Statistical analysis	Reported potential the risk of bias
Kale et al., 2010	No	Yes	yes	Yes	No	high
Kumar et al., 2011	No	yes	yes	yes	No	high
Patter et al., 2014	yes	yes	yes	yes	yes	low
Sudhakar et al., 2015	Yes	yes	yes	yes	yes	low

Tooth in the line of fracture and its implication on malunion and non-union

The incidence of nonunion was assessed in one study with 30 fractures divided into 15 in the transbuccal group and 15 in the extraoral group. The incidence of non-union in both groups was 0%.

Infection

A total of 107 fractures enrolled in four studies evaluated the incidence of infection, 48 fractures in the transbuccal approach group and 59 fractures in the extraoral group. In the transbuccal group, the incidence of infection was 8.1% (range, 0% to 20%) whereas in the extraoral group the incidence of infection was 11.7% (range, 0 % to 20%).

Malocclusion

Four studies with 107 fractures divided into 48 in the transbuccal and 59 in the extraoral group assessed the incidence of malocclusion. In the transbuccal group, the incidence of malocclusion was 4.8% (range, 0% to 12.5%)whereas in the extraoral group the incidence of malocclusion was 22.5% (range, 0% to 50%).

Mouth opening (trismus)

Three studies analysed the incidence of trismus postoperatively with 62 fractures divided into 33 in the transbuccal group and 29 in the extraoral group. The incidence of trismus was 0% in both groups.

Table 3: Summary of the intervention effect

	Approach		Transbuccal							Extraoral						
Author /year	Transoral /transbucal	Extraoral	INF%	MO%	PR%	NU /MU%	TR%	SC%	FW%	INF%	MO%	PR%	NU /NU%	TR%	SC%	FW%
Kale et al.,2010	10	4	0	0	_	_	0	0	0	0	50	_	_	0	100	0
Kumar et al.,2011	15	30	0	6.7	0	_	_	_	_	16.6	0	3.3	_	_	_	_
Pattar et al.,2014	8	10	12.5	12.5	0	_	0	0	_	10	40	10	_	0	10	_
Sudhakar et al., 2015	15	15	20	0	_	0	0	VRS 3.6	13.3	20	0	_	0	0	VRS 6.7	53

INF = Infection, MO = Malocclusion, PR = Plate removal ,NU/MU = Non union /Malunion, TR = Trismus, SC = Scar, FW = Facial weakness.

Plate removal

The incidence of plate removal was reported in two studies with 63 fractures divided into 23 in the transbuccal group and 40 fractures in the extraoral group. The incidence of plate removal in the transbuccal group was 0% whereas the incidence of plate removal in the extraoral group was 6.7% (range, 3.3% to 10%).

Discussion

The surgical approach in the management of mandibular fractures has been an ongoing point of debate some authors advocating the transbuccal approach and others the extraoral approach still others advocate a combination approach [18]. To the best of our knowledge, there is no systematic literature review comparing the transbuccal and extraoral approaches for mandibular angle fractures.

The extraoral approach provides easy access and direct visualisation, but it is associated with marginal mandibular nerve injury and an often visible scar [12]. In a study by Toma et al. [8] no significant difference in the complication rate was reported between the transoral and extraoral approaches for the treatment of mandibular fractures, including body, angle, and ramus. Angle fractures are more difficult to treat with the transoral approach than anterior mandibular fractures and they have a higher incidence of complications such as infection and non-union.

It has been shown that when the surgeon shifts from the transoral approach to the extraoral approach intra-operatively the complication rate increases, Therefore, a preoperative decision about the surgical approach should be made. The extraoral approach theoretically provides a cleaner wound by separating the sterile skin from the contaminated oral cavity [8]. The extraoral approach also allows direct visualisation of both medial and lateral cortices to assist with proper reduction [19]. Unfortunately, the extraoral route may cause an unsightly scar [8, 12, 17].

The transbuccal approach is usually advocated because it results in no external scar and allows direct visualisation and confirmation of the proper occlusion during placement of the bone plates [12]. Despite the advantages of this approach, it is through a contaminated area that might increase the risk of infection. Transbuccal trocar instrumentation is a sensitive technique and the surgeon has to be familiar with the armamentarium and be skilled in the use of the trocar cannula. In the literature, there is some debate about identifying a safe and accurate technique for transbuccal incisions [20]. It has been suggested that the surgeon's inexperience will lead to additional facial incisions, especially when access is severely limited due to the nature of the masseteric region, and there is a risk of damaging the facial nerve [21, 22].

From an aesthetic point of view, the extraoral route can cause an obvious unsightly scar. Three studies [9, 10, 12] assessed the incidence of the postoperative scar and two of these studies found that the mean extraoral scar was 55 %, while transbuccal approach showed no obvious unsightly scar. While the remaining study evaluated the scar using the Vancouver scar rating scale which showed a value of 3.6 with the transbuccal approach and 6.73 in the extraoral approach patients. Some authors attributed hypertrophic scar formation to abnormal healing processes. Although the processes leading to hypertrophic scar formation are not yet clarified, altered apoptotic behaviour was believed to be a significant factor [23].

Facial nerve injury is a common complication encountered with the extraoral approach. Two of the included studies assessed facial nerve function postoperatively and found that the incidence of postoperative transient facial nerve weakness in the transbuccal group was 6.6% and in the extraoral approach, the incidence was 26.5%. The complication could be attributed to the blunt trauma caused due to soft tissue retraction and tissue dissection [9].

Nonunion and delayed union usually result from infection or conditions that decrease the blood supply after mandibular fracture treatment [24]. The incidence of nonunion and malunion is between 1% and 2% in the literature [25]. What to do with a tooth in the line of fracture is always a question. Regarding

its implication on malunion and non-union, tooth in the line of fracture has been implicated among causes of non-union in mandibular fractures [26]. one of the investigated study [9] assessed nonunion was 0% in both groups.

Infection is the most common complication with mandibular fractures, especially those at the angle. Infections evaluated in all included studies, Infections were 8.1% with the transbuccal approach and 11.7% with the extraoral approach which could be due to increased operative time and improper patient maintenance and wound dehiscence [9]. Some authors claim that infection is attributable to poor oral hygiene, inappropriate post-operative instructions, longer operative time and surgical technique but not the hardware used, others blame fixation hardware [27]. Successful treatment of mandible fractures depends on undisturbed healing in the correct anatomical position under stable conditions. Failure to achieve this leads to infection, malocclusion or nonunion [2 4 . Some authors claim that the use of a single miniplate leads to more infections than when two- mini plates are employed [28, 29]. However, the process of putting the second miniplate at the lower border means increased periosteal stripping, bacterial contamination and added hardware on the mandible, which theoretically can increase the possibility of infection [17, 25].

Plate removal was much higher in the extraoral approach than with the transoral approach (6.7% versus 0%). The need for plate removal was attributed to infection and wound dehiscence [10]. Four studies reported a 4.8% malocclusion rate with the transbuccal approach and 22.5% with the extraoral approach. In analysing the cause of malocclusion the patients had an associated second fracture on the contralateral side and this may be a confounding factor. In some cases, malocclusion was the result of a sub-optimal reduction at operation or inadequate stability after treatment [24, 25]. Mouth opening (trismus), three studies analysed the incidence of trismus postoperatively. The incidence of trismus was 0% in both groups. Two studies did not mention the duration of surgery. Therefore, an appropriate comparison regarding the mean operation time was not possible. However, the dissection through multiple tissue layers and the closure with the extraoral approach obviously increases the duration of surgery.

The results of this study suggest that transbuccal approach shows fewer complications than extraoral approach when used for the treatment of mandibular angle fractures.

References

1. Booth PW, Schendel SA, Hausamen JE. Maxillofacial surgery, 2nd ed, vol. 1. London, UK: Churchill Livingstone, 2007; p. 74–76.

2. Gear AJ, Apasova E, Schmitz JP, Schubert W. Treatment modalities for mandibular angle fractures. J Oral Maxillofac Surg. 2005;63(5):655-63. http://dx.doi.org/10.1016/j.joms.2004.02.016 PMid:15883941

3. Zix J, Lieger O, Iizuka T. Use of straight and curved 3-dimensional titanium miniplates for fracture fixation at the mandibular angle. J Oral Maxillofac Surg. 2007;65(9):1758-63. http://dx.doi.org/10.1016/j.joms.2007.03.013 PMid:17719394

4. Iizuka T, Lindqvist C, Hallikainen D, Paukku P. Infection after rigid internal fixation of mandibular fractures: A clinical and radiologic study. J Oral Maxillofac Surg. 1991; 49(6):585-93. http://dx.doi.org/10.1016/0278-2391(91)90340-R

5. Lamphier J, Ziccardi V, Ruvo A, Janel M. Complications of mandibular fractures in an urban teaching center. J Oral Maxillofac Surg. 2003;61(7):745-9. http://dx.doi.org/10.1016/S0278-2391(03)00147-2

6. Ellis E 3rd. Treatment methods for fractures of the mandibular angle. J Craniomaxillofac Trauma. 1996; 2(1):28-36. PMid:11951472

7. Guimond C, Johnson JV, Marchena JM. Fixation of mandibular angle fractures with a 2.0-mm 3- dimensional curved angle strut plate. J Oral Maxillofac Surg. 2005;63(2):209-14. http://dx.doi.org/10.1016/j.joms.2004.03.018 PMid:15690289

8. Toma VS, Mathog RH, Toma RS, Meleca RJ. Transoral versus extra-oral reduction of mandible fractures: A comparison of complication rates and other factors. Otolaryngol Head Neck Surg. 2003;128(2): 215–19. http://dx.doi.org/10.1067/mhn.2003.59 PMid:12601317

9. Sudhakar GV, Rajasekhar G, Dhanala S, Vura N, Ramisetty S. Comparison of Management of Mandibular Angle Fractures by Three Approaches. J Maxillofac Oral Surg. 2015;14(4):979-85. http://dx.doi.org/10.1007/s12663-015-0779-0 PMid:26604473

10. Pattar P, Shetty S, Degala S. A Prospective Study on Management of Mandibular Angle Fracture. J Maxillofac Oral Surg. 2014;13 (4): 592-8. http://dx.doi.org/10.1007/s12663-013-0542-3 PMid:26225033 PMCid:PMC4518788

11. Gulses A, Kilic C, Sencimen M. Determination of a safety zone for transbuccal trocar placement: an anatomical study. Int J Oral Maxillofac Surg. 2012;41(8):930-33. http://dx.doi.org/10.1016/j.ijom.2012.02.013

12. Kale TP, Baliga SD, Ahuja N, Kotrashetti SM. A comparative study between transbuccal and extra-oral approaches in treatment of mandibular fractures. J Maxillofac Oral Surg. 2010;9(1):9-12. http://dx.doi.org/10.1007/s12663-010-0026-7 PMid:23139558 PMCid:PMC3453684

13. Wan K, Williamson RA, Gebauer D, Hird K. Open reduction and internal fixation of mandibular angle fractures: does the transbuccal technique produce fewer complications after treatment than the transoral technique? J Oral Maxillofac Surg. 2012; 70(11):2620-8. http://dx.doi.org/10.1016/j.joms.2012.07.051 PMid:22959879

14. Von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP, for the STROBE Initiative. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. Lancet. 2007; 370(9596):1453–7. http://dx.doi.org/10.1016/S0140-6736(07)61602-X

15. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA, Thacker SB. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis of Observational Studies in Epidemiology (MOOSE) group. JAMA. 2000; 283(15):2008–12. http://dx.doi.org/10.1001/jama.283.15.2008 PMid:10789670

16. Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 2009; 6(7): e1000097. http://dx.doi.org/10.1371/journal.pmed.1000097 PMid:19621072 PMCid:PMC2707599

17. Kumar S, Prabhakar V, Rao K, Brar R. A comparative review of

treatment of 80 mandibular angle fracture fixation with miniplates using three different techniques. Indian J Otolaryngol Head Neck Surg. 2011 ;63(2):190-2. http://dx.doi.org/10.1007/s12070-011-0236-4 PMid:22468259 PMCid:PMC3102161

18. Singh V, Khatana S, Bhagol A. Superior border versus inferior border fixation in displaced mandibular angle fractures: prospective randomized comparative study. Int J Oral Maxillofac Surg. 2014; 43(7):834-40. http://dx.doi.org/10.1016/j.ijom.2013.09.009 PMid:24636170

19. Cillo JE Jr, Ellis E 3rd. Treatment of patients with double unilateral fractures of the mandible. J Oral Maxillofac Surg. 2007;65(8):1461-9. http://dx.doi.org/10.1016/j.joms.2006.08.013 PMid:17656269

20. Wales CJ, Carter LM. A safe and accurate technique for transbuccal incision. Br J Oral Maxillfac Surg. 2007; 45(2):177. http://dx.doi.org/10.1016/j.bjoms.2006.03.004 PMid:16621207

21. Cole P, Rottgers SA, Cameron H, HollierJr LH. Improving the minimally invasive approach to mandible angle repair. J Craniofac Surg. 2008;19(2):525–7. http://dx.doi.org/10.1097/SCS.0b013e3180a77340 PMid:18362737

22. Zide MF, Kent JN. Indications for open reduction of mandibular condyle fractures. J Oral Maxillofac Surg. 1983;41(2):89–98. http://dx.doi.org/10.1016/0278-2391(83)90214-8

23. Shetty V, Bertolami CN. Wound Healing. In Peterson's Principles Of Oral and Maxillofacial Surgery: Second Edition, Chapter 1, 2004; 1: 9.

24. Vineeth K, Lalitha RM, Prasad K, Ranganath K, Shwetha V, Singh J. A comparative evaluation between single noncompression titanium miniplate and three dimensional titanium miniplate in treatment of mandibular angle fracture - a randomized prospective study. J Craniomaxillofac Surg. 2013;41(2):103-9. http://dx.doi.org/10.1016/j.jcms.2012.05.015 PMid:22809633

25. Siddiqui A, Markose G, Moos KF, McMahon J, Ayoub AF. One miniplate versus two in the management of mandibular angle fractures: a prospective randomised study. Br J Oral Maxillofac Surg. 2007;45(3):223-5. http://dx.doi.org/10.1016/j.bjoms.2006.08.016 PMid:17110006

26. Mendonca D, Kenkere D. Avoiding occlusal derangement in facial fractures: An evidence based approach. Indian Journal of Plastic Surgery: Official Publication of the Association of Plastic Surgeons of India. 2013;46(2):215-20. http://dx.doi.org/10.4103/0970-0358.118596 PMid:24501457 PMCid:PMC3901902

27. Singh V, Puri P, Arya S, Malik S, Bhagol A. Conventional versus 3- dimensional miniplate in management of mandibular fracture: a prospective randomized study. Otolaryngol Head Neck Surg. 2012;147(3):450–5. http://dx.doi.org/10.1177/0194599812449437 PMid:22647925

28. Levy FE, Smith RW, Odland RM, Marentette LJ. Monocortical miniplate fixation of mandibular fractures. Arch Otolaryngol Head Neck Surg. 1991;117 (2):149-54. http://dx.doi.org/10.1001/archotol.1991.01870140037002 PMid:1991053

29. Valentino J, Levy FE, Marentette LJ. Intraoral Monocortical miniplating of mandible fractures. Arch Otolaryngol Head Neck Surg. 1994;120(6):605-12. http://dx.doi.org/10.1001/archotol.1994.01880300021003 PMid:8198783

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):489-492.
http://dx.doi.org/10.3889/oamjms.2016.100
eISSN: 1857-9655
Stomatology – Case Report

Managing the Cutaneous Sinus Tract of Dental Origine

Edvard Janev*, Enis Redzep

Faculty of Dentistry, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia

Citation: Janev E, Redzep E. Managing the Cutaneous Sinus Tract of Dental Origine. Open Access Maced J Med Sci. 2016 Sep 15; 4(3):489-492. http://dx.doi.org/10.3889/oamjms.2016.100

Keywords: Fistula; cutaneous sinus tract; periapical dental infections; root canal.

***Correspondence:** Edvard Janev. Faculty of Dentistry, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia. E-mail: edijanev@hotmail.com

Received: 28-Jun-2016; **Revised:** 08-Jul-2016; **Accepted:** 24-Aug-2016; **Online first:** 06-Sep-2016

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: Draining cutaneous sinus tract in chin area may be caused by chronic periapical dental infections. Misdiagnosis of these lesions usually leads to destructive invasive treatment of the sinus tract that is not correct and curative.

CASE REPORT: A 31-year-old male patient referred to us with a chronically draining lesion on his chin. The lesion previously was misdiagnosed by medical doctors and had undergone two times surgery with a focus on the skin lesion and had received antibiotic therapy for a prolonged period of time. After clinical and radiologic examination the dental origin of the lesion was evident and proper endodontic and surgical treatment was performed. Three months later, after the treatment, the lesion showed total healing and reoccurrence occurred.

CONCLUSION: The key to successful treatment of cutaneous sinus tract of dental origin must be in appropriate communication between the dentist and the physician in order to achieve correct diagnosis and therapy in such cases.

Introduction

The successful treatment of cutaneous sinus tract of dental origin depends on the diagnosis of the source which may be very challenging. These lesions present a diagnostic problem and misdiagnosis leads to incorrect and unsuccessful treatment. Very often the possibility of an odontogenic origin is overlooked because most of the patients do not experience any dental symptoms.

Treatment with systemic antibiotics results in temporary cessation of the drainage which returns immediately after antibiotic treatment is over. The diagnosis may be challenging for several reasons:

1. The cutaneous lesions do not always arise in close proximity to the underlying infection and only half of all patients ever recall having had a toothache.
2. The sinus tract appears most commonly on the chin or jaw line but they also can appear elsewhere on the face and neck [1, 2].
3. Lesions have been reported to occur as far away from oral cavity as the chest, tight or sacrum [2-5].
4. Because cutaneous lesions can mimic other disorders, several inappropriate surgeries and courses of antibiotics are commonly used before definite therapy is instituted [2, 6, 7].

Patients with extraoral drainage from periapical pathosis may be unaware of any dental problem and tend to seek treatment from physicians, who may not give high priority to chronic dental infections.

The differential diagnosis should include:

- Infected pylar or epidermal cyst
- Carbuncle
- Pyogenic granuloma
- Suppurative lymphadenitis
- Foreign body reaction
- Thyroglossal tract fistula
- Branchial cleft fistula
- Actinomycosis
- Basal cell and squamous cell carcinoma [8-11].

Dental etiology of these lesions can be confirmed by:

- Tracing the sinus tract to its origin with gutta percha or other radioopaque material
- Pulp vitality testing
- Periapical films
- Panoramic films

Case Report

A 31-year-old male patient referred to our clinic with a chronically draining lesion on his chin. His history revealed that he had this lesion for more than 5 months and had undergone two times surgery and received antibiotics for prolonged period of time.

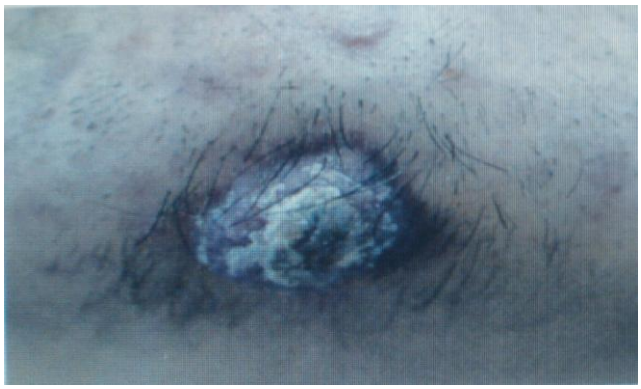

Figure 1: Skin lesion located on chin area

Dental history revealed no pain or any dental symptoms but he recalls to a direct blunt trauma to the anterior mandibular region. The periapical radiograph showed a large radiolucent area around lower right first incisor. There was no electric or thermal pulp testing performed on the same tooth. Neither percussion nor palpation revealed any abnormality.

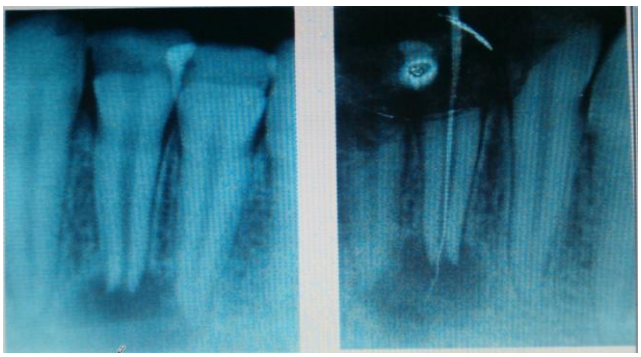

Figure 2: Rtg diagnosis of chronical periodontitis periapical and root canal treatment

The tooth was treated with calcium hydroxide and glycerine and antibiotics for 14 days. After the initial filling of the root canal, an apicoectomy and sinus excision was performed. Three months postoperative control revealed no sinus fistula or exudates from chin or from the mucosa.

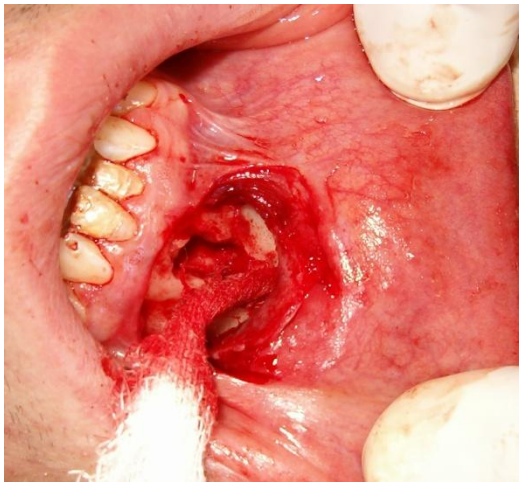

Figure 3: Surgical removal of periapical lesion with apical resection

Discussion

If correctly diagnosed and treated the tract is expected to disappear within 7 to 14 days.Systemic antibiotic therapy will result in a temporary reduction of the drainage and apparent healing [12]. This tract however will recur immediately the AB therapy is completed unless the initial source is not eliminated [12].

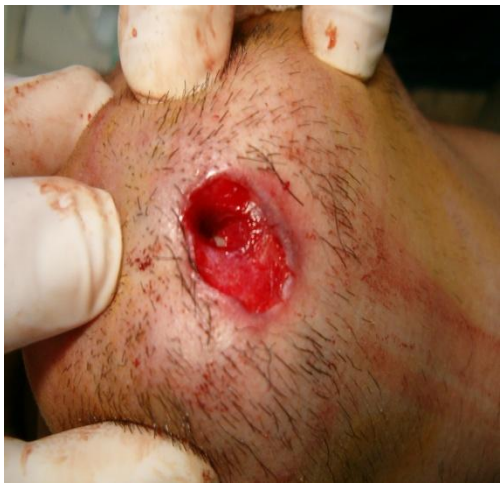

Figure 4: Curettage of the fistula

Extraoral cutaneous sinus tracts are usually lined with granulomatous tissue with a lumen containing a purulent exudate. The exudate is composed mainly of PMNL [13, 14]. Unlike intraoral tracts, extraoral tracts heal with granulation tissue

leaving a cutaneous scar [15]. The patients may have to undergo a revision of the scar.

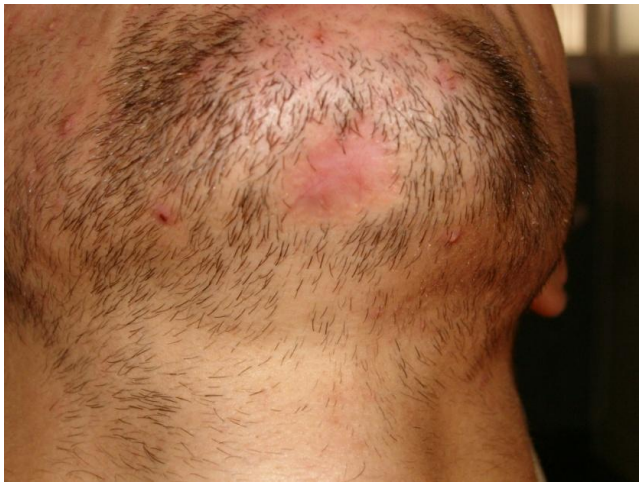

Figure 5: Total recovery in mental extraoral area a few weeks later

Eighty of reported cases of odontogenic origin are associated with mandibular teeth [16]. The sinus tract usually disappears in 5 to 14 days after the root canal system has been thoroughly cleansed [17]. An intraoral and extraoral sinus can develop depending on the path of the inflammation dictated by surrounding muscular attachments and facial planes [18].

The majority of sinuses arisen are intraoral [19, 20]. A retained root fragment can be the cause in edentulous patients [21, 22]. Most infections are polymicrobial and culture often yields growth of anaerobes or facultative anaerobes such as streptococcal spices [2, 19, 23, 24]. Due to two time's surgical interventions and a prolonged antibiotic usage, we did not see relevant to have an antibiogram as it would not reflect the exact picture of the flora.

Johnson et al. believe that the application to heat to the face may contribute to the cutaneous exit of these sinus tracts since it is well known that the heat causes vasodilatation and increase blood flow to the local area [12]. Caliskan and colleagues presented three cases of cutaneous sinus tracts that were treated with CaOH and glycerine mixture intensionally placed beyond the apex. They performed microbiological culturing and found a mixed assortment of both obligate and facultative anaerobic bacteria identified as representatives of both endodontic abscesses and skin infections [25].

Conventional root canal therapy and sometimes extraction of the tooth are effective in achieving healing of cutaneous sinus tracts in a few weeks.In general, it is not necessary to treat the skin lesion,except for esthetic reason [26]. Al-Kandari reported completely healing of the sinus tract after proper root-canal treatment without surgical treatment in three months leaving a small scar [27].

In this case, the skin lesion was treated surgically because the patient had undergone two times surgical intervention with the focus on the skin lesion and had a bigger defect on his chin.Also, the time of healing is shortened with additional surgical removal and apical resection.

In conclusion, the key to successful treatment of cutaneous sinus tract of dental origin must be in appropriate communication between the dentist and the physician in order to achieve correct diagnosis and therapy in such cases. Basic principles of root canal treatment should be used judiciously to create a favourable environment while effectively eliminating the pathogens and giving the body's immune, healing and repair mechanism a chance for the desired result.

References

1. Marasco PV, Taylor RG, Marks MW. Dentocutaneous fistula. Ann Plast Surg. 1992;29:205-210. http://dx.doi.org/10.1097/00000637-199209000-00003 PMid:1524369

2. Karp MP, Bernat JE, Cooney DR. Dental diseases masquerading as suppurative lesions of the neck. J Pediatric Surg. 1982 ;17:532-536. http://dx.doi.org/10.1016/S0022-3468(82)80103-6

3. Stoll HL, Solomon HA. Cutaneous sinuses of dental origin. JAMA. 1963 ;184:120-138 http://dx.doi.org/10.1001/jama.1963.03700150074012

4. Endelman J. Oral and Dental Pathology. St.Lous, CV. Mosby Co., 1920.

5. Gurdin M, Pangman WJ. Dermal sinuses of dental origin with a report of three cases. Plastic. Reconstruction Surg. 1953 ;11:444-453. http://dx.doi.org/10.1097/00006534-195306000-00004

6. Cohen PR, Elezri YD. Cutaneous odontogenic sinus simulating a basal cell carcinoma. Plastic. Reconstruction Surg.1990;86:123-127. http://dx.doi.org/10.1097/00006534-199007000-00020 PMid:2193312

7. Fatouris PN. A cautionary tale: case report. Ausrt Dent J. 2000;45:53-54. http://dx.doi.org/10.1111/j.1834-7819.2000.tb00243.x

8. Kun H, Chung WK, Se L. Cutaneous sinus tract from remaining tooth fragment of edentulous maxillary. J Craniofac Surg. 2000;11 (3):254-257. http://dx.doi.org/10.1097/00001665-200011030-00010

9. Held JL, Junacov MJ, Barber RJ. Cutaneous sinus tract of dental origin:a diagnosis requiring clinical and radiological correlation. Cutis. 1989;43:22-24. PMid:2914462

10. Son TW, Chang BK, Yoo TV. Sinus tract of dental origin with pyogenic granuloma. Korean J Dermatol. 1980;18:253-258.

11. Koo Bg, Cha JS, Park JK. Two cases of cutaneous fistula of dental origin. Korean J Dermatol. 1983;21:703-705.

12. Johnson BR, Remeikis NA, Van Cura NA. Diagnosis and treatment of cutaneous facial sinus tracts of dental origin. J Am Dent Assoc. 1999;130:832-836. http://dx.doi.org/10.14219/jada.archive.1999.0307 PMid:10377641

13. Nestor C, Sunil K, Ilan R. Extraoral sinus tract misdiagnosed as an endodontic lesion. J Endod. 2003;29(12):841-843. http://dx.doi.org/10.1097/00004770-200312000-00015 PMid:14686820

14. Valderhaug J. A histologic study of experimentally produced intra-oral odontogenic fistula in monkeys. Int J Oral Surg. 1973;2:54-61. http://dx.doi.org/10.1016/S0300-9785(73)80003-1

15. McWalter GM, Alexander JB, del Rio CE, Knott JW. Cutaneous sinus tract of dental etiology. Oral Surg Oral Med Oral Path. 1988;66:608-614. http://dx.doi.org/10.1016/0030-4220(88)90384-2

16. Cioffi GA, Terezhalmy GT, Parlette HL. Cutaneous draining sinus tract:an odontogenic etiology. J Am Acad Dermatol. 1986;14:94-100. http://dx.doi.org/10.1016/S0190-9622(86)70012-1

17. Spear KL, Sheridan PJ, Parry HO. Sinus tract to the chin and jaw of dental origin. J Am Acad Dermatol. 1983;8:486-492. http://dx.doi.org/10.1016/S0190-9622(83)70053-8

18. Canatore JL, Klein PA, Lieblich LM. Cutaneous dental sinus tract, a common misdiagnosis: a case report and review of the literature. Cuts. 2002;70:264-267.

19. Foster KH, Primack PD, Kulid JC. Odontogenic cutaneous sinus tract. J Endodontics. 1992;18:304-306. http://dx.doi.org/10.1016/S0099-2399(06)80959-3

20. Yang ZP, Lai YL. Healing of a sinus tract of periodontal origin. J Endodontics. 1992;18:178-180. http://dx.doi.org/10.1016/S0099-2399(06)81414-7

21. Orlow SJ, Watsky KL, Bolognia JL. Skin Bones II. J Am Acad Dermatol. 1991;25:447-462. http://dx.doi.org/10.1016/0190-9622(91)70226-R

22. Tidwell E, Jenkins JD, Ellis CD. Cutaneous odontogenic sinus tract to the chin: a case report. Int Endodontic J. 1997; 352-355. PMid:9477827

23. Swift JQ, Gulden WS. Antibiotic therapy: managing odontogenic infections. Dent Clin N Am. 2002;46: 623-633. http://dx.doi.org/10.1016/S0011-8532(02)00031-9

24. Weiger R, Manncke B, Werner H. Microbial flora or sinus tracts and root canals of non-vital teeth. Endo Dent Traumatol. 1995;11:15-19. http://dx.doi.org/10.1111/j.1600-9657.1995.tb00673.x

25. Caliskan MK, Sen BH, Ozinel MA. Treatment of extraoral sinus tracts from traumatized teeth with apical peropdontitis. Endod Dent Traumatol. 1995;11:115-120. http://dx.doi.org/10.1111/j.1600-9657.1995.tb00471.x PMid:7641626

26. Grosman II. Endodontic Practice. 9th ed.Philadelphia: Lea&Fabriger Co., 1978:89-96.

27. Al-Kandari AM, Al-Quoud OA, Ben-Nadji A. Quintescence Int. 1993; 10:729-733.

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):493-498.
http://dx.doi.org/10.3889/oamjms.2016.066
eISSN: 1857-9655
Public Health

Allostatic Load Assessment for Early Detection of Stress in the Workplace in Egypt

Ola Sayed Ali[1], Nadia Badawy[2], Sanaa Rizk[2], Hend Gomaa[2], Mai Sabry Saleh[2*]

[1]Department of Biochemistry, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt; [2]Department of Environmental and Occupational Medicine, National Research Center, Cairo, Egypt

Citation: Ali OS, Badawy N, Rizk S, Gomaa H, Saleh MS. Allostatic Load Assessment for Early Detection of Stress in the Workplace in Egypt. Open Access Maced J Med Sci. 2016 Sep 15; 4(3):493-498. http://dx.doi.org/10.3889/oamjms.2016.066

Keywords: Allostatic load; stress; job satisfaction;occupational stress; chronic diseases.

***Correspondence:** Mai Sabry Saleh. Department of Environmental and Occupational Medicine, National Research Center, Cairo, Egypt. E-mail: nouranomer@gmail.com

Received: 11-Apr-2016; **Revised:** 25-May-2016; **Accepted:** 26-May-2016; **Online first:** 16-Jun-2016

Competing Interests: The authors have declared that no competing interests exist.

Abstract

AIM: Workplace stress is hazardous for its harmful impact on employees' health and organizational productivity. The aim of the study is to apply the Allostatic Load Index (ALI) which is a multi-component measure for health risk assessment and early detection of stress among workers in Egypt.

METHODS: Sixty-two working adults randomly selected from two different working environments in Egypt were included in the study. Participants completed a self-reported questionnaire for socio-demographic and work variables. Andrews and Withey test for Job Satisfaction was filled and 3 ml blood samples were collected. Markers assessed for Allostatic Load were serum cortisol, c-reactive protein, dehydroepiandrosterone-sulphate, total thyroxine, total cholesterol, triglycerides, low-density lipoprotein, high-density lipoprotein, total cholesterol to high-density lipoprotein ratio, systolic and diastolic blood pressures, waist to hip ratio and body mass index. The risk quartile method was used for calculation of ALI. ALI value of four or more indicates high Allostatic Load.

RESULTS: Job satisfaction scale defined about a quarter of the study population (24%) to be dissatisfied with Allostatic Load of 2.4 as the mean value. Population percentage with ALI ≥4 reached 12.9% with 100% of them females. A significant association was found between Allostatic Load of primary mediators and age, the presence of chronic diseases, place of work and female gender.

CONCLUSION: Female gender and the old age of the Egyptian workforce under study are at higher risk of chronic diseases. Using an alternative way -for example, the cut-point method- instead of the risk quartiles for dichotomization of markers used in ALI calculation could be more precise for early detection of stress among healthy individuals.

Introduction

Workplace stress is an issue of growing interest in the field of Occupational health. It is defined by the Canadian Centre for Occupational Health and Safety [1] as the harmful physical and emotional responses that can result from conflicts between job demands on the employee and the amount of control an employee has over meeting these demands. Unfortunately, the decreased ability of workers in developing countries to recover from job stressors due to lack of awareness could seriously contribute to undesirable health conditions [2] beside the negative impact on workplace productivity and profit.

Allostatic load (AL) model [3], is a powerful way to assess physiological dysregulations in the state of prolonged secretion of stress hormones [4], stress hormones in conjunction with pro- and anti-inflammatory cytokines represent the AL biomarkers referred to as the primary mediators (primary stage). Secondary outcomes (secondary stage), include metabolic (e.g., insulin, glucose, total cholesterol, high-density lipoprotein, cholesterol, triglycerides, visceral fat depositing), cardiovascular (e.g., systolic and diastolic blood pressure), and immune (e.g. fibrinogen, C-reactive protein) parameters that reach sub-clinical levels. The final stage of AL progression is allostatic overload; in which physiological dysregulations lead to disordered, diseased, and deceased endpoints referred to as tertiary outcomes. The AL model proposes that by measuring the multi-systemic interactions among primary mediators and effects, in conjunction to sub-clinically relevant biomarkers representing secondary outcomes,

biomedical advances can be made in the detection of individuals at high risk of tertiary outcomes [5].

The aim of the present study is to apply the multiple biochemical measures of AL for early detection of workers at high risk of debilitating drawbacks of workplace stress in Egypt. Working environment related variables including job satisfaction and some socio-demographic factors will be studied as predisposing factors to workplace stress.

Subjects and Methods

A sample of 62 Egyptian working adults (18 males and 44 females) completed the study. The mean age of the study sample participants was 40 years ranging from 20 to 59 years. Thirty-five of them work in the different departments of the Faculty of Pharmacy (Girls), Al-Azhar University, Egypt and 27 works at the pediatric oncology outpatient clinic, National Cancer Institute (NCI), Egypt .Ethical comity at the National Research Center approved the study design and procedure was done under its guidance.

All participants were asked to fill a self-reported questionnaire including socio-demographic data (gender, age, social status, residence and history of chronic diseases) and some work related variable encompassing presence or absence of a second work, number of networking hours per day, total number of working years and job nature (worker or employee). Andrews and Withey Job Satisfaction Scale [6] was included in the questionnaire.

A 3 ml whole blood sample was taken from the study participants for biochemical analysis of AL biomarkers. The blood was collected in a plain red top venipuncture tube without additives or anti-coagulants for serum. Blood was allowed to clot for serum samples. Centrifugation of the specimen was done to separate the serum from the cells. Samples were refrigerated at -4 degrees Celsius for few days before analysis.

For determination of AL, biochemical markers measured as primary mediators were serum cortisol and dehydroepiandrosterone-sulphate (DHEA-s), c-reactive protein (CRP) and total thyroxine (tT4). Secondary outcome biomarkers were total cholesterol (TC), HDL-cholesterol, LDL-cholesterol and triglycerides (TG).

Serum cortisol and tT4 were assayed by an enzyme-linked immunosorbent assay (ELISA) technique using Immunospect kit (CA). DHEA-s and CRP were estimated in serum using ELISA kit manufactured by DRG diagnostics, Germany. TC, TG, and HDL-cholesterol in plasma were determined by a kinetic method using the Bio-diagnostic kit (Egypt) according to the methods described by Allain et al. [7], Fassati and Principe [8] and Burstein [9], respectively. LDL was estimated with reference to measured values of TC, HDL, and TG according to the Modified Friedewald Formula (MFF): LDL-C (mg/dl) = Non-HDL-C × 90% - TG × 10% [10].

Systolic blood pressure (SBP), diastolic blood pressure (DBP) and anthropometric measures; body mass index (BMI) and waist-to-hip ratio (WHR) were also measured to be included in the AL score. SBP and DBP were calculated as the average of two seated blood pressure readings taken about one minute apart, using a mercury sphygmomanometer [11]. WHR was calculated based on waist circumference (measured at its narrowest point between the ribs and iliac crest) and hip circumference (measured at the maximal buttocks) [12] and BMI was calculate from measured data as weight in kilograms divided by height in squared meters [11]. Total cholesterol to high-density lipoprotein cholesterol (TC/HDL) was calculated for determination of Allostatic load index (ALI) .

All parameters chosen for determination of ALI were chosen according to the literature [13-14]. For each biomarker, the high-risk threshold was calculated and each participant was assigned a point for each biomarker that was beyond the threshold. The high-risk threshold was defined as below the 25th percentile for DHEA-s and HDL and above the 75th percentile for all other markers according to each measurement's distribution within the population under study (Table1). The points were summed to generate the ALI, with a range from 0 to 13. According to similar research, an ALI of four or greater was used to define a high AL [15-16].

Descriptive statistics were represented as mean and standard deviation for studied variables and measures using the statistical package for social sciences, version 16 for windows (SPSS Inc., USA). Pearson Chi-square and student t-test were used for comparing data.

Table 1: Criteria for contribution from individual biological parameters for ALI calculation

Highest quartile
SBP (≥ 150 mm Hg)
DBP (≥ 100 mm Hg)
WHR (≥ 1.5)
TC/HDL (≥ 10.4)
Serum cortisol (≥ 15.85 μg/dl)
CRP (≥ 7.6 mg/l)
Total thyroxin (≥12.4 μg/dl)
BMI (≥38.1)
TG (≥231 mg/dl)
TC (≥253 mg/dl)
LDL (≥171 mg/dl)
Lowest quartile
HDL cholesterol (≤ 49.8 mg/dl)
DHEA-S (≤ 1.02 μg/ml)

Results

Descriptive data of study population as represented in Table 2 shows that percentage of females exceeds males in the study with 71% and 29%, respectively. Most of the study population are married (69.4%), don't have a second job (81%), live in urban residence (82%), work for more than five hours per day (76%) and work as employees (71%).

Nearly neither quarter of the population (24%) under study shows to be dissatisfied or neither satisfied nor dissatisfied with their job.

Table 2: Distribution of the study variables

Study Variables	Frequency (%)
Gender	
Male	18 (29%)
Female	44 (71%)
Age	
<40	27 (44%)
≥40	35 (56%)
Work Place	
Al-Azhar	35 (56.5%)
NCI	27 (43.5%)
Social Status	
Married	43 (69.4%)
Others	19 (30.6%)
Other Job	
Present	11 (18%)
Absent	50 (81%)
Chronic Diseases	
Present	24 (38.7%)
Absent	38 (61.3%)
Residence	
Urban	51 (82%)
Rural	8 (13%)
Daily Working Hours	
≤ 5	13 (21%)
> 5	47 (76%)
Working Years	
≤ 10	24 (38.7%)
> 10	35 (56.5%)
Job Nature	
Employee	44 (71%)
Worker	18 (29%)
Job Satisfaction	
<20	47 (76%)
≥20	15 (24%)

Table 3 shows the means with standard deviations together with minimum and maximum values of the different biomedical measures under study, ALI for all measures, ALI for primary mediators and job satisfaction scale. Mean value for cortisol (11.4 µg/dl) lies nearly at the middle of the normal range (5-23 µg/dl) as well as cholesterol (183 mg/dl) which has a normal range of 150-225 mg/dl.

As for DHEA-s (0.8 µg/mlL) the mean value is very near to the lower normal (0.56 µg/ml) while CRP (4.9 mg/l), tT4 (9.8 µg/dl), TG (122 mg/dl), HDL (61 mg/dl) and LDL (96 mg/dl) means greatly approach the highest values within their normal ranges; 0.068-8.2 mg/l, 5-13 µg/dl, 40-140 mg/dl for women and 60-165 mg/dl for men, 30-70 mg/dl for men and 35-85 mg/dl for women and >120 mg/dl, respectively. Mean values for systolic (122.8) and diastolic (81) blood pressures are normal while BMI mean (31.5) exceeds obesity threshold (30). Total ALI (2.4) showed to be higher than ALI for primary mediators (1.44).

Table 3: Descriptive statistics of biomedical measures in the study

Variable (N)	Mean ± SD	Minimum	Maximum
Cortisol (62)	11.4 ± 3.7	4.3	19.7
DHEA-s (62)	0.8 ± 0.6	0.2	3.5
CRP (62)	4.9 ± 3.3	0.5	10.1
T4 (62)	9.8 ± 2.7	4.7	15.1
TC (62)	183 ± 66	140	284
TG (62)	122 ± 52	48	292
HDL (62)	61 ± 28	22	99
LDL (62)	96 ± 54	1	228
TC/HDL (62)	3.5 ± 2.2	1.2	13.5
SBP (62)	122.8 ± 14.6	90	170
DBP (62)	81 ± 8.3	60	113
BMI (62)	31.5 ± 6.6	18	48
WHR (62)	0.88 ± 0.15	0.6	1.85
Job Satisfaction	15.4 ± 4.4	8	25
ALI (62)	2.4 ± 1.2	0	5
ALI of Primary Mediators(62)	1.44 ± 0.92	0	4

Mean values of ALI didn't show to be significantly different with respect to work related and socio-demographic variables considered in the study (Table 4). On the other hand gender (p = 0.036), age (p = 0.013), workplace (p = 0.000) and presence of chronic diseases (p = 0.014) significantly affected ALI of primary mediators as shown in Table 4. Females (1.6), higher age group (1.7), participants working at Faculty of Pharmacy Al-Azhar University (1.8) and participants with chronic diseases (1.8) showed higher values of ALI for primary mediators than males (1.1), lower age group (1.1), participants working at NCI (0.9) and participants not suffering from chronic diseases (1.2), respectively.

Table 4: Comparing means for Allostatic load according to studied socio-demographic and work-related variables of the study population

Study Variables	Allostatic Load Index	ALI of Primary Mediators
Gender		
Male	2.0 ± 0.8	1.1 ± 0.8*
Female	2.5 ± 1.3	1.6 ± 0.9
Age		
<40	2.1 ± 1.3	1.1 ± 0.9*
≥40	2.5 ± 1.2	1.7 ± 0.9
Work Place		
Al-Azhar	2.5 ± 1.1	1.8 ± 0.9**
NCI	2.1 ± 1.3	0.9 ± 0.68
Social Status		
Married	2.3 ± 1.2	1.4 ± 0.9
Others	2.5 ± 1.2	1.5 ± 0.9
Other Job		
Present	2.4 ± 1.1	1.5 ± 0.8
Absent	2.3 ± 1.2	1.4 ± 1.0
Chronic Diseases		
Present	2.7 ± 1.2	1.8 ± 1.0*
Absent	2.1 ± 1.2	1.2 ± 0.8
Residence		
Urban	2.4 ± 1.2	1.5 ± 0.9
Rural	2.1 ± 0.8	1.4 ± 0.9
Daily Working Hours		
≤ 5	2.5 ± 1.1	1.7 ± 1.0
> 5	2.3 ± 1.2	1.4 ± 0.9
Working Years		
≤ 10	2.3 ± 1.5	1.2 ± 1.0
> 10	2.4 ± 1.0	1.6 ± 0.9
Job Nature		
Employee	2.4 ± 1.3	1.5 ± 1.0
Worker	2.3 ± 1.1	1.4 ± 0.7
Job Satisfaction		
<20	2.4 ± 1.2	1.4 ± 0.85
≥20	2.1 ± 1. 4	1.57 ± 1.13

*p ≤ 0.05; **p ≤ 0.01.

No significant difference was detected between the population sample showing high ALI (≥ 4) and those with low ALI (< 4) regarding the different variables tested in the study except for gender where 100% of the population with high ALI was found to be females (Table 5).

Table 5: Relation between Allostatic Load Index (ALI) and study variables

Variables (N)		ALI		Chi-square	P-value
		< 4 N = 54	≥ 4 N = 8		
Gender (62)	Male	33%	0%	3.76	0.015*
	Female	66%	100%		
Age (62)	<40	44%	37.5%	0.137	0.710
	≥40	56%	62.5%		
Social status (62)	Married	70%	62.5%	0.203	0.675
	others	30%	37.5%		
Chronic Diseases (62)	Present	37%	50%	0.494	0.487
	Absent	63%	50%		
Residence (59)	Urban	81%	87.5%	1.246	0.140
	Rural	15%	0%		
Work Place (62)	Al-Azhar	56%	62.5%	0.137	0.710
	NCI	44%	37.5%		
Working Years (59)	<10	37%	50%	0.892	0.350
	≥10	59%	37.5%		
Working Hours (60)	≤ 5	22%	12.5%	0.254	0.599
	> 5	76%	75%		
Other Job (61)	Present	18.5%	12.5%	0.191	0.650
	Absent	79.5%	87.5%		
Job Nature (62)	Employee	70%	75%	0.072	0.785
	Worker	30%	25%		
Job Satisfaction	<20	76%	75%	0.003	1.00
	≥20	24%	25%		

*p≤0.05.

Discussion

Upon assessment of AL for our randomly selected sample of working adults in Egypt, ALI was found to be 2.4 as the mean value. Fortunately, this value of AL score is lower than values obtained by the similar cross-sectional studies on the workforce like those done in Canada (2.69) [17], Germany (3.15) [18] and China (3.69-4.54) [19]. At the time where only one study in Netherland showed a lower range of means of AL (1.7-7-2.03) for a population sample of males working as telecom managers [20] compared to our results.

However, according to results of the assessed markers, risk quartile thresholds of TC (253 mg/dl), TG (231 mg/dl), LDL (171 mg/dl), TC/HDL (≥ 10.4), DBP (≥ 100 mmHg), WHR (≥ 1.5) and BMI (38.1) highly jumped over the corresponding threshold ranges detected in similar studies as reported by Mauss and his colleagues [21] and even exceeded the normal ranges. As reported in their review, ranges of threshold values showed to be 177.9–249.0 mg/dl, 101.5–141.75 mg/dl, 116.0–137.3 mg/dl, 3.71, 71.2–95.0 mm Hg, 0.83–0.97, 25.2–28.5, respectively which is too much lower. The threshold for DHEA-s according to lowest risk quartile was ≤1.02 µg/ml that is also lower than the corresponding thresholds reported (13.3–51.5 µg/dl).

Such results reflect a seriously bad general health condition for our study population compared to others and render the mean value for AL deceiving and reflecting a false indication of the good state of health. Moreover, in our consideration, if the same data obtained from the present study were recomputed but using the cut-point method [22] for calculation of AL, a higher mean value for AL will be detected. Using the quartile method allowed a high percentage of the population to skip being rated on the ALI despite their absolute values for certain measures exceeded the normal range which could be indication of serious load (see for BMI as an example where the population with the range 30-38 were rated with 0 for AL since the highest quartile threshold is 38.1). This could be regarded as a disadvantage of the risk quartile method for dichotomization of AL measures, and invites more research studying the best indicative way for ALI calculation.

Work-related variables under study including job satisfaction didn't show significant influence on AL score in agreement with findings of Johansson and his colleagues [23]. No associations were detected, except for a place of work where population working at Al-Azhar University suffered from significantly higher ($p<0.01$) AL of primary mediators (1.8) compared to those working at the NCI (0.9) at $p<0.01$. AL of primary mediators reflects the allostatic state phase of the AL sequence [24] which is the state preceding AL and allostatic overload. The importance of detection of primary mediators lies on the fact that they represent nonclinical measures that provide additional warning signs of bad health above and beyond that shown by standard clinical measures as stated by Goldman et al. [25]. Similarly, ALI mean values for Al-Azhar University (2.5) showed to be higher than that of NCI workers mean (2.1) but, non-significantly, which ensures the worth health conditions of the former over the later.

Job satisfaction is a crucial indicator for measuring work wellbeing [26]. About quarter of the study population (24%) appeared to score more than or equals to twenty with a mean value of 15.4 for the whole population. This value for job satisfaction could be indicating a trend of dissatisfaction or at least carelessness of the issue. No association was detected between ALI and job satisfaction scores as demonstrated by the present work in contrast with the study hypothesis which assumed there would be a direct association between the two measures due to the reported effect of job satisfaction on physical and psychological wellbeing [27].

Many studies reported a direct association between high AL and increased age [14, 28-29]. Age appeared to affect AL prominently in our results where AL was 2.5 for the higher age group (≥ 40 years) than the lower age group (2.1), also AL for primary mediators was significantly higher ($p = 0.013$) in the older group (1.7) than the other group (1.1). The adverse effect of age on AL could be attributed to the

fact that AL measures the cumulative biological risk normally increased with age as stated by Crimmins et al., [30].

Although both males (29%) and females (71%) were represented in the study population yet, all the percentage of the sample (12.9%) that showed high AL (ALI ≥ 4) were females. Moreover, means of both ALI and that of primary mediators showed to be higher in females (2.5 and 1.6, respectively) compared to males (2.0 and 1.1, respectively) with p = 0.036 for AL of primary mediators. These results are in contrast with Schnorpfeil et al., (2003) and Li (2007) [18, 28] who recorded a positive association between AL and the male gender and may indicate sever life conditions and health state for women in Egypt.

The presence of chronic diseases also showed to be associated with high AL (2.7) compared to population free from chronic diseases (2.1). Significantly higher AL of primary mediators (1.8 vs. 1.1, p = 0.014) for a population with chronic diseases was also detected. These results are in agreement with the reported significantly increased AL with decreased physical health for Latino day workers in the USA as stated by De Castro et al., [31], the decreased self-rated health recorded by Naswall [32] in Sweden and the increased physical complaints as detected by Juster and Lupien [17] in Canada.

In conclusion, despite diversity among prior research on AL, regarding inclusion of biomarkers, method of calculation of AL, type of study and population under examination which makes it difficult to compare results of different studies, yet a growing confidence in the great worth of using the multi-component AL measure as predictor for health risk assessment is emphasized. According to our study, results signified bad health conditions for our working adults, especially women.

Work-related variables like effort-reward imbalance, work safety, job control, job demands and burnout that proved direct association with AL in previous studies made on various communities are needed to be investigated in Egypt and other developing countries. Socio-economical status as reported by De Castro et al. together with age and gender as discussed earlier also represent important predictors of stress and health deterioration.

Re-launching similar studies over larger population for screening purposes in Egypt and trying other methods for calculation of risk thresholds are highly recommended putting in mind that the present work could be regarded as a pilot exploratory cross-sectional study.

References

1. Canadian Centre for Occupational Health and Safety. Workplace stress-general, 2000. http://www.ccohs.ca/oshanswers/psychosocial/stress.html

2. Harris A, Ursin H, Murison R, Eriksen HR. Coffee, stress and cortisol in nursing staff. Psychoneuroendocrinology. 2007;32:322-330. http://dx.doi.org/10.1016/j.psyneuen.2007.01.003 PMid:17350175

3. Juster RP, McEwen BS, Lupien SJ. Allostatic load biomarkers of chronic stress and impact on health and cognition. Neurosci Biobehav Rev. 2010;35(1):2-16. http://dx.doi.org/10.1016/j.neubiorev.2009.10.002 PMid:19822172

4. McEwen BS. Interacting mediators of allostasis and allostatic load: towards an understanding of resilience in aging. Metabolism. 2003;52:10. http://dx.doi.org/10.1016/S0026-0495(03)00295-6

5. McEwen BS. Allostasis and allostatic load: implications for neuropsychopharmacology. Neuropsychopharmacology. 2000;22:108. http://dx.doi.org/10.1016/S0893-133X(99)00129-3

6. Andrews FM, Withey SB. Social indicators of well-being: American's perceptions of life quality. New York: Plenum Press, 1976. http://dx.doi.org/10.1007/978-1-4684-2253-5

7. Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem. 1974;20(4):470-5. PMid:4818200

8. Fossati P, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin Chem. 1982;28(10):2077-2280. PMid:6812986

9. Burstein M, Scholnick HR, Morfin R. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. Lipid Res. 1970;11:583-595.

10. Chen Y, Zhang X, Pan B, Jin X, Yao H, Chen B, Zou, Y, Ge J Chen H. A modified formula for calculating low-density lipoprotein cholesterol values. Lipids in Health and Disease. 2010;9:52. http://dx.doi.org/10.1186/1476-511X-9-52 PMid:20487572 PMCid:PMC2890624

11. Seplaki CL, Goldman N, Glei D, Weinstein MA. Comparative analysis of measurement approaches for physiological dysregulation in an older population. Exp Gerontol. 2005;40(5):438-449. http://dx.doi.org/10.1016/j.exger.2005.03.002 PMid:15919596

12. Lohman TG, Roche AF, Martorell R. Anthropometric Standardization. Champaign, IL: Human Kinetics Books, 1988. PMCid:PMC279682

13. Read S, Grundy E. Allostatic load – a challenge to measure multisystem physiological dysregulation. National Center of Research Methods, 2012. http://eprints.ncrm.ac.uk/2879/1/NCRM_workingpaper_0412.pdf

14. Seeman TE, Singer BH, Rowe JW, Horwitz RI, McEwen BS. Price of adaptation - Allostatic load and its health consequences - MacArthur studies of successful aging. Archives of Internal Medicine. 1997;157(19):2259-2268. http://dx.doi.org/10.1001/archinte.1997.00440400111013 PMid:9343003

15. Geronimus AT, Hicken M, Keene D, Bound J. Weathering and age patterns of allostatic load scores among blacks and whites in the United States. Am J Public Health. 2006;96(5):826–833. http://dx.doi.org/10.2105/AJPH.2004.060749 PMid:16380565 PMCid:PMC1470581

16. Nelson KM, Reiber G, Kohler T, Boyko EJ. Peripheral arterial disease in a multiethnic national sample: the role of conventional risk factors and allostatic load. Ethn Dis. 2007;17(4):669-675. PMid:18072377

17. Juster RP, Lupien S. A sex- and gender-based analysis of allostatic load and physical complaints. Gend Med. 2012;9:11–23. http://dx.doi.org/10.1016/j.genm.2012.10.008 PMid:23158957

18. Schnorpfeil P, Noll A, Schulze R, Ehlert U, Frey K, Fischer JE. Allostatic load and work conditions. Soc Sci Med. 2003; 57:647–656. http://dx.doi.org/10.1016/S0277-9536(02)00407-0

19. Sun J, Wang S, Zhang J, Li W. Assessing the cumulative effects of stress: The association between job stress and allostatic load in a large sample of Chinese employees. Work and Stress. 2007;2:333–347. http://dx.doi.org/10.1080/02678370701742748

20. Langelaan S, Bakker AB, Schaufeli WB, van Rhenen W, van Doornen LJP. Is burnout related to allostatic load? Int J Behav Med. 2007;14:213–221. http://dx.doi.org/10.1007/BF03002995 PMid:18001236

21. Mauss D, Li J, Schmidt B, Angerer P, Jarczok MN. Measuring allostatic load in the workforce: a systematic review. Industrial Health. 2015; 53:5–20. http://dx.doi.org/10.2486/indhealth.2014-0122 PMid:25224337 PMCid:PMC4331190

22. Lipowicz A, Szklarska A, Malina RM. Allostatic load and socioeconomic status in Polish adult men. J Biosoc Sci. 2014;46:155–167. http://dx.doi.org/10.1017/S0021932013000345 PMid:23806750

23. Johansson G, Huang Q, Lindfors P. A life-span perspective on women's careers, health, and well-being. Soc Sci Med. 2007;65:685–697. http://dx.doi.org/10.1016/j.socscimed.2007.04.001 PMid:17493728

24. McEwen BS. Protection and damage from acute and chronic stress: Allostasis and allostatic overload and relevance to pathophysiology of psychiatric disorders. Annals of the New York Academy of Sciences. 2004;1032:1-7. http://dx.doi.org/10.1196/annals.1314.001 PMid:15677391

25. Goldman N, Turra CM, Glei DA, Seplaki CL, Lin Y, Weinstein M. Predicting mortality from clinical and nonclinical biomarkers. Journal of Gerontology. 2006;61A(10):1070–1074. http://dx.doi.org/10.1093/gerona/61.10.1070

26. Spector PE, Jex SM. Relations of job characteristics from multiple data sources with employee affect, absence, turnover intentions, and health. Journal of Applied Psychology. 1991;76:46–53. http://dx.doi.org/10.1037/0021-9010.76.1.46

27. Judge TA, Church AH. Job satisfaction: Research and practice. In C. L. Cooper & E. A. Locke (Eds.), Industrial and organizational psychology: Linking theory with practice (pp. 166–198). Oxford, UK: Blackwell, 2000. http://dx.doi.org/10.1037/10519-162

28. Li Wea. Job stress related to lycol-lipid allostatic load, adiponectin and visfatin. Stress and Health. 2007;23:257−266. http://dx.doi.org/10.1002/smi.1145

29. Juster RP, Moskowitz DS, Lavoie J, D'Antono B. Sex-specific interaction effects of age, occupational status, and workplace stress on psychiatric symptoms and allostatic load among healthy Montreal workers. Stress. 2013;16:616–629. http://dx.doi.org/10.3109/10253890.2013.835395 PMid:23952366

30. Crimmins EM, Johnston M, Hayward M, Seeman T. Age dysregulation. Exp Gerontol. 2003;38(7):731–734. http://dx.doi.org/10.1016/S0531-5565(03)00099-8

31. De Castro AB, Voss JG, Ruppin A, Dominguez CF, Seixas NS. Stressors among Latino day laborers. A pilot study examining allostatic load. AAOHN J. 2010;58:185–196. http://dx.doi.org/10.3928/08910162-20100428-01 PMid:20507008 PMCid:PMC2964275

32. Näswall K, Lindfors P, Sverke M. Job insecurity as a predictor of physiological indicators of health in healthy working women: an extension of previous research. Stress Health. 2012;28: 255–263. http://dx.doi.org/10.1002/smi.1430 PMid:22170746

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):499-503.
http://dx.doi.org/10.3889/oamjms.2016.075
eISSN: 1857-9655
Public Health

Analysis of Marketing Strategy for Food Supplements and Over-The-Counter Medicines

Marjan Dzeparoski[1*], Suzana Trajkovic-Jolevska[2]

[1]Bionika Pharmaceuticals Ltd., Skopje, Republic of Macedonia; [2]Faculty of Pharmacy, Ss. Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia

Citation: Dzeparoski M, Trajkovic-Jolevska S. Analysis of Marketing Strategy for Food Supplements and Over-The-Counter Medicines. Open Access Maced J Med Sci. 2016 Sep 15; 4(3):499-503. http://dx.doi.org/10.3889/oamjms.2016.075

Keywords: marketing; marketing strategy; analysis; food supplements; over-the-counter medicines.

***Correspondence:** Marjan Dzeparoski. 1Bionika Pharmaceuticals Ltd., Skopje, Republic of Macedonia. E-mail: marjan.dzeparoski@bionikapharm.com

Received: 08-Jun-2016; **Revised:** 26-Jun-2016; **Accepted:** 27-Jun-2016; **Online first:** 04-Jul-2016

Competing Interests: The authors have declared that no competing interests exist.

Abstract

Marketing strategy is correlated with the regulations for the corresponding product category. Accordingly, there is a big difference in the marketing strategy of food supplements and over-the-counter medicines. In this paper are presented 2 different marketing strategies of a new small pharmaceutical company in two studies. The findings of studies analysis can be used for developing marketing strategies in the wider sense and other products, for other small to medium sized companies in other countries of interest with similar regulations and help them understand how to position and promote themselves and their products.

Introduction

Marketing strategy differs depending on the product, pharmaceutical company and market situation. The process consists of 3 phases: planning, implementation and control [1-3]. The strategy can relate to therapeutic groups, product line or individual product [4]. In this paper, 2 different marketing strategies of the new small pharmaceutical company in two studies will be presented. In Study One will be explored marketing strategy for food supplements (product line). In Study Two will be explored and created a marketing strategy for over-the-counter (OTC) medicine (product strategy) for a non-EU pharmaceutical company (Macedonia) to enter the EU market (Slovenia), based on the comprehensive research analysis. Today pharmaceutical companies must sell smarter and deliver greater value with smaller sales teams.

Methods

In both studies are used qualitative and literature research. Additionally, in Study One are used questionnaires for wholesalers and pharmacies and competitors analysis. In Study Two are used situation analysis (Political, Economic, Regulatory, Technological, Social/PERTS & SWOT analysis) and interviews with company employees.

Results

Study One

Products are positioned as food supplements that support the health of patients. Starting points of the strategy are:

- Brand names of food supplements - easy to

remember;

- Visual differentiation through packaging – impressiveness;
- Abstract display of elements/effects on packaging;
- Modern and clear design - associated with modern sophisticated technology.

The concept for the brand name is to mark the effect which food supplements have on the human body (some names are a blend of ingredients that are contained or the organ for which they are intended, others mark the effect).

On the packages is displayed modern graphic typography concept, letters contribute to playfulness, visual impressiveness and authenticity, complemented by body parts that affect the supplements. Organs are presented in an interesting cubist manner, by emphasising the tissue as a creator of every organ and this gives original minimalist outlook that sets it apart as unique packaging sample.

Communication objectives are: creating company awareness, promoting a line of food supplements, further education of target audience for their action and the way of usage.

Target audiences are professionals - doctors, pharmacists, associations and non-governmental organisations, and the general public - medias.

Limited launch phase (preparatory phase) is represented by testing the products with special target groups (physicians), building company credibility and presentation of products among experts.

Introduction phase of food supplements is represented by presenting the company and announcement of products – public relations, launch, positioning, point of sale and point of purchase support, creating awareness (cooperation with portals that have headings for health) through mass electronic media - TV and print media campaigns for the professionals and general public.

PR activities:

1. Presentation of the company - company brand through an introduction to general public and specific audiences, by demonstrating capacity and technology through interviews with selected media.

Objective: presentation to the public and target audiences, promoting investment and highlighting the contribution of the company to the economy and quality of life.

Target audience: representatives of medias, companies (partners, suppliers, customers), experts, general public.

2. Building credibility - strengthening the company position by initiating important topics related to contemporary trends in company's interest at target audiences.

3. Products presentation - communication-related to certain products and advice for their positive characteristics using "third party".

Objective: to inform the public about products advantages and characteristics, promotion, strengthening confidence in products recommended by physicians and pharmacists.

Target audience: the general public.

According to the presented data, it is obvious that registration of food supplements is not demanding as the registration process of medicines, so their replacement with other food supplements is also easier and predicted. Usually, they are hit products which have a shorter life cycle, about 4-5 years, which is not the case with the medicines. Requirements in terms of investments in the manufacturing facilities, equipment and staff are also much smaller in terms of requests for necessary good pharmaceutical practices that are imperative for the pharmaceutical product manufacturers. Manufacturers of food supplements need only an HACCP certificate. Food supplements pricing is free and is not regulated by law.

Study Two

Marketing plan for the OTC product is developed based on the tabulated data from situation analysis - PERTS analysis (Table 1) and SWOT analysis (Table 2).

PERTS analysis model is exploring the external factors, particularly useful for international markets.

Table 1: PERTS analysis – macro environment

External factors	Opportunities	Threats
Political	Reimbursement list and health care policies are subject to change	Removal from reimbursement list
Economic	Economical situation is improving Medicine market grows	Economic crisis Change of exchange rate
Regulatory	Obtaining marketing authorization	Regulatory changes
Technological	Growth by expanding production with semisolid and liquid pharmaceutical dosage forms, medical devices and cosmetics	Competition New innovative competitors technology
Social	Direct-to-Consumer pharmaceutical advertising Good relationships with pharmacists and physicians	Limited economic opportunities Dependence on personal relationships with pharmacists and physicians Consumer brand affection

SWOT analysis is an analysis of both, internal and external company factors (Table 2).

Based on two presented analyses, we found weak and critical points of the marketing plan and process optimisation of the medicine launch (ML) is proposed. Optimisation process of ML is important to eventually reduce or exclude certain risks that can cause failure or poor results. They would have a lasting negative effect on the positioning and the

image of the company and products in markets where growth in sales is expected.

Table 2: SWOT analysis – micro & macro environment

STRENGTHS	WEAKNESSES
Experienced staff Trained sales representatives Reputation for quality	Competition is well established and employees are well educated Limited resources Not so innovative products
OPPORTUNITIES	**THREATS**
Expansion of product portfolio Medicine market grows Reimbursement list and health care policies are subject to change Economical situation is improving	Reduction of prices of competitors products Decreased interest among distributors due to competitive products Decreased interest among key practitioners Removal from reimbursement list

Critical points

There are more activities and key points which are important for the smooth development of ML, which may cause difficulties or halt the process if not monitored carefully. Involved are the finance department, education, legal resources, registration & pharmacovigilance, quality control, production, research & development, procurement & marketing. Some of the activities are well organised, but some need to be organised better:

1. Marketing/Management

Communication is imperative, is very important between pharmaceutical company from Macedonia and the Slovenian partner responsible for the marketing of OTC medicine, as well as managing the employees in each of the companies. Employees should know to whom to address for certain issues. Also, the perception of Slovenian market is important, because although there are similarities, there are social and cultural differences between Macedonia and Slovenia that influence decision-making [5].

- *Project leader*. Important is to appoint the project leader who will allocate tasks, monitor the activities and implementation of timelines and budget frames. This will facilitate to get answers quickly and to know always in which phase is ML.

- *Goals.* Goals motivate employees to achieve them. Goals should be realistic and achievable, in order not to cause opposite effect among employees, but also should not be too low. They should be measurable and specific for certain time period.

- *Communication*. It is defined in the plan for ML and consists of meeting for ML, limited launch phase (LLP), local promotion of medicine, full launch phase (FLP) and choice of marketing materials.

- *Sales representatives.* OTC medicine is scheduled to be sold through sales representatives, as well as direct marketing and advertising for the general public. Success is depending on whether sales representatives are motivated enough.

- *Traceability and communication*. It is very important for the team to be willing to achieve good results. It is necessary to monitor and communicate the success or failure, to know at what stage ML is and whether earlier stages were successful.

- *Price*. Price formation is complex and depends on regulation, international markets and expectations of the company. Pricing of OTC medicines in Macedonia is free, but for example in Slovenia [6] and in other countries price is regulated by law.

- *LLP centres*. Sales representatives should select key physicians and centres that will be included in the limited launch phase. The country manager should decide which of the selected centres are crucial and will support OTC medicine.

- *Marketing materials.* Once marketing sectors agreed on the selection of promotional materials, the materials will be translated into Slovenian. Slovenian partner has full responsibility for this activity.

- *Organising events*. Marketing team participates in events organisation by selecting hotels, transport, advertising materials and other activities or hires PR and travel agencies.

2. Education

Training level depends on the importance of ML. Education will be carried out internally at the pharmaceutical company and externally in the business partner premises.

Internal education is the education of sales representatives and includes presentations for OTC medicine and therapy, and marketing training, as well. For all companies, it is important to have experienced sales representatives who are continually educated. Training should be interesting and useful for the participants.

External education is the education of the local partner sales representatives. The external educational program will be provided for Slovenian physicians and pharmacists, as well. This way, the awareness for OTC medicine will be created. Participants will acquire information about the way and duration of use, packaging, and will have the opportunity to test the medicine.

3. Legislation/Regulation

Laws: legal sector should check earlier legal requirements for contracts with distributors, their validity and possibility of automatic extending. The legal sector could explore the possibility for certain exclusivity because the law on anti-monopoly behaviour for medicines does not allow exclusivity.

Regulation: EU GMP certificate is a prerequisite for obtaining medicines marketing authorization in EU. Without this certificate, it is not possible medicines to be released in EU, also in some non-EU countries which have implemented EU legislation [7-9]. The process of obtaining EU-GMP certificate is complex, laborious and expensive, requires great employee's efforts and lasts minimum one year.

Propositions for optimising ML

- *Communication and employee management.* Managing staff and communication with the responsible employee is very important for companies; especially in ML. Communication can be improved by determining contact responsible person for each part. The person manages the flow of communications and forwards them to the appropriate person. This way the performance and speed are improved. PR agency can be engaged, committed to publishing texts for OTC medicine, ML and the timetable for the launch. Changing this way, communication is better and employee involvement is greater.

- *Creating campaign*. It is very important to create a campaign for a certain problem that can be cured through the use of OTC medicine. The use of medicine can be maximised, where it has not been used before or it has been used to a lesser extent (an indication for which the medicine has not been developed originally, but for which the medicine offers an appropriate solution) [10]. Awareness can be created through meetings or interviews with physicians, pharmacists, consumers, sales representatives, and through direct marketing and advertising to the general public. This will create customers satisfaction, offering a solution to their needs.

- *Internal delays*. Internal delays may be caused by differences in priorities of both partners and include late decision making, timelines are not always respected, the last moment meeting cancellations. Problems can be overcome if more independence is given to the Slovenian business partner in decision making or by involving more company employees in the activities.

- *Marketing materials.* Different type of promotional materials can be used in marketing. In this case, it is important ML, therefore appropriate materials for ML have to be chosen. It is important because not all marketing materials have the same importance in different approaches of marketing strategies. Marketing materials should be ordered and delivered on time.

- *Production/Procurement, Distribution.* Although forecasts and orders can be given in time, there may be delays in transportation /delivery of the product (e.g., delay in clearance), problems can arise in production, delay in delivery of any material which enters the manufacturing process of medicine, leading to time prolongation in planned deliveries. One solution could be storing larger stock of necessary raw materials, which requires more storage space and brings higher costs. A better solution would be closely monitoring of all activities and full involvement by the responsible persons from the company. This would make possible more objective and effective product distribution (certain product quantities for specific region) based on the regional sales data.

Because of the size of investment and expectations, the approach in marketing strategy development is much more serious, more complex and broader for OTC medicines. The presented data show that LLP and FLP reference centers are selected very carefully, critical points are set and monitored, proposals for optimization of ML are given, in order to avoid any marketing strategy delay or failure, which would have long lasting negative effect on the positioning and image of the pharmaceutical company and products in markets where sales growth is expected. As the product is OTC medicine, the approach must be supported with much more scientific data and therefore patients and healthcare professionals take them very seriously.

In conclusion, marketing strategy is correlated with the regulations for the corresponding product category. Registration of food supplements is easier and much quicker than registration of medicines. As a result, there is a big difference in the marketing strategy of food supplements and OTC medicines. Studied findings can be used for developing marketing strategies in the wider sense and other products, for other small to medium sized companies in other countries of interest with similar regulations.

References

1. Kotler P. Marketing Management: analysis, planning and control. Zagreb, Information, 1988/1989.

2. Kotler P, Keler KL. Marketing Management. thirteenth ed., Skopje, DATAPONS DOOEL, 2009.

3. Corey RE. Marketing Strategy – An Overview. Boston, Harvard Business School Publishing, 2003.

4. Dogramatzis D. Pharmaceutical Marketing A Practical Guide. Denver, Interpharm Press, 2002.

5. Hamilton R. Slovenia: Recovery Continues To Edge Ahead. Emerging Europe Monitor South East Europe. 2014; 21(6): 7.

6. Rulebook on determining the price of medicines for usage in human medicine (Official Gazette of RS, no. 32/15 and 15/16). Available at http://www.uradni-list.si/1/content?id=121647&part=u&highlight=pravilnik+o+dolo%25C4%258Danju+cen+zdravil+v+humani+medicini#!/Pravilnik-o-dolocanju-cen-zdravil-za-uporabo-v-humani-medicini. Accessed 26 April 2016.

7. Law on medicines and medical devices ("Official Gazette of the Republic of Serbia" No. 30/2010 and 107/2012).

8. Law on Medicines ("Official Gazette of Montenegro" No 56/11 and No 06/13).

9. LAW No. 105/2014 ON DRUGS AND PHARMACEUTICAL SERVICE (Promulgated by Decree no. 8680, dated 15.08.2014 of the President of the Republic of Albania, Bujar Nishani).

10. Boston Consulting Group. Breaking Through the Noise. September, 2014. Available at: http://www.pharmatalents.es/assets/files/BreakingTheNoiseMCMBCG.pdf. Accessed 20 January 2015.

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):504-509.
http://dx.doi.org/10.3889/oamjms.2016.093
eISSN: 1857-9655
Public Health

A Worksite Health Education Workshop as Empowerment Intervention for Health Promotion in the National Research Centre of Egypt

Nagat Mohamed Amer[1], Zeinab M. Monir[2], Mai Sabry Saleh[1], Heba Mahdy-Abdallah[1*], Salwa Farouk Hafez[1]

[1]Environmental Research Division, Environmental and Occupational Medicine Department, National Research Centre, Cairo, Egypt; [2]Medical Research Division, Department of Child Health, National Research Centre, Cairo, Egypt

Citation: Amer NM, Monir ZM, Saleh MS, Mahdy-Abdallah H, Hafez SF. A Worksite Health Education Workshop as Empowerment Intervention for Health Promotion in the National Research Centre of Egypt. Open Access Maced J Med Sci. 2016 Sep 15; 4(3):504-509. http://dx.doi.org/10.3889/oamjms.2016.093

Keywords: Occupational stress; health education; health promotion; empowerment; workshop.

***Correspondence:** Heba Mahdy-Abdallah, Assistant Professor of Industrial Medicine. Environmental and Occupational Medicine Department. National Research Centre. EL Buhouth Street, Dokki, Cairo, Egypt. E-mail: hebamahdy_2003@hotmail.com, hebamahdy2003@yahoo.com

Received: 28-Jul-2016; **Revised:** 10-Aug-2016; **Accepted:** 12-Aug-2016; **Online first:** 28-Aug-2016

Competing Interests: The authors have declared that no competing interests exist.

Abstract

AIM: The study aimed to assess worksite health education workshops as a successful tool for health promotion of employees.

MATERIAL AND METHODS: A one day workshop was held for individuals engaged in research activities in the National research Centre of Egypt at the worksite. Its main objective was to highlight the nature, causes, symptoms and management of job stress. Participants were asked to fill a personality assessment sheet, a self-reported questionnaire for job satisfaction. Other questionnaires for assessment of falsification of type and some socio-demographic data were filled by the attendants. A concise survey was introduced at the end of the workshop for feedback collection.

RESULTS: Attendants of the workshop were 36 subjects mainly females (94.4%). Mean age was 40.5 years with 63.9% of participants at their postdoctoral studies stage. Participants were at midway in the scale of job satisfaction (3.3) and did not suffer from falsification (0.3). The feedback survey score (11.5) showed great acceptance for the intervention. Special interest in the topic of stress was reported by 35.1% of attendants who found it the best item in the workshop and the interactive manipulation came next as declared by 18.9% of the participants.

CONCLUSION: Worksite health education workshops seem to be a successful practice for empowerment in the Egyptian workplace.

Introduction

Empowerment is a process by which people, organisations and communities gain mastery over their affairs [1]. Individual empowerment -in particular- which is also known as psychological empowerment, focuses more on people feeling and actually having a sense of control over their lives. As reported, the ability to create such 'sense of control' could show a direct effect on improving an individual's mental and physical health [2]. Moreover, evidence from research ensures the effectiveness of empowerment interventions –in general- in their ability to produce improved health impacts [3, 4].

In the context of the conceptual theory and applications, 'empowerment' is closely related to 'health promotion'. Health promotion is defined as the process of enabling people to increase control over, and to improve, their health [5]. Health education represents one discipline of 'health promotion' [6] which include educational efforts to influence lifestyles that guard against ill-health [7].

According to the WHO [8] people in developing countries are subjected to increasing work-related stress mainly due to globalisation and changes in the nature of work. For that reason, the WHO [8,9] encourages interventions that improve awareness with hazards of stress and promote suitable ways of coping with demanding working conditions. On practical basis, a variety of health deficits caused by stress, especially psychological ones were found to be improved by enhancing self-awareness and self-efficacy as well as changing the

irrational thoughts as part of health education programs [10].

In consequence, we aimed to introduce a workshop for health education against workplace stress for the specific category of workers: scientific researchers as an empowerment intervention for health promotion. Feedback was collected after the intervention for evaluation of the process and assessment of the response of the study group.

Methodology

A health educative *one day workshop* with title: "Let your job be your friend" was held by the Principal Investigator, Co-Investigator and some of the team members of the internal project funded by the National Research center (NRC) of Egypt entitled: "Increasing productivity of young researchers in NRC through learning style preference assessment and investigation of some related professional factors". The workshop was held in the main auditorium of the NRC. Attendants were asked to fill a survey at the end of the workshop to record their feedback. Ethical committee approval was taken in advance.

Participants

Attendants of the workshop were 36 subjects (2 males and 34 females) working at the different departments and divisions of the NRC. All participants completed the whole workshop and showed a high level of interaction.

Description of the workshop

The workshop first explained its objectives and presented a short scientific background to the audience as well as some instructions to help them obtaining maximum benefit from the intervention introduced.

Topics of the workshop encompassed an introduction to workplace stress and its hazardous effect on health, hormones involved in stress induction, stress management and coping strategies and finally subjective stressors according to personality traits and hazardous effect of falsification of the type in the work environment. Interactive manipulation of the various topics was the main feature of the intervention.

Measures and scales

Participants in the workshop were asked to fill a personality assessment sheet modelled after the Myers-Briggs Type Indicator (MBTI) and guided by Cognitive Style Inventory [11]. The questionnaire investigated the 16 different personalities of the MBTI where each personality is represented by four letters representing the four famous dichotomies: (Extrovert (E)/Introvert (I), Sensing (S) /Intuition (N), Thinking (T)/Feeling (F) and Judging (J)/Perceiving (P).

The self-reported questionnaire for job satisfaction designed by Andrews and Withey [12] was used in the workshop to assess the degree to which attendants liked their jobs. It consisted of five questions asking about the different aspects featuring an individual's working environment (job, co-workers, work itself, the amount of work and facilities provided). A Likert scale ranging from one (delighted) to seven (terrible) described their degree of satisfaction with work. The average score was then calculated to measure overall state of job satisfaction ranging from 1 indicating the state of delight and 7 indicating feeling terrible.

Another questionnaire was presented to participants for identification of falsification of type inside the work environment [13]. The questionnaire consisted of 14 statements describing one's feelings and behaviours towards his workplace. Participants were asked to respond to all the sentences with 'Agree' (scores for 1), 'don't agree' (scores for zero) or 'sometimes yes and sometimes no' (scores for half). The total score was calculated by summing up all responses and calculating the average value. The scale of falsification ranged from zero to 1 where zero represents the absence of falsification and 1 mean complete falsification of type at work.

Double translation-retranslation and face validation were done for all the tools in use. Participants were asked to score the different questionnaires to assess themselves and participate in the workshop. Demographic data were finally collected which included age, gender, educational level (academic degree), address district (near or far from work), residence, monthly income, position and if someone suffers from any chronic disease (hypertension, diabetes …).

Feedback collection

For the purpose of feedback collection, a short survey was designed up of five forced choice questions with allowed answers yes, no or don't know and three open questions in which participants were asked to mention the best thing in the workshop, to give their own recommendations, and to add a positive and/or negative comments if present.

The forced choice questions were:

Q1. If this workshop is held again I will encourage my friends to attend it.

Q2. If this workshop is extended for more than

one day I will try to attend it.

Q3. There are many workshops speaking about the same topic of this workshop.

Q4. The topic of the workshop is new for me.

Q5. This workshop manipulates its topic in an innovative manner.

Calculating the number of points for each forced choice question was as follows: Yes accounted for two points and, one point for don't know, and zero for the answer (no), except the third question, were, answering with no gets 2 points and zero for yes. Two points were added to the score for each positive comment, and 2 points were subtracted corresponding to each negative comment.

Statistical analysis

Statistical analysis was carried out using the statistical package for social sciences, version 16 for windows (SPSS Inc., USA). Descriptive statistics including frequency distribution, mean and standard deviation: to describe different characteristics and the studied scores. One sample t-test was used to test whether the average of the sample differs significantly from a population mean. Two groups comparison was done using Independent sample t-test. Pearson Correlation (r) was used to test the association between two variables. A P value less than 0.05 was considered as statistically significant.

Results

As shown in Table 1, total participants in the workshop reached 36 from different divisions in the NRC. Research members from the Environmental Research division represented the highest contribution in the workshop (19.4%) followed by the division of Food Industry and Nutrition, Pharmaceutical Industries division and Genetic Engineering and Biotechnology division with the percentage of 11.1 % each.

The average age of years for the included staff was 40.5 ± 11.5, ranging between 24 and 62 years. Females (n = 34) highly exceeded males (n = 2) with percentages 94.4% and 5.6%, respectively which obeys normal distribution of researchers working in the NRC. Monthly income for most of the sample (70.6%) exceeded 5000 Egyptian pounds with 63.9% of the population sample holding Medical Degree (MD) or Doctoral Degree of Philosophy (PhD). Participants were nearly equally distributed over the different positions in the NRC with the slight increase among researchers (31.4%). Urban residence (97.1%) greatly predominated rural residence (2.9%) and 40% of the sample showed to suffer from chronic diseases. As for personality types, not all the 16 MBTI types were represented by the sample but only 12 of them as shown in Table 1.

Table 1: Socio-demographic, some work stress related factors and personality types in the studied group

		Number	Percentage %
Gender	Male	2	5.6
	Female	34	94.4
Divisions	Agriculture and Biology	3	8.3
	Chemical Industries	3	8.3
	Engineering	2	5.6
	Environmental Sciences	8	22.2
	Food Industry and Nutrition	4	11.1
	Genetic Engineering and Biotechnology	4	11.1
	Inorganic Chemical Industries and Mineral Resources	3	8.3
	Medical Sciences	1	2.8
	Pharmaceutical Industries	4	11.1
	Physics	1	2.8
	Textile Industries	1	2.8
	Veterinary	1	2.8
	Human Genetics & Genome	1	2.8
Income	<3000 LE	2	5.6
	<5000 LE	9	25
	>5000 LE	25	69.4
Highest Degree	Bachelor	9	25.0
	Master of Science	4	11.1
	Medical or Philosophy Degree	23	63.9
Position	Specialist	6	16.7
	Assistant Researcher	8	22.2
	Researcher	11	30.6
	Assistant Professor	6	16.7
	Professor	5	13.8
Residence	Urban	34	94.4
	Rural	2	5.6
Place of Work	Near from home	16	44.4
	Far from home	20	55.6
Chronic Diseases	Present	14	38.9
	Absent	22	61.1
Personality Type	ISTJ	8	22.2
	ISFJ	3	8.3
	INFP	1	2.8
	INTJ	1	2.8
	INFJ	1	2.8
	ISFP	1	2.8
	INTP	1	2.8
	ESTP	1	2.8
	ESTJ	3	8.3
	ESFJ	11	30.6
	ENFJ	3	8.3
	ESFP	2	5.6

The ESFJ personality predominated (30.6%) followed by the ISTJ (22.%) while the INFP, INTJ, INFJ, ISFP, INTP and ESTP were the least represented (2.8% each). The mean score of falsification of the type for the workshop participants as shown in Table 2 reached 0.3 (SD = 0.19) with a good indication of the nearly absence of falsification within the sample.

Table 2: Falsification of type, job satisfaction and feedback survey scores of participants

	Minimum	Maximum	Mean ± SD
Falsification of Type	0.00	0.78	0.3 ± 0.19
Job Satisfaction	0.24	4.60	3.3 ± 0.85
Feedback Survey Scores	6	14	11.5 ± 2.06

As for job satisfaction, the mean score showed a general state of satisfaction with work (3.3). At the same time, the survey showed a mean score of 11.5 which indicates a high percentage of acceptance (82%) for the workshop which is described in details in tables (3) and (4). Data in Table 3 describes frequencies of responses to force choice questions of the survey with yes, no or don't know. As shown, the mean percentage of positive responses ('yes' for all questions except the third which takes 'no') is 82.2%.

Table 3: Frequency table for responses to force choice items of the feedback survey with Yes, No or Don't know

Serial	Items	Number of respondents	YES No. (%)	NO No. (%)	Don't know No. (%)
1	If this workshop is held again I will encourage my friends to attend it.	35	35 (100%)	0	0
2	If this workshop is extended for more than one day I will try to attend it.	35	30 (85.7%)	1 (2.9%)	4 (11.4%)
3	There are many workshops speaking about the same topic of this workshop.	35	1 (2.9%)	17 (48.6%)	17 (48.6%)
4	The topic of the workshop is new for me.	34	30 (88.2%)	3 (8.8%)	1 (2.9%)
5	This workshop manipulates its topic in an innovative manner.	35	31 (88.6%)	1 (2.9%)	3 (8.6%)

Positive comments (42.9%) as represented in Table 4 highly exceeded negative comments (11.1%) where the former included an appraisal of the topic (new, interesting and important) primarily and its manipulation (simplified, interactive and interesting) secondarily. Recommendations were offered by 51% of participants and encompassed more illustrations, follow-up, extended time for the workshop, copy of the material content and more active participation from attendants while 94.1% stated their opinion on the best thing in the workshop as detailed in Table 4.

Table 4: Descriptive data showing responses of participants to open-ended questions in the feedback survey

Survey Items		Number	Percentage %
Best item in the workshop		33	89.1
	The main topic (interesting, novel, new information)	13	35.1
	Manipulation of the topic	7	18.9
	Stress management	5	13.5
	Personality topic	3	8.1
	Performance and skills of trainers	5	13.5
Recommendations		18	51.4
	More illustrations	3	8.3
	Follow-up	3	8.3
	Extend the duration of the workshop	6	16.7
	Provide copy of the material content	5	13.5
	More active participation from attendants	1	2.8
Positive Comments		15	42.9
Negative Comments		4	11.1

The response to the workshop according to personality differences between participants through comparison of mean scores of the survey is shown in Table 5. The Feeling have shown to be significantly more responsive and convinced by the workshop than the Thinking type

As for the study of Pearson correlation, a negative correlation was found between falsification of type and both income ($p = 0.039$) and degree of job satisfaction ($p = 0.002$), which means that falsification increases in case of low income and decreased job satisfaction.

Upon comparing means of scores of falsification of the type using student t-test, it showed significantly to have a lower mean (0.25 ± 0.2) with ($p = 0.041$) for a participant who are MD or PhD holders compared to those who have only a bachelor or master degree (0.4 ± 0.16).

Table 5: Comparing means for feedback survey scores between participants with different personality traits

MBTI Dichotomies		N	Mean ± SD	p value
Orientation to World	Introverted	16	10.81 ± 2.37	0.09
	Extroverted	19	12.00 ± 1.63	
Take in Information	Sensing	28	11.64 ± 1.66	0.29
	Intuitive	7	10.71 ± 3.30	
Make Decisions	Thinking	14	10.50 ± 2.59	0.02*
	Feeling	21	12.10 ± 1.34	
Adapt to the World	Judging	29	11.62 ± 1.86	0.31
	Perceiving	6	10.67 ± 2.94	

*Significance at p-value ≤ 0.05.

Discussion

The job represents one of the major stressors for many people [14]. According to NIOSH [15], job stress is more strongly associated with health complaints than financial or family problems. Work-related stress in developing countries –in particular- is even made worse by a broad spectrum of factors outside the work environment [8]. Identifying stressors and learning how to face them crucially help in reducing distress in people lives [16]. As noted by the Canadian Mental Health association [17], employees lack the ability to recognise signs and symptoms of deteriorating workplace stress which subject them to serious physiological and psychological conditions in addition to the negative impact that could be exerted on the workplace productivity and profit. For that reason, we tried to implement our workshop for employees' empowerment which we consider as *public health intervention* that intends to protect the psychological health and prevent illness [18].

Despite the great diversity of empowerment practices at the workplace [19], we preferred for our intervention to be a one-day health education workshop at the worksite since worksite interventions proved to have the capacity to reach a big proportion of the working population [14]. Moreover, health education is highly encouraged in particular as it represents an important part of the health promotion activities currently occurring in the WHO Eastern Mediterranean Region countries including Egypt [7].

A survey was introduced to participants at the end of the workshop for feedback collection as a method for evaluation of the process as it is difficult to accurately determine the impact on the individual health outcomes later on [4]. The survey was designed in light of the definition of evaluation which regards it as "the systematic examination and assessment of features of an initiative and its effects, in order to produce information that can be used by those who have an interest in its improvement or effectiveness" [20].

According to the presented evaluation, we

found a great degree of acceptance regarding the intervention introduced. It was represented by the high mean values (11.5 ± 2.1) of the survey score that indicates the positive opinion of participants, 88.2% were interested in the topic and 88.6% found the manipulation of the material interesting and innovative. Moreover, 100% of the participant declared their willingness to re-attend the intervention if repeated. A percentage of 85.7 of them preferred that the workshop could have been extended for a longer period. These results ensure a great deal of serious need for such kind of interventions and highlight the importance of paying attention to the problem of work-stress in our community. As reported by the WHO [8] very little data is available from developing countries concerning the magnitude of causes and consequences of work-related stress. This could explain the urgent need for such kind of health education intervention as shown from our results.

As part of the process evaluation, we had to screen some of the individual characteristics of participants who received our intervention. Personality, age, gender, level of education, and family situation are reported to influence an individual's ability to cope with work demands and may also interact with risk factors at work and either exacerbate or buffer their effects [8]. Another main interest in such screening was to test job satisfaction and the prevalence of stress according to falsification of type together with some work-related factors which might influence them. According to statistical analysis although the sample showed good job satisfaction (3.3) and low value on the scale of falsification (0.3), yet significant difference was detected that revealed the presence of some kind of stress exerted on researchers who haven't yet received their PhD/MD (0.4) compared to PhD/MD holders (0.25) at $p < 0.05$. Falsification of type -which reflects signs and symptoms of stress in response to not convenient working conditions- also showed significant negative correlation at $p < 0.05$ with low income among researchers. Researchers in this stage (specialists and assistant researchers) are mostly subjected to some or all of the following factors which are highly reported to be sources of work stress: low income, high job demands, time pressure, a lack of control over workload and work processes, lack of social support from colleagues and/or supervisors (occasional), lack of participation in decision-making, job insecurity, lack of opportunity for growth, lack of advancement or promotion, irregular working hours and others.

As for the relation between personality type and response to the workshop, feeling style score in the survey significantly showed better satisfaction and convenience with the intervention than thinking style. This could be attributed to the nature of the thinking style that is not easily influenced and always tends to weigh pros and cons to judge things. No significant differences appeared among the rest of types which is a good sign of general acceptance. In this same context, one important point is the prominent role of individual differences among workers according to personality type in predicting whether certain job specifications will result in stress or not and specifying the kind of stressors that are the most irritating [8]. The workshop was able to clarify this point and introduced some tips that focus on the individual and promote ways of coping with demanding working conditions according to one's nature. Participants were responsive with the idea and 8.1% chose such topic as the best thing in the workshop.

Recommendations as introduced by the attendants varied between the need of more illustrations, follow-up, extended time for the workshop, copy of the material content and more active participation from attendants. Negative comments were as follows: the need of more practical examples from life, time management, the need for more illustrations and the need of illustrative videos for personality types. As for positive comments they mainly stated both the topic and its manipulation were interesting and innovative.

Upon interpretation of the process evaluation results, we can clearly conclude a great deal of acceptance to such kind of interventions in our Egyptian community. Stress at work is proved to be an important topic and its management is a serious need. More developed programs with longer duration and variety of topics would be of crucial importance and are expected to receive a big flow of participants.

Acknowledgement

This work was a part of a project funded by the National Research Centre.

References

1. Rappaport J. Terms of empowerment/exemplars of prevention: toward a theory for community psychology. American Journal of Community Psychology.1987;15(2),121-48. http://dx.doi.org/10.1007/BF00919275 PMid:3604997

2. Koelen MA, Lindstrom B. Making healthy choices easy choices: the role of empowerment. European Journal of Clinical Nutrition. 2005; 59(1),S10-S6. http://dx.doi.org/10.1038/sj.ejcn.1602168 PMid:16052175

3. Laverack G. Improving health outcomes through community empowerment: a review of the literature. Journal of Health, Population and Nutrition. 2006;24(1),113-20. PMid:16796158

4. Wallerstein N. What is the Evidence on Effectiveness of Empowerment to Improve Health? Report for the Health Evidence Network (HEN), 2006.

5. World Health Organization (WHO). Ottawa Charter for Health

Promotion. Geneva,1986.

6. Woodall J, Raine G, South J. et al. Empowerment and Health & Well-being: Evidence Review. Centre for Health Promotion Research, Leeds Metropolitan University, 2010.

7. World Health Organization (WHO). Health education: theoretical concepts, effective strategies and core competencies, printed by WHO Regional Office for the Eastern Mediterranean, Cairo 2012. Retrieved from http://applications.emro.who.int/dsaf/EMRPUB_2012_EN_1362.pdf

8. World Health Organization (WHO). Raising Awareness of Stress at Work in Developing Countries A modern hazard in a traditional working environment (Protecting Workers' Health series No. 6). Geneva, 2007.

9. World Health Organization (WHO). AIMS Report on Mental Health System in (Egypt), WHO and Ministry of Health, Cairo, Egypt, 2006. http://www.who.int/occupational_health/publications/raisingawarenessofstress.pf

10. Crepaz N, Passin W F, Herbst JH et al., Meta-analysis of cognitive behavioral interventions on HIV-positive persons' mental health and immune functioning. Health Psychology. 2008; 27(1), 4-14. http://dx.doi.org/10.1037/0278-6133.27.1.4 PMid:18230008

11. Cognitive Style Inventory© Ross Reinhold, at www.PersonalityPathways.com http://www.personalitypathways.com/type_inventory.html

12. Andrews FM and Withey SB. Social indicators of well-being: American"s perceptions of life quality. New York: Plenum Press,1976. http://dx.doi.org/10.1007/978-1-4684-2253-5

13. Benziger K. The Physiological and Psycho-Physiological Bases for Jungian Concepts: An Annotated Bibliography KBA, 1996.

14. Center for Engineering & Occupational Safety and Health (CEOSH). Employee Health Promotion Disease Prevention Guidebook Published by VHA,2011. Retrieved from http://www.publichealth.va.gov/docs/employeehealth/health-promotion-guidebook.pdf

15. National Institute for Occupational Safety and Health (NIOSH). Stress at Work, 2009. Retrieved from: http://www.cdc.gov/niosh/docs/99-101/.

16. National Institutes of Health (NIH). Stress and Anxiety, 2008. Retrieved from: http://www.nlm.nih.gov/medlineplus/ency/article/003211.htm.

17. Canadian Mental Health Association. Stress in the Workplace: A General Overview of the Causes, the Effects, and the Solutions, 2005. http://www.cmhanl.ca/pdf/Work%20Place%20Stress.pdf

18. Rychetnik L, Frommer M, Hawe P et al. Criteria for evaluating evidence on public health interventions. Epidemiol Community Health. 2002;56,119–127. http://dx.doi.org/10.1136/jech.56.2.119 PMCid:PMC1732065

19. Whatley L. Women Empowerment. Wikepedia.org 2012. Retrieved from: http://www. selfgrowth.com/articles/Articles_Women_Empowerment.html.

20. World Health Organization (WHO). Health promotion glossary. Geneva, 1998. http://www.who.int/hpr/NPH/docs/hp_glossary_en.pdf.

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):510-516.
http://dx.doi.org/10.3889/oamjms.2016.071
eISSN: 1857-9655
Review Article

The Role of Levosimendan in Patients with Decreased Left Ventricular Function Undergoing Cardiac Surgery

Marija Bozhinovska[1*], Gordana Taleska[1], Andrej Fabian[2,3], Maja Šoštarič[1]

[1]Clinical Department of Anesthesiology and Perioperative Intensive Therapy, Division of Cardiac Anesthesiology and Intensive Therapy, University Clinical Center Ljubljana, Zaloska 7, 1000 Ljubljana, Slovenia; [2]Institute of Physiology, Medical Faculty, University of Ljubljana, Slovenia; [3]Department of Vascular Neurology and Neurological Intensive Therapy, University Clinical Centre Ljubljana, Slovenia

Citation: Bozhinovska M, Taleska G, Fabian A, Šoštarič M. The Role of Levosimendan in Patients with Decreased Left Ventricular Function Undergoing Cardiac Surgery. Open Access Maced J Med Sci. 2016 Sep 15; 4(3):510-516. http://dx.doi.org/10.3889/oamjms.2016.071

Keywords: Cardiac surgery; Levosimendan; Ventricular function; Heart failure; Myocardial dysfunction.

***Correspondence:** Marija Bozhinovska, MD. Zaloska 7, SI-1000 Ljubljana, Slovenia. Tel: + 386 70 321 247. E-mail: marija.bozinovska@kclj.si

Received: 18-Jun-2016; **Revised:** 21-Jun-2016; **Accepted:** 25-Jun-2016; **Online first:** 28-Jun-2016

Competing Interests: The authors have declared that no competing interests exist.

Abstract

The postoperative low cardiac output is one of the most important complications following cardiac surgery and is associated with increased morbidity and mortality. The condition requires inotropic support to achieve adequate hemodynamic status and tissue perfusion. While catecholamines are utilised as a standard therapy in cardiac surgery, their use is limited due to increased oxygen consumption. Levosimendan is calcium sensitising inodilatator expressing positive inotropic effect by binding with cardiac troponin C without increasing oxygen demand. Furthermore, the drug opens potassium ATP (KATP) channels in cardiac mitochondria and in the vascular muscle cells, showing cardioprotective and vasodilator properties, respectively. In the past decade, levosimendan demonstrated promising results in treating patients with reduced left ventricular function when administered in peri- or post- operative settings. In addition, pre-operative use of levosimendan in patients with severely reduced left ventricular ejection fraction may reduce the requirements for postoperative inotropic support, mechanical support, duration of intensive care unit stay as well as hospital stay and a decrease in post-operative mortality. However, larger studies are needed to clarify clinical advantages of levosimendan versus conventional inotropes.

Introduction

Patients undergoing open heart surgery with cardiopulmonary bypass (CPB) experience global perioperative heart ischemia followed by reperfusion. This leads to different degrees of myocardial dysfunction due to free radical formation, impairment of the coronary vasculature and calcium overload [1, 2]. If severe enough this can cause postoperative low cardiac output syndrome (LCOS), a life-threatening complication with a prevalence of about 10% and a mortality of 17% [3].

Acute worsening of the left heart failure is commonly treated pharmacologically with intravenous positive inotropic agents, or mechanical support in severe cases [4]. Currently, beta-adrenergic agonists and phosphodiesterase inhibitors are most frequently used in clinical practice [4, 5]. Beta-adrenergic agents increase cardiac output (CO), cardiac index (CI) and stroke volume index by increasing intracellular cyclic adenosine monophosphate (cAMP) production and calcium influx to myocytes [6]. Phosphodiesterase inhibitors, like milrinone, do the same by inhibiting cAMP degradation [7]. This results in increased cellular energy demands and oxygen consumption, can trigger arrhythmias and can even be cardiotoxic [8, 9].

Levosimendan is a newer inotropic agent belonging to a class of calcium sensitizers that

increase myocardial contractility by increasing the sensitivity of troponin C for calcium without increasing myocardial oxygen consumption [10]. Unlike some calcium sensitizers, levosimendan increases myocardial contractility without impairing diastolic cardiac function [11]. It displays vasodilator properties as well [12].

This review is focused on the impact of levosimendan in patients with left heart failure undergoing open heart surgery. Pharmacologic features of this drug will be briefly presented as well.

Used Literature

The data sources used in this article are PubMed, Excerpta Medica, Reference Update, BIOSIS, Science Citation Index and Index Medicus. The abstract and papers were found by searching ([levosimendan] and [surgery OR bypass OR valve OR CABG]) were selected to the timing of the use of levosimendan and the type of intervention.

Pharmacology of Levosimendan

Levosimendan is calcium sensitising inodilator that binds to troponin C, thereby enhancing myofilament responsiveness to calcium. This mechanism enhances myocardial contractility, maintaining the energy cost of contraction and intracellular concentration of calcium at a near normal level [1, 13]. Vasodilation is achieved by opening adenosine triphosphate (ATP) - sensitive K_{ATP} channels on smooth muscle cells of systemic and coronary vascular bed. Activation of K_{ATP} channels on cardiac mitochondria explains its cardioprotective effects [14].

Cardioprotective effects of levosimendan are short term (e. g. pre- and postconditioning, anti-ischemic, anti-stunning), and long term (e.g. anti-remodeling, anti-inflammatory and anti-apoptotic) [1]. Preconditioning refers to the ability of levosimendan to protect the heart from ischemia-reperfusion injury by preventing calcium overload, saving high energy phosphates and stabilising membrane potential due to increased potassium influx in cardiac mitochondria through activation of K_{ATP} channels [1, 14, 15].

In animal models, myocardial infarct size was significantly decreased after global ischemia-reperfusion in both levosimendan pretreatment (45 ± 2%) and ischemic preconditioning (38 ± 2%) compared with controls (52 ± 2%) ($p < 0.05$). Additionally, recent ex-vivo model study of cardiac ischemia-reperfusion showed that levosimendan pretreatment can decrease the infarct size and improve cardiac function [16].

The pharmacokinetics of levosimendan appears to be similar in patients with heart failure and healthy volunteers and remains relatively unaltered by gender, age and organ dysfunction [17]. Usually, a bolus dose of levosimendan of 6 to 24 μg/kg is used followed by continuous infusion of 24 h of 0.05-0.2 μg/kg in patients with heart failure NYHA classification II-IV [15, 18]. After a single dose or intravenous infusion, plasma concentration of levosimendan increases in a dose-dependent manner [4]. Approximately 97-98% is bound to plasma albumins, and its plasma half-life is one hour. Levosimendan is extensively metabolised through two pathways before excretion. The main pathway is conjugation into inactive metabolites, while the minor pathway is gut reduction into intermediate metabolite (OR-1855) and subsequent acetylation into an active metabolite (OR 1896) [4].

The OR-1896 exhibits similar haemodynamic influence as levosimendan and a half-life of approximately 80 hours [19]. This most likely explains the sustained improvements in ventricular performance and natriuretic peptide levels compare to traditional inotropes [20, 21]. The most often reported adverse effect is a headache, observed in 10 % of the healthy volunteers receiving levosimendan [4]. Other side effects that probably appear due to the vasodilatatory effects of levosimendan include dizziness, nausea and palpitations. Levosimendan acutely reduces systemic vascular resistance and ventricular filling pressure. Consequently, in some patients with heart failure, it lowers blood pressure and vasopressors are required in the first few hours or days of administration [22].

Effects of Levosimendan in Patients with Heart Failure

Levosimendan has been used in the treatment of heart failure (HF) over the past decade. Studies have shown clinical improvement in terms of hemodynamic parameters, cardiac function, shorter overall in-hospital stay, while data for long-term mortality is still controversial [23].

In a Randomized study of safety and effectiveness of Levosimendan in patients with left ventricular failure after acute myocardial infarction (RUSSLAN), 504 patients were randomised to receive either infusion of levosimendan or placebo [24]. Patients in levosimendan group experienced reduced short-term and long term mortality at 14 days (11.7% vs. 19.6%, $p = 0.031$) and on 180 days (22.6% vs.

31.4%, p = 0.053) compared to placebo group [11,24].

In the randomised multicenter evaluation of intravenous Levosimendan efficacy (REVIVE II) study 600 patients with acute decompensated heart failure (ADFH), were treated with levosimendan (299 patients) or placebo (301 patients) infusion in addition to standard therapy. 58 patients in the levosimendan group and only 44 patients in the placebo group got improved at 6 h, 24 h and 5 days. By contrast, 82 patients in the placebo but only 58 patients in the levosimendan group got worse (p = 0.015) [10]. In respect of mortality at 90 days, there was no difference between the groups [25].

In SURVIVE study (Survival of patients with acute HF in need of intravenous inotropic support), which randomised 1327 patient in a double-blind fashion to either dobutamine or levosimendan, short-term (31 days) and long-term (180 days) mortality was evaluated in patients with severe ADHF [26]. Patients received bolus of levosimendan 12 μg/kg in 10 min, followed by 0.1-0.2 μg/kg/min continuous infusion for 24 h [10]. Mortality rate was evaluated at 5 days, 2 weeks, 1 and 6 months and the results showed reduced mortality for levosimendan group by 27%, 14%, 13%, and 6.4% respectively, compared to dobutamine group, however with no statistically significant difference [27].

Levosimendan in Cardiac Surgery

Evidence shows the benefit of coronary surgery over medical treatment in patients suffering from both decreased left ventricular function and coronary artery disease [20, 28]. Patients undergoing cardiac surgery with high-risk profile require peri-operative and post-operative inotropic support and levosimedan seems to be an attractive option in such patients [23]. Different meta-analyses have suggested mortality benefits in patients undergoing cardiac surgery receiving levosimendan. Landoni and co-workers analysed 45 randomised clinical trials including 4580 patients and found reduced mortality with an OR of 0.35 (95% CI 0.18-0.71, p = 0.003) in patients treated with levosimedan [29]. Maharaj and co-workers analyzed 17 studies including 729 patients after coronary revascularization treated with levosimendan versus control, and found mortality benefit with an OR of 0.40 (95% confidence interval (CI) 0.21 to 0.76, *P* = 0.005) [28].

Harrison and co-workers analysed 14 randomised controlled trials including 1155 patients, evidence showed decreased mortality rate in patients intraoperatively treated with levosimendan (risk difference −4.2%, 95% CI −7.2% to −1.1%, p = 0.008), in patients with reduced ejection fraction (EF) these benefits were greatest [28].

Lim and co-workers analysed 14 studies of patients with reduced left ventricular ejection fraction (LVEF) undergoing cardiac surgery. Results showed reduced early mortality (5.5% vs. 9.1%), reduced Intensive care unit stay (ICU) 1.01 (1.61–0.42) days and reduced postoperative acute renal injury (ARI) (7.4% vs. 11.5%) in patients treated with levosimendan [31].

Reports from several clinical studies have compared levosimendan versus milrinone, placebo, dobutamine or intra-aortic balloon pump (IABP)[23]. Patients with preserved or reduced left ventricular function received levosimendan in pre, intra or post-operative setting (period).

Pre-Operative Use of Levosimendan in Cardiac Surgery

In a trial by Levin and co-workers, 252 high-risk patients with severely reduced LVEF scheduled for coronary artery bypass grafting (CABG), were randomized to receive either levosimendan or placebo infusion 24 hours pre-operatively. Levosimendan was started with a loading dose of 10 μg/kg infusion for 1 h than followed by continuous infusion of 0.1 μg/kg/ min infusion for 23 h. Reduced mortality was seen in levosimendan group compared to placebo (3.9% vs. 12.8%; P = 0.05), decreased incidence of LCOS (7.1% vs. 20.8%; P = 0.05) and complicated weaning from CPB (2.4% vs. 9.6%; P = 0.05). In patients treated with levosimendan results showed lower requirement for other inotropes (7.9% vs. 58.4%; P = 0.05), vasopressors (14.2% vs. 45.6%; P = 0.05), and lower requirements for IABP (6.3% vs. 30.4%; P = 0.05) compared to placebo group [32].

In a randomised, double-blind, placebo-controlled study by Leppikangas and co-workers, 24 patients were enrolled and received either levosimendan or placebo infusion 24 hours before surgery. Results showed improved hemodynamic parameters in levosimendan group compared to placebo as well as higher levels of levosimendan's metabolites compared to other studies that use levosimendan in intra-operative setting [33].

Intra-operative Use of Levosimendan

Several trials assessed the effect of levosimendan started after induction of anaesthesia or before initiation of CPB in patients with preoperatively

impaired LVEF. Although the difference in mortality rate on patients treated with levosimendan compared to placebo or other inotrops showed no statistical significance difference, secondary end points such as ICU stay, hospital stay, mechanical ventilation, requirements for mechanical support and other inotrops were significantly decreased [34, 35].

In the randomised double-blind trial, Triapepe and co-authors compared placebo versus levosimendan in 106 patients scheduled for elective CABG. Patients receive levosimendan slow i.v. (24 μg kg (-1)) or placebo over 10 min before initiation of CPB. Results showed significant reduction of the length of ICU stay and tracheal intubation time, lower level of postoperative troponin I ($P < 0.0001$) and higher cardiac index ($P < 0.0001$), without a statistically significant difference in the study groups in respect of 30-day mortality [36].

The effect of levosimendan on the postoperative outcome with respect to the time of administration (early versus late) was assessed in 159 patients with impaired LVEF less than 35%, in a trial conducted by Treskatsh and co-workers. Patients that received levosimendan intra-operatively up to the first hour after ICU admission showed better survival (49/70) compared to patients treated with levosimendan infusion after the first hour of ICU admission (41/89). The survivors in the late start group showed increased incidence of in-hospital mortality ($p = 0.004$) and increased 1-year mortality ($p = 0.027$). Additionally, the incidence of renal dysfunction ($p = 0.002$), requirements for renal replacement therapy (RRT) ($p = 0.032$) and duration of mechanical ventilation ($p = 0.002$) were significantly increased in the late start group [37].

Järvelä and co-workers randomized 24 patients with severe aortic stenosis and left ventricular hypertrophy scheduled for aortic valve replacement (AVR). Levosimendan group received bolus, followed by i.v. continuous infusion (0.2 μg/kg/min), and started after induction of anaesthesia. LVEF was maintained in levosimendan group while it decreased in the placebo group, without significant difference [34].

Benefits of Levosimendan compared to IABP were assessed in a prospective randomised trial of 90 patients with coronary artery disease and LVEF less than 35%, scheduled for surgery [35]. Patients in levosimendan group had significantly shorter ICU stay compared to other groups ($p < 0.01$); six hours after surgery level of cardiac troponin (cTnI) was increased in all groups but significantly lower in the levosimendan group. Concentration of cTnI at the first postoperative day was 4.67 ng/mL (1.04 to 8.20), 2.40 ng/mL (1.17 to 5.14), and 2.99 ng/mL (1.52 to 7.40) and levels decreased at second post-operative day. Furthermore, levosimendan improved hemodynamic parameters and contributed to lower cTnI levels compared to preoperative IABP [35].

Elahi et al. also evaluated the impact of preoperative use of either IABP or levosimendan on patients outcome, and data showed that in cardiac surgery patients with LCOS both improved cardiac function, although provided no evidence to suggest weather IABP or levosimendan is superior in treatment of patients with LCOS with regard to morbidity and mortality [38].

In a trial by Lahtinen et al. 207 patients scheduled for valve ± coronary surgery were randomised into placebo or levosimendan group and the infusion was initiated after induction of anaesthesia. Results showed reduced the number of patients with HF in levosimendan group (risk ratio 0.26, 95% CI0.16-0.43, $p = 0.001$) but they were more likely to require noradrenalin and less likely to require IABP compared to placebo group. No significant difference in in-hospital and six months mortality between the groups [39].

The Use of Levosimandan in Post-Operative Course

Several studies have evaluated the effect of levosimendan on patients with LCOS (defined as CI < 2.2 l/min/m^2, mixed venous saturation < 60%, pulmonary wedge pressure > 18 mmHg) after cardiac surgery. Levin and colleagues randomised 253 parents with LCOS after surgery, to receive either levosimandan or dobitamine. Patients in levosimendan group showed reduced requirements for inotrops, vasopressors, IABP use, as well as decrease postoperative mortality (7.1 vs. 15.9%) [23, 40].

Alvarez and colleagues randomised 41 patients after open heart surgery on CPB to either levosimendan or dobutamine administered in the treatment of post operative LCOS. Although both drugs are effective in LCOS treatment, short term hemodynamics was improved, levosimendan showed significantly greater effects in improving CI (2.9 (0.3) l/min/m^2 vs. 2.4 (0.2) l/min/m^2, $p = 0.05$) as well as significantly reduced systemic and pulmonary vascular resistances, also decreased pulmonary capillary wedge and central venous pressure(CVP) [41]. Al–Shawaf and colleagues conducted a randomised trial comparing levosimendan vs milrinone for the management of LCOS in diabetic patients after cardiac surgery with LVEF < 35%. Significantly higher mixed venous saturation, cardiac indices, lower pulmonary capillary wedge pressures and oxygen extraction rates were found In levosimendan group. Therefore, it was suggested that levosimendan was more effective in treating hemodynamic worsening of post operative LCOS [42].

Treatment of Levosimandan in Patients with Right Heart Failure

Several trials have investigated the effects of levosimendan on patients with biventricular HF and cardiogenic shock related to acute myocardial infarction, and demonstrated reduced right ventricle (RV) afterload and increased RV contractility [1, 10]. Russ and colleagues evaluated the effect of levosimandan versus conventional therapy (dobutamine and norepinephrine) in patients with cardiogenic shock due to myocardial infarction treated with percutaneous coronary intervention (PCI). Results showed significant increase in cardiac power index in both left and right ventricle (2.1 ± 0.1 to 3.0 ± 0.2, $p < 0.01$) and (0.14 ± 0.19 to 0.18 ± 0.12, $p < 0.001$) respectively, and also decreased pulmonary vascular resistance [43]. In a study, Yulmas and colleagues randomised 40 patients with significant biventricular systolic dysfunction to receive either levosimendan or dobutamine and in levosimendan group doppler echocardiographic markers of systolic function and tricuspid annulus were significantly improved (15% ± 12% vs. 2% ± 6%, $P < 0.001$) versus dobutamine [44].

Postoperative Kidney Function in Patients Treated with Levosimendan

Postoperative renal dysfunction in patients after cardiac surgery is an important complication and independently is associated with increased mortality, morbidity, prolonged ICU and prolonged overall in-hospital stay [45]. Several trials have investigated the effect of levosimendan in patients with LCOS after cardiac surgery the results showed that the drug can increase renal blood flow (RBF), CO and by this features its reno-protective effects were recognised [46, 47]. In one of the latest meta-analysis evaluating the renal effect of levosimendan continuous infusion 0.1 – 0.2 μg/kg/min after loading dose of 6-24 μg/kg for 24 h or only loading dose 24 μg/kg in 1h versus placebo and/or other inotropic drugs in 13 trials and 1345 patients, the authors found that levosimendan reduces the incidence of postoperative AKI, RRT, postoperative mortality, duration of mechanical ventilation and ICU stay. In a prospective, double-blind clinical trial Baysal and colleagues randomised 128 patients undergoing mitral valve surgery with LVEF less 45 % were to receive either levosimendan 6 μg/kg/min as a loading dose then 0.1 μg/kg/min after aortic clamp removal compared to standard inotropic therapy. Patients in the levosimendan group showed improved postoperative renal clearance and higher values of estimated glomerular filtration rate (eGFR) on day 1 and day 3 compared to control group $p = 0.0001$, $p = 0.0009$ respectively and the need of RRT was reduced [48].

In a retrospective observational study, Balzer and colleagues analysed 46 patients with reduced LVEF treated with levosimendan as a continuous infusion 0.1 μg/kg/min with respect to the timing of it's administration during surgery early versus late start in ICU. Intra-operatively 61% of the patients received levosimendan versus 39% of patients treated with levosimendan in ICU and the results showed significantly reduced creatinine plasma levels $p= 0.009$, reduced incidence of postoperative renal dysfunction (67.9% vs. 94.4%, $p = 0.033$) and also reduced the duration of the RRT [49].

Knezevic and al. evaluate the reno-protective role of levosimendan versus standard inotropic therapy, in 94 patients included for heart transplantation. In the first week after transplantation, patients in levosimendan group showed increased eGFR (62% vs.12%, $p = 0.002$) with lower incidence of AKI (28% vs. 6%, $p = 0.01$). In the first tree months, 19% of patients with AKI died versus 8% of patients without AKI ($p = 0.37$). In the study population, the one-year survival rate was 87%, without a statistically significant difference in mortality rate between the groups (11% in levosimandan vs. 15% in control, $p = 0.54$)[50].

Cost-Effectiveness of Treatment with Levosimendan

Economic and health analysis of treatment with levosimendan in acute decompensated heart failure showed that it is cost effective. However, in cardiac surgery, there is limited data regarding it's cost effective. In an observational study with 292 patients with acute heart failure, Fedele and colleagues preformed cost-effectiveness analysis of treatment with levosimendan versus the standard treatment with dobutamine. It was found that per capita cost of treatment with levosimendan is higher in the initial hospitalisation, but when re- hospitalisations were considered costs, they were significantly lower [23, 51]. Therefore, available data imply that treatment with levosimendan in cardiac surgery positively affects the elements that are likely to increase the costs of treatment: shorter ICU stay, fewer requirements for IABP and renal replacement therapy [1]. Furthermore, improved indirect indicators may show the advantage of levosimendan in cost-effectiveness over dobutamine, particularly when administered in high-risk patients [23].

In conclusion, clinical studies show that levosimendan enhances the cardiac function and

improves the hemodynamic, pulmonary and general condition in patients after cardiac surgery in both reduced and normal LVEF. Pre operative use of levosimendan in high-risk patients with severely reduced LVEF may reduce the requirements for inotropic support, mechanical support, duration of intensive care unit stay as well as hospital stay and decrease post operative mortality. The greatest benefits may be seen in high-risk patients where levosimandan is applied in preoperative setting 24 h before surgery.

References

1. Toller W, Heringlake M, Guarracino F , Algotsson L, Alvarez, J, Argyriadou H, et al. Preoperative and perioperative use of levosimendan in cardiac surgery: European expert opinion. Int J Cardiol. 2015;184:323-36. http://dx.doi.org/10.1016/j.ijcard.2015.02.022 PMid:25734940

2. Suleiman MS, Zacharowski K, Angelini G D.Inflammatory response and cardioprotection during open-heart surgery: the importance of anaesthetics. Br J Pharmacol. 2008;153(1)21-33. http://dx.doi.org/10.1038/sj.bjp.0707526 PMid:17952108 PMCid:PMC2199383

3. Malliotakis P, Xenikakis T, Linardakis M, Hassoulas J. Hemodynamic effects of Levosimednan for low cardiac output after cardiac surgery: A case series. Hellenic J Cardiol. 2007;48: 80-88. PMid:17489345

4. Raja SG, Rayen BS. Levosimendan in cardiac surgery: current best available evidence. Ann Thorac Surg. 2006;81(4):1536-46. http://dx.doi.org/10.1016/j.athoracsur.2005.08.073 PMid:16564321

5. Amsallem E, Kasparian C, Haddour G, Boissel J P, Nony P. Phosphodiesterase III inhibitors for heart failure. Cochrane Database of Syst. Rev. 2005; (1): CD002230. http://dx.doi.org/10.1002/14651858.cd002230.pub2

6. Overgaard CB, Dzavík V. Inotropes and vasopressors: review of physiology and clinical use in cardiovascular disease. Circulation. 2008;118(10):1047-56. http://dx.doi.org/10.1161/CIRCULATIONAHA.107.728840 PMid:18765387

7. Packer M. The search for the ideal positive inotropic agent. N Engl J Med. 1993;329:201–2. http://dx.doi.org/10.1056/NEJM199307153290310 PMid:8515793

8. Lee JC, Downing SE. Cyclic AMP and the pathogenesis of myocardial injury. Res Commun Chem Pathol Pharmacol. 1980;27:305–18. PMid:6245436

9. Ferrick KJ, Fein SA, Ferrick AM, et al. Effect of milrinone on ventricular arrhythmias in congestive heart failure. Am J Cardiol. 1990;66:431–4. http://dx.doi.org/10.1016/0002-9149(90)90699-2

10. Nieminen MS, Fruhwald S, Heunks LM, Suominen PK, Gordon AC, Kivikko M, et al. Levosimendan: current data, clinical use and future development. Heart Lung Vessel. 2013;5:227–245. PMid:24364017 PMCid:PMC3868185

11. Antoniades C, Tousoulis D, Koumallos N, Marinou K, Stefanadis C. Levosimendan: beyond its simple inotropic effect in heart failure. Pharmacol Ther. 2007;114(2):184-97. http://dx.doi.org/10.1016/j.pharmthera.2007.01.008 PMid:17363065

12. Caimmi PP, Kapetanakis EI, Beggino C, Molinari C, Giustini G, Crosio E, et al. Management of acute cardiac failure by intracoronary administration of levosimendan. J Cardiovasc Pharmacol. 2011;58:246–53. http://dx.doi.org/10.1097/FJC.0b013e318220e491 PMid:21654504

13. Kamath SR, Jaykumar I, Matha S. Levosimendan. Indian Pediatr. 2009;46:593–596. PMid:19638658

14. Pathak A, Lebrin M, Vaccaro A, Senard JM, Despas F. Pharmacology of levosimendan: inotropic, vasodilatory and cardioprotective effects. J Clin Pharm Ther. 2013;38(5):341-9. http://dx.doi.org/10.1111/jcpt.12067 PMid:23594161

15. Kasikcioglu HA, Cam N. A review of levosimendan in the treatment of heart failure. Vasc Health Risk Manag. 2006;2(4):389-400. http://dx.doi.org/10.2147/vhrm.2006.2.4.389

16. Lepran I, Pollesello P, Vajda S, Varró A, Papp G J. Preconditioning effects of levosimendan in a rabbit cardiac ischemia–reperfusion model. J. Cardiovasc. Pharmacol. 2006;48:148–152. http://dx.doi.org/10.1097/01.fjc.0000246151.39758.2a PMid:17086092

17. Ng TM. Levosimendan, a new calcium-sensitizing inotrope for heart failure. Pharmacotherapy. 2004;24:1366–84. http://dx.doi.org/10.1592/phco.24.14.1366.43145

18. Jonsson N E, Antila S, McFadyen L, Lehtonen L, Karlsson M. Population pharmacokinetics of levosimendan in patients with congestive heart failure. Br J Clin Pharmacy. 2003;55(6):544-551. http://dx.doi.org/10.1046/j.1365-2125.2003.01778.x PMCid:PMC1884248

19. Moreno N, Tavares-Silva M, Lourenço AP, Pinto J O, Henriques-Coelho T, Leite-Moreira AF. Levosimendan: The current situation and new prospects. Rev Port Cardiol. 2014;33(12):795-800. http://dx.doi.org/10.1016/j.repc.2014.05.009 PMid:25459636

20. Eriksson HI, Jalonen JR, Heikkinen LO, Kivikko M, Laine M, Leino KA, et al. Levosimendan facilitates weaning from cardiopulmonary bypass in patients undergoing coronary artery bypass grafting with impaired left ventricular function. Ann Thorac Sure. 2009;87:448–454. http://dx.doi.org/10.1016/j.athoracsur.2008.10.029 PMid:19161758

21. Kivikko M, Lehtonen L, Colucci WS. Sustained hemodynamic effects of intravenous levosimendan. Circulation. 2003;107:81–6. http://dx.doi.org/10.1161/01.CIR.0000043245.00859.11 PMid:12515747

22. Nieminen MS, Buerke M, Cohen-Solál A, Costa S, Édes I, Erlikh A, Franco F, et al. The role of levosimendan in acute heart failure complicating acute coronary syndrome: A review and expert consensus opinion. Int J Cardiol. 2016;218:150-157. http://dx.doi.org/10.1016/j.ijcard.2016.05.009 PMid:27232927

23. Shi WY, Li S, Collins N, Cotten DB, Bastian BC, James AN, Mejia R. Peri- operative Levosimendan in Patients Undergoing Cardiac Surgery: An Overview of the Evidence. Heart Lung Circ. 2015;24(7):667-72. http://dx.doi.org/10.1016/j.hlc.2015.03.007 PMid:25862519

24. De Luca L, Colucci WS, Nieminen MS, Massie BM, Gheorghiade M. Evidence-based use of levosimendan in different clinical settings. Eur Heart J. 2006;27:1908-20. http://dx.doi.org/10.1093/eurheartj/ehi875 PMid:16682381

25. Delaney A, Bradford, C, McCaffrey J, Bagshaw S M, Lee R. Levosimendan for the treatment of acute severe heart failure: A meta-analysis of randomised controlled trials. Int J Cardiol. 2010;138(3):281-9. http://dx.doi.org/10.1016/j.ijcard.2008.08.020 PMid:18817994

26. Mebazaa A, Nieminen MS, Filippatos G S, Cleland JG, Salon JE, Thakkar R, Padley RJ, Huang B, Cohen-Solal A. Levosimendan vs. dobutamine: outcomes for acute heart failure patients on beta-blockers in SURVIVE. Eur J Heart Fail. 2009;11:304–311. http://dx.doi.org/10.1093/eurjhf/hfn045 PMid:19158152 PMCid:PMC2645051

27. Cohen-Solal A, Logeart D, Huang B, et al.Lowered B type natriuretic peptide in response to levosi mendan or dobutamine treatment is associated with improved survival in patients with severe acutely decompensated heart failure. J Am Coll Cardiol. 2009;53:23432348. http://dx.doi.org/10.1016/j.jacc.2009.02.058 PMid:19539144

28. Harrison R W, Hasselblad V, Mehta R H, Levin R, Harrington RA, Alexander JH. Effect of levosimendan on survival and adverse events after cardiac surgery: a meta-analysis. J Cardiothorac Vasc Anesth. 2013;27:1224–32.

http://dx.doi.org/10.1053/j.jvca.2013.03.027 PMid:24050857

29. Landoni G, Mizzi A, Biondi-Zoccai G, et al., Reducing mortality in cardiac surgery with levosimendan: a meta-analysis of randomized controlled trials. J Cardiothorac Vasc Anesth. 2010;24:51–57. http://dx.doi.org/10.1053/j.jvca.2009.05.031 PMid:19700350

30. Maharaj R, Metaxa V. Levosimendan and mortality after coronary revascularisation: a meta-analysis of randomised controlled trials. Crit Care. 2011;15:R140. http://dx.doi.org/10.1186/cc10263 PMid:21651806 PMCid:PMC3219012

31. Lim JY , Deo SV, Rababa'h A, Altarabsheh SE, Cho YH, Hang D, et al. Levosimendan reduces mortality in adults with left ventricular dysfunction undergoing cardiac surgery: a systematic review and meta-analysis. J Card Surg. 2015;30:547–554. http://dx.doi.org/10.1111/jocs.12562 PMid:25989324

32. Levin R, Degrange M, Del Mazo C, Tanus E, Porcile R. Preoperative levosimendan decreases mortality and the development of low cardiac output in high-risk patients with severe left ventricular dysfunction undergoing coronary artery bypass grafting with cardiopulmonary bypass. Exp Clin Cardiol. 2012;17:125–30. PMid:23620700 PMCid:PMC3628425

33. Leppikangas H, Järvelä K, Sisto T, Maaranen P, Virtanen M, Lehto P, et al. Preoperative levosimendan infusion in combined aortic valve and coro- nary bypass surgery. Br J Anaesth. 2011;106:298–304. http://dx.doi.org/10.1093/bja/aeq402 PMid:21258075

34. Järvelä K, Maaranen P, Sisto T, Ruokonen E. Levosimendan in aortic valve surgery: car- diac performance and recovery. J Cardiothorac Vasc Anesth. 2008;22:693–698. http://dx.doi.org/10.1053/j.jvca.2008.01.024 PMid:18922425

35. Lomivorotov VV, Boboshko VA, Efremov SM, et al. Levosimendan versus an intra-aortic balloon pump in high-risk cardiac patients. J Cardiothorac Vasc Anesth. 2012;26:596–603. http://dx.doi.org/10.1053/j.jvca.2011.09.006 PMid:22051419

36. Tritapepe L, De Santis V, Vitale D, Guarracino F, Pellegrini F, Pietropaoli P, et al. Levosimendan pre-treatment improves outcomes in patients undergoing coronary artery bypass graft surgery. Br J Anaesth. 2009;102: 198–204. http://dx.doi.org/10.1093/bja/aen367 PMid:19151048

37. Treskatsch S, Balzer F, Geyer T, Spies CD, Kastrup M, Grubitzsch H, et al. Early levosimendan administration is associated with decreased mortality after cardiac surgery. J Crit Care. 2015;30(4):859.e1-6. http://dx.doi.org/10.1016/j.jcrc.2015.03.008 PMid:25837801

38. Elahi MM, Lam J, Asopa S, Matata BM. Levosimendan versus an intra-aortic balloon pump in adult cardiac surgery patients with low cardiac output. J Cardiothorac Vasc Anesth. 2011; 25(6):1154–62. http://dx.doi.org/10.1053/j.jvca.2011.01.001 PMid:21376634

39. Lahtinen P, Pitkänen O, Pölönen P, et al. Levosimendan reduces heart failure after cardiac surgery – a prospective, randomized, placebocontrolled trial. Crit Care Med. 2011;39:2263-2270. http://dx.doi.org/10.1097/CCM.0b013e3182227b97 PMid:21666445

40. Levin R, Porcile R, Salvagio F, Del Mazo C, Botbol A, Tanus E, et al. Levosimendan reduces mortality in post-operative low cardiac output syndrome after coronary surgery. Circulation. 2009;120:S987–8.

41. Alvarez J, Bouzada M, Fernández AL, et al. Hemodynamic effects of levosimendan compared with dobutamine in patients with low cardiac output after cardiac surgery. Rev Esp Cardiol. (Engl. Ed.). 2006;59:338–345. http://dx.doi.org/10.1016/S1885-5857(06)60770-6

42. Al-Shawaf E, Ayed A, Vislocky I, Radomir B, Dehrab N, Tarazi R. Levosimendan or milrinone in the type 2 diabetic patient with low ejection fraction undergoing elective coronary artery surgery. J Cardiothorac Vasc Anesth. 2006;20:353–357. http://dx.doi.org/10.1053/j.jvca.2006.02.012 PMid:16750735

43. Russ MA, Prondzinsky R, Carter JM, Schlitt A, Ebelt H, Schmidt H, Lemm H. et al. Right ventricular function in myocardial infarction complicated by cardiogenic shock: Improvement with levosimendan. Crit Care Med. 2009;37(12):3017–3023. http://dx.doi.org/10.1097/CCM.0b013e3181b0314a PMid:19661807

44. Yilmaz M B, Yontar C, Erdem A, Karadas F, Yalta K, Turgut O, et al. Comparative effects of levosimendan and dobutamine on right ventricular function in patients with biventricular heart failure. Heart Vessels. 2009;24(1):16–21. http://dx.doi.org/10.1007/s00380-008-1077-2 PMid:19165563

45. Niu ZZ, Wu SM, Sun WY, Hou WM, & Chi YF. Perioperative levosimendan therapy is associated with a lower incidence of acute kidney injury after cardiac surgery: a meta- analysis. J Cardiovasc Pharmacol. 2014;63(2):107-12. http://dx.doi.org/10.1097/FJC.0000000000000028 PMid:24126568

46. Zorlu A, Yucel H, Yontar OC, Karahan O, Tandogan I, Katrancioglu N, et al. Effect of levosimendan in patients with severe systolic heart failure and worsening renal function. Arq Bras Cardiol. 2012;98:537–43. http://dx.doi.org/10.1590/S0066-782X2012005000048 PMid:22641390

47. Zhou C, Gong J, Chen D, Wang W, Liu M, Liu B. Levosimendan for prevention of acute kidney injury after cardiac surgery: A Meta-analysis of controlled trials. Am J Kidney Dis. 2016;67(3):408-16. http://dx.doi.org/10.1053/j.ajkd.2015.09.015 PMid:26518388

48. Baysal A, Yanartas M, Dogukan M , Gundogus N , Kocak T, Koksal C. Levosimendan improves renal outcome in cardiac surgery: a randomised trial. J Cardiothorac Vasc Anesth. 2014;28:586–594. http://dx.doi.org/10.1053/j.jvca.2013.09.004 PMid:24447501

49. Balzer F, Treskatsch S, Spies C, Sander M, Kastrup M, Grubitzsch H, et al. Early administration of levosimendan is associated with improved kidney function after cardiac surgery, a retrospective analysis. J Cardiothorac Surg. 2014;9:167. http://dx.doi.org/10.1186/s13019-014-0167-8 PMid:25399779 PMCid:PMC4240807

50. Knezevic I, Poglajen G, Hrovat E, Oman A, Pintar T, et al. The effects of levosimendan on renal function early after heart transplantation: results from a pilot randomized trial. Clin Transplant. 2014;28(10):1105-11. http://dx.doi.org/10.1111/ctr.12424 PMid:25053182

51. Fedele F, et al. Cost-effectiveness of levosimendan in patients with acute heart failure. J Cardiovasc Pharmacol. 2011; 58(4):363-6. http://dx.doi.org/10.1097/FJC.0b013e318224e0a2 PMid:21697728

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):517-522.
http://dx.doi.org/10.3889/oamjms.2016.086
eISSN: 1857-9655
Review Article

Etiopathogenesis of Differentiated Thyroid Carcinomas

Tanja Makazlieva*, Olivija Vaskova, Venjamin Majstorov

Institute of Pathophysiology and Nuclear Medicine "Akademik Isak Tadzer", Medical Faculty, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia

Citation: Makazlieva T, Vaskova O, Majstorov V. Etiopathogenesis of Differentiated Thyroid Carcinomas. Open Access Maced J Med Sci. 2016 Sep 15; 4(3):517-522. http://dx.doi.org/10.3889/oamjms.2016.086

Keywords: Papillary thyroid carcinoma; Follicular thyroid carcinoma; radiation exposure; MAPK signalling network; PI3K-AKT pathway.

***Correspondence:** Tanja Makazlieva. Institute of Pathophysiology and Nuclear Medicine "Akademik Isak Tadzer", Medical Faculty, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia. E-mail: tmakazlieva@gmail.com

Received: 20-Jun-2016; **Revised:** 20-Jul-2016; **Accepted:** 22-Jul-2016; **Online first:** 02-Aug-2016

Competing Interests: The authors have declared that no competing interests exist.

Abstract

INTRODUCTION: Thyroid malignomas are a heterogeneous group of neoplasm consisting of most frequent differentiated encountered carcinomas, papillary and follicular thyroid carcinoma, then medullary thyroid carcinoma originating from neuroendocrine calcitonin-producing C-cells and rare forms of thyroid lymphomas arising from intrathyroidal lymphatic tissue, thyroid sarcomas and poorly differentiated anaplastic thyroid carcinoma. There are increasing numbers of epidemiological studies and publications that have suggested increased incidence rate of thyroid carcinomas. We have read, analysed and compare available reviews and original articles investigating different etiological factors in the development of thyroid carcinomas through Google Scholar and PubMed Database.

DISCUSSION: Aetiology involved in the development of thyroid carcinomas is multifactorial and includes external influences, as well as constitutional predispositions and genetic etiological factors. The actual effect of environmental and constitutional factors is on promoting genetic and epigenetic alterations which result in cell proliferation and oncogenesis. Until now are identified numerous genetic alterations, assumed to have an important role in oncogenesis, with MAPK and PI3K-AKT as crucial signalling networks regulating growth, proliferation, differentiation and cell survival/apoptosis.

CONCLUSION: This new molecular insight could have a crucial impact on diagnosis and also on improving and selecting an appropriate treatment to the patients with thyroid malignancies.

Introduction

Classification of thyroid malignomas

Thyroid malignomas (TM) are a heterogeneous group of neoplasm, which according to histopathological features are grouped in neoplasm originating from epithelial follicular cells, the differentiated thyroid carcinomas with the most frequent variants, papillary and follicular carcinoma and medullary thyroid carcinoma originating from neuroendocrine calcitonin-producing C-cells. Very rare forms of primary thyroid neoplasm are thyroid lymphomas arising from intrathyroidal lymphatic tissue and thyroid sarcomas, developing probably from cells of the intrathyroidal connective tissue [1, 2]. In separate category is poorly differentiated anaplastic thyroid carcinoma, which according to prognosis is one of the most aggressive tumours, but is very rare with only 1 – 2 % of all TM [3].

Papillary thyroid carcinoma (PTC) is the most common variant of TM, with the representation of around 80% according to the most studies, but this category of TM isn't homogenous. There are several histopathological variants of PTC described which are: typical variant, follicular variant, microcarcinoma, tall cell, oncocytic, columnar cell, diffuse sclerosing, solid, clear cell, cribriform morular, macrofollicular, PTC with fasciitis – like stroma, relatively rare and newly described Warthin – like PTC, mixed papillary and medullary carcinoma and papillary with dedifferentiation in anaplastic carcinoma [4-7].

Category of well-differentiated TM includes also follicular carcinoma (FC). According to appropriate chapter of *WHO Classification of Tumours of Endocrine Organs,* FC is defined as malignant epithelial tumours with follicular cell differentiation,

without the presence of specific diagnostic nuclear characteristics for PTC. Data from the study of Manuel Sobrinho-Simões et al. indicate declining incidence of this category TM from entirely not known reasons [8]. Frequent detection of follicular variant of PTC and worldwide conducted projects for correction of iodine deficiency are assumed main reasons for detected decline in the incidence rate of FC. According to the epidemiological data, FC is accounting for about 10% of all thyroid carcinomas, but the biggest diagnostic challenge is exact discrimination between follicular adenomas (FA) from minimally invasive follicular thyroid carcinomas and encapsulated follicular variant of PTC [8]. Among other variants of TM, FC includes also Oncocytic Hurthle cell variant [2, 9, 10].

Epidemiology

Epidemiological data indicate that TM are the most frequent endocrinological neoplasm and according to the latest evaluations they participate with around 1 % in all malignomas [11-13]. An epidemiological study from US National Cancer Institute од Surveillance, Epidemiology and End Results (SEER) program for the period 1975 to 2012 year registered 13.5 newly diagnosed cases of thyroid carcinomas per 100 000 people per year. The mortality rate from thyroid carcinomas was 0.5 deaths per 100 000 persons, per year and is estimated that approximately 1.1 % of the population will be diagnosed with TM at some point in life, extrapolated from statistical data for the American population for the period 2011 – 2012 [14]. According to the data published in guidelines of American Thyroid Association (ATA, 2015) incidence rate per year for well-differentiated thyroid carcinomas almost tripled from only 4.9/100 000 in 1975 to 14.3/100 000 in 2009. Analysis showed that almost all increase is due to the rise in the incidence rate of PTC thus 39% of the cases detected in 2008/09 were < 1cm [15]. Carlo La Vecchia et al. using available data from WHO, published detailed epidemiological study regarding incidence, prevalence and mortality rate of TM in countries from Europe, North, Central and South America and Asia. Main conclusions from the study were that the highest increase in mortality rate was detected in countries of Central America and Asia and in countries of East and Central Europe and lowest mortality in countries of Western Europe and North America. Valuable acknowledgement from this study was that there is a continuous decrease in overall mortality from TM, although in the same time increase in incidence rate of thyroid carcinomas in all countries included in epidemiological analysis through all continents was detected. Investigations were in the direction of detecting potential exposure to geographical risk factors responsible for the change in incidence and mortality rate. Some of the regions with the high mortality rate included territories with iodine deficiency in the past, with also highest incidence of benign thyroid disorders, goitre and benign thyroid nodules/adenomas, which are considered as one of the most significant risk factors for the development of TM [16]. Conclusions from this and similar studies suggest that increased incidence of thyroid carcinomas may be also due to the improvement in diagnostic tools and early detection of small microcarcinomas less than 1 cm in diameter. According to others one of the dominant risk factors is increased exposition to radiation, mostly due to inappropriate use in diagnostic and therapeutic purposes [17, 18].

Discussion

Environmental etiological factors

Etiological factors involved in the development of TM are multifactorial and include external influences and constitutional predispositions and genetic etiological factors [1]. Because of the heterogeneous character of this neoplasm, according to originating cells, histopathological features, genetic factors, differences in therapeutic protocols and prognosis, the focus of this review will be on the etiopathogenesis of well-differentiated thyroid carcinomas, originated from epithelial follicular cells. The most studied risk factor in the oncogenesis of TM is radiation exposition during childhood, confirmed in numerous publications that significantly increase the risk for development of thyroid carcinomas. Unfortunately first observations about the connection between radiation exposition of neck during childhood and increased risk for development of thyroid carcinomas are studies of children which in period from 1940 till 1950 underwent local irradiation treatment of the head and neck for infection and inflammation of tonsillar and nasopharyngeal region and also irradiation therapy of acne and thymus [19]. Long term follows up of this children, showed the frequent occurrence of nodules in the thyroid gland and an insignificant number of these children was diagnosed thyroid carcinoma [20, 21]. Later gathered data of population from Hiroshima and Nagasaki atomic bombing were in concordance with these first observations. Furukawa et al. published one of the most thorough analyses on this issue. His study is authentic and gives important contribution because of the fact that evaluation and follow-up of a large number of exposed subjects in period 1958 – 2005 were conducted including 105,401 exposed persons. Using linear dose – response model, increased the relative risk of 1.28 (95% confidence interval 0.95-2.7) for thyroid carcinoma after radiation exposition of 1 Gy was calculated at the age of 60 years, after acute exposition in childhood at 10 years age. Results of

this study showed that increased relative risk for development of thyroid carcinoma in a population that was exposed to radiation in early childhood; persist even after a long latent period of 50 years, although this relative risk is very low [22, 23]. Facts for the deleterious effect of radiation exposition in the development of thyroid carcinoma in population from Hiroshima and Nagasaki later were confirmed after Chernobyl nuclear power plant accident. Increased incidence rate was detected even in the first 3 – 4 years after the accident, especially in the youngest population in the age group up to the age of 4 years. Highest incidence increment of paediatric thyroid carcinomas was detected in the region of Chernobyl and in place Gomel in Belarus as a result of exposition to ^{131}I radioisotope and the additional predisposing factor was iodine deficiency of the population [24]. Schonfeld et al. suggested that latent period between exposition and manifestation of the disease was at least 5 years, while in the study of Cardis et al. incidence increment after irradiation in Chernobyl was detected after only 3 years. The relative risk for development of TM was highest after a period of 20 years, and it reduces after this period. The evaluated relative risk exists at mean exposition dose of 10 cGy and above this exposition dose up to 1500 cGy exists linear dependence between irradiation and risk for development of TM. Higher expositions above 1500 cGy are associated with a reduction in relative risk, probably because of the cytotoxic effect of this high exposition doses. Besides irradiation, important constitutional factor was the age of the children, it was detected that age above 15 years is accompanied with reduced risk for development of TM after exposition to irradiation [19].

Another environmental factor in the etiopathogenesis of TM is assumed to be iodine diet intake (nutritive iodine deficiency or sufficiency) [23]. It is well-known fact that low but also high intake of iodine may result in changes of TSH. Experimental animal studies showed that both conditions could be stimulating cancerogenic factors. Studies with prolonged nutritive iodine deficiency with simultaneous use of known cancerogenic agents such as chemical mutagens resulted in a significantly higher incidence of TM in experimental animal studies. According to this survey iodine deficiency has impact more like promoter, not like direct cancerogenic agent through inducing increase of TSH, and TSH stimulation stimulates autocrine/paracrine regulated thyroid EGF (Epidermal growth factor) stimulated proliferation, lowering in the same time TGFβ1 (Transforming growth factor β1), which acts as a negative factor of thyroid cell proliferation, which results in increased angiogenesis and promoting tumor growth [25]. A number of studies showed a high incidence of follicular and anaplastic carcinomas in population from iodine deficiency regions. Studies with increased iodine ingestion and risk of TM are inconclusive. Rossing et al. in a study conducted among Asian women originating from Philippines and Japan but living in America, detected higher incidence in those born in Asia and later settled in America in comparison to those born in America. Authors concluded that this difference in incidence rate may be the result of environmental influences (perhaps due to nutritive differences), which act in the later stages of cancerogenesis and that those influences are reversible [26].

Constitutional etiological factors

Pre-existing benign thyroid disease is also one of the risk factors for the development of TM. According to several case-control studies and prospective studies, both benign thyroid nodular/multinodular disease and goitre, autoimmune disorders (Graves's disease and Hashimoto disease) are described as conditions for increased risk for developing TM, although biases cannot be excluded [27].

Because of significant difference in incidence rate of TM between females and males after puberty and in reproductive period, it was suggested possible oestrogen effects in the development of thyroid carcinomas. Cheng GG et al, in their in vitro study, demonstrated that oestrogen receptors are present on thyroid cancer cell lines [28].

Obesity is another risk factor detected through numerous case-control studies. The exact pathophysiological mechanism is not entirely known, but an increase of TSH and interplay of TSH and insulin – like growth factor 1 inactivation of MAPK and PI3K pathways in obese patients may be enrolled in pathogenesis [29].

High incidence of papillary carcinoma is registered in patients with familial adenomatous polyposis and Cowden's disease (multiple hamartoma Sy). Thyroid carcinomas are mostly sporadic, but in 5% are with familial appearance, with determined few loci in predisposing genes in this families [30].

Genetic alterations

Further elucidation of oncogenesis of thyroid carcinomas can be achieved with recent developments in molecular biology and identification of genetic abnormalities in patients with TM. Some identified genetic alterations in patients with thyroid carcinomas are changes in tyrosine kinase domain of RET gene in 15 – 33 %, RAS mutations detected in 10% and B-RAF mutations in 40-60% of cases. Several molecular alterations were identified in last year's, which included genetic and epigenetic alterations in signalling pathways, like MAPK (Mitogen-activated protein kinases are known also as ERK – extracellular signal-regulated kinases) pathway responsible for tumour initiation and PI3K-AKT signalling pathway for progression and

dedifferentiation of thyroid carcinomas. Molecular mechanisms involved in the pathogenesis of TM can be divided into genetic and epigenetic alterations. Genetic alterations can be also classified as nuclear genetic mutations, genetic rearrangements and loss of heterogeneity, while epigenetic changes can be DNA mutilation, histone modification and genetic silencing through microRNA. Nuclear genetic mutations include activation of MAPK pathway. Activation of this pathway in thyroid carcinomas is through RET/PTC rearrangement, RAS mutations and BRAF mutations. MAPK pathway is a network of three kinases which successfully activates one another through sequential phosphorylation in response to various stimulating factors, like growth factors, cytokines, neurotransmitters, cellular stress and cell adhesion. This complex activating network participates in the regulation of numerous cellular processes, among which is control of cell growth, differentiation, cellular adaptation to chemical and physical stress. In RAF-RAS/MEK/ERK pathway signalling transduction starts with activation of small molecule GTPase RAS, through receptor tyrosine kinases, G – protein coupled receptors and/or integrins. These membrane proteins are a group of signalling complexes which activates RAS proteins through changes in RAS – GTP, active or RAS – GDP, inactive form. RAS mutations cause inactivation of GTP are resulting in permanent active RAS- GTP form. There are 3 isoforms of RAS: HRAS, KRAS and NRAS found in thyroid tumours. RAS mutations are usually found in follicular thyroid adenomas, suggesting that those mutations occur early in the development of premalignant lesions and additional genetic alteration can trigger malignant transformation to follicular adenomas. Presumably, KRAS and concomitant PTEN deletion can induce malignant transformation in the aggressive form of follicular thyroid carcinoma. Mutations and deletions of tumour suppressor PTEN gene are genetic alterations which activate PI3K-AKT pathway and are a genetic basis for follicular thyroid carcinoma in Cowden's Sy [31]. While RAS mutations are characteristic for follicular thyroid neoplasms, RET/PTC proto-oncogenes are almost exclusively found in PTC. Development of TM very similar to PTC in humans was detected in experimental models of mice with RET/PTC expression. There are variations in the prevalence of RET/PTC oncogenes in PTC, but the highest frequency was detected in tumours occurring in children after radiation exposition [32]. At least 10 different RET/PTC oncogenes are described as a result of the fusion of tyrosine kinase domain on RET 5' part of different genes. RET/PTC1 and RET/PTC3 are most common types of all rearrangement with >90% participation [33]. Another relatively often detected genetic alteration, which is experimentally proven in around 45% of PTC is T1799A transverse point mutation of BRAF (mutation of the gene for B- a type of RAF kinase), manifesting with an expression of BRAF - V600E mutated protein, resulting in successive activation of serine/threonine kinase. One extensive multicentric study showed a strong association of BRAF - V600E mutated protein with the worst prognostic outcome and more aggressive form of PTC, usually associated with tall cell variant of PTC and lost of iodine avidity and, because of that, resistance on radioiodine treatment [34].

Besides changes in MAPK pathway important for oncogenesis are changes in the PI3K-AKT signalling pathway, which stimulates cell proliferation. PTEN tumour suppressor gene (phosphate and tensin homolog), localised on the 10q23 chromosome is an inhibitor of this pathway, acting as a natural restrictor of cell proliferation, preventing the tumour growth [35]. Various changes, like mutations, deletion and silencing of this gene are described. TP53 is tumour suppressor gene localised to chromosome 17 and it is considered that dedifferentiation in the evolution of the tumours may be due to the mutations of this gene. Genetic rearrangement like translocation results in creating a new protein with oncogenic features. W Chien et al in their study *Molecular biology of thyroid cancer*, indicate the presence of chromosomal translocation t(2;3)(q13;p25), PAX8/PPARγ rearrangement, with a fusion of PAX8, thyroid-specific transcription factor to PPARγ, nuclear hormone receptor involved in differentiation of cells. This genetic rearrangement was found in 36% of FC (Follicular thyroid carcinoma), 11% of Follicular adenoma and 13 % of Follicular variant of PTC [36, 37]. Understanding of molecular processes contributed to the thorough investigation of molecular pathogenesis in well-differentiated thyroid carcinomas.

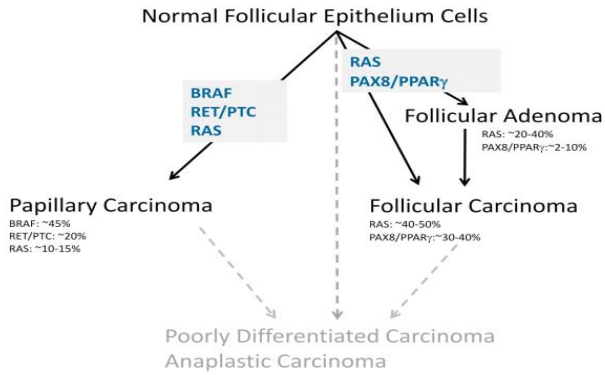

Figure 1: Molecular mechanisms in Thyroid carcinomas. (Asuragen Inc. White Paper: Molecular Pathogenesis of Thyroid Cancer)

In conclusion we can resume that in the development of TM complex mechanisms of interaction between environmental factors, radiation exposition, especially in early childhood, nutritive factors with particular emphasis on iodine intake, chemical mutagen agents and on the other hand internal constitutional predispositions age, sex, obesity and previous thyroid disorders as multinodular goiter could have impact. The actual effect of these environmental and constitutional factors is to promote

genetic and epigenetic alterations which result in cell proliferation and oncogenesis. Till now numerous genetic alterations are identified that have an important role in oncogenesis, with MAPK and PI3K-AKT being crucial signalling networks regulating growth, proliferation, differentiation and cell survival/apoptosis. Changes in a different segment of this pathways result in activation of oncogenesis in TM. Different disorders, such as inflammatory, immunological and degenerative processes, could initiate impairment of complex signalling networks and this new molecular insight could have a crucial impact on improving and selecting appropriate treatment for patients with TM [38].

References

1. De Groot LJ, Larsen PR, Hennemann G. The thyroid and its diseases, sixth edition. Churchill Livingstone, December 12, 1995.

2. Scopa CD. Histopathology of Thyroid Tumors. An Overview. Hormones. 2004;3(2):100-110. http://dx.doi.org/10.14310/horm.2002.11118 PMid:16982584

3. Kebebew E, Greenspan FS, Clark OH, Woeber KA, McMillan A. Anaplastic thyroid carcinoma. Treatment outcome and prognostic factors. Cancer. 2005;103(7):1330-5. http://dx.doi.org/10.1002/cncr.20936 PMid:15739211

4. Lloyd RV, Buehler D, Khanafshar E. Papillary thyroid carcinoma variants. Head Neck Pathol. 2011;5(1):51-6. http://dx.doi.org/10.1007/s12105-010-0236-9 PMid:21221869 PMCid:PMC3037461

5. Li Volsi VA. Papillary thyroid carcinoma: an update. Modern Pathology. 2011;24:S1–S9. http://dx.doi.org/10.1038/modpathol.2010.129 PMid:21455196

6. Girardi FM, Barra MB, Zettler CG. Variants of papillary thyroid carcinoma: association with histopathological prognostic factors. Braz J Otorhinolaryngol. 2013;79(6):738-44. http://dx.doi.org/10.5935/1808-8694.20130135 PMid:24474487

7. Al-Brahim N, Asa SL. Papillary Thyroid Carcinoma An Overview. Arch Pathol Lab Med. 2006;130:1057–1062. PMid:16831036

8. Sobrinho-Simoes M, Eloy C, Magalhães J, Lobo C, Amaro T. Follicular thyroid carcinoma. Modern Pathology. 2011;24:S10–S18. http://dx.doi.org/10.1038/modpathol.2010.133 PMid:21455197

9. McHenry CR, Phitayakorn R. Follicular Adenoma and Carcinoma of thyroid gland. The Oncologist. 2011;16:585-593. http://dx.doi.org/10.1634/theoncologist.2010-0405 PMid:21482585 PMCid:PMC3228182

10. D'Avanzo A, Treseler P, Ituarte PHG, Wong M, Streja L, Greenspan FS, et al. Follicular Thyroid Carcinoma: Histology and Prognosis. Cancer. 2004;100:1123–9. http://dx.doi.org/10.1002/cncr.20081 PMid:15022277

11. Ruggeri RM, Campenni A, Baldari S, Trimarchi F, Trovato M. What is new on thyroid cancer biomarkes. Biomark Insights. 2008;3:237-252. PMid:19578508 PMCid:PMC2688342

12. Burns WR, Zeiger MA. Differentiated thyroid cancer. Semin Oncol. 2010;37(6):557-66. http://dx.doi.org/10.1053/j.seminoncol.2010.10.008 PMid:21167375

13. Davies L, Welch HG. Increasing Incidence of Thyroid Cancer in the United States, 1973-2002. JAMA. 2006;295(18):2164-2167. http://dx.doi.org/10.1001/jama.295.18.2164 PMid:16684987

14. Howlader N, Noone AM, Krapcho M, Garshell J, Miller D, Altekruse SF, Kosary CL, Yu M, Ruhl J, Tatalovich Z, Mariotto A, Lewis DR, Chen HS, Feuer EJ, Cronin KA (eds). SEER Cancer Statistics Review, 1975-2012, National Cancer Institute. Bethesda, MD, http://seer.cancer.gov/csr/1975_2012/, based on November 2014 SEER data submission, posted to the SEER web site, April 2015.

15. Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, et al. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid [Internet]. Mary Ann Liebert Inc; 2016;26(1):1–133.

16. La Vecchia C, Malvezzi M, Bosetti C, Garavello W, Bertuccio P, Levi F, et al. Thyroid cancer mortality and incidence: A global overview. Int J Cancer. 2015;136:2187–2195. http://dx.doi.org/10.1002/ijc.29251 PMid:25284703

17. Moriss LG, Sikora AG, Tosteson TD, Davis L. The increasing incidence of thyroid cancer: the influence of access to care. Thyroid. 2013;23(7):885-91. http://dx.doi.org/10.1089/thy.2013.0045 PMid:23517343 PMCid:PMC3704124

18. Hodgson NC, Button J, Solorzano CC. Thyroid Cancer: Is the Incidence Still Increasing? Ann Surq Oncol. 2004;11(12):1093-97. http://dx.doi.org/10.1245/ASO.2004.03.066 PMid:15576834

19. Favus MJ, Schneider AB, Stachura ME, Arnold JE, Rio UY, Pinski SM, et al. Thyroid Cancer Occurring as a Late Consequence of Head-and-Neck Irradiation — Evaluation of 1056 Patients. N Engl J Med. 1976; 294:1019-1025. http://dx.doi.org/10.1056/NEJM197605062941901 PMid:1256510

20. Refetoff S, Harrison J, Karanfilski B, Kaplan EL, De Groot LJ, Bekerman C. Continuing Occurrence of Thyroid Carcinoma after Irradiation to the Neck in Infancy and Childhood. N Engl J Med. 1975; 292:171-175. http://dx.doi.org/10.1056/NEJM197501232920402 PMid:122783

21. Tucker MA, Morris Jones PH, Boice JD, Robison LL, Stone BJ, Stovall M, Jenkin RDT. Therapeutic Radiation at a Young Age Is Linked to Secondary Thyroid Cancer. Cancer Research. 1991; 51:2885-88. PMid:1851664

22. Furukawa K, Preston D, Funamoto S, Yonehara S, Ito M, Tokuoka S, et al. Long-term trend of thyroid cancer risk among Japanese atomic bomb survivors: 60 years after exposure. Int J Cancer. 2013; 132(5):1222–26. http://dx.doi.org/10.1002/ijc.27749 PMid:22847218 PMCid:PMC3910094

23. Schonfeld SJ, Lee C, de González AB. Medical exposure to radiation and thyroid cancer. Clin Oncol (R Coll Radiol). 2011;23(4):244-50. http://dx.doi.org/10.1016/j.clon.2011.01.159 PMid:21296564

24. Cardis E, Hatch M. The Chernobyl accident — an epidemiological perspective, Clin Oncol (R Coll Radiol). 2011;23(4): 251–260. http://dx.doi.org/10.1016/j.clon.2011.01.510 PMid:21396807 PMCid:PMC3107017

25. Knobel M, Medeiros-Neto G. Relevance of Iodine Intake as a Reputed Predisposing Factor for Thyroid Cancer. Arq Bras Endocrinol Metabol. 2007;51(5):701-12. http://dx.doi.org/10.1590/S0004-27302007000500007 PMid:17891233

26. Horn-Ross PL, Moriss JS, Lee M, West DW, Whittemore AS, McDougall IR, et al. Iodine and Thyroid Cancer Risk among Women in a Multiethnic Population: The Bay Area Thyroid Cancer Study. Cancer Epidemiol Biomarkers Prev. 2001;10(9):979–985. PMid:11535551

27. Schumberger M, Pacini F, Tutle RM. Thyroid tumors, Forth edition, Paris: Institute Medico-Educatif, 2015.

28. Chen GG, Vlantis AC, Zeng Q, Van Hasselt CA. Regulation of cell growth by estrogen signaling and potential targets in thyroid cancer. Curr Cancer Drug Targets. 2008;8:367-77. http://dx.doi.org/10.2174/156800908785133150 PMid:18690843

29. Belfiore A, La Rosa GL, La Porta GA, Giuffrida D, Milazzo G, Lupo L, et al. Cancer risk in patients with cold thyroid nodules: relevance of iodine intake, sex, age and multinodularity. AmJ Med. 1992;93:363-69. http://dx.doi.org/10.1016/0002-9343(92)90164-7

30. Schlumberg MJ. Papillary thyroid carcinoma. Orphanet encyclopedia, March 2004.

31. Xing M. Molecular pathogenesis and mechanisms of thyroid cancer. Nat Rev Cancer. 2013;13(3): 184-199. http://dx.doi.org/10.1038/nrc3431 PMid:23429735 PMCid:PMC3791171

32. Wiench M, Wloch J, Oczko M, Gubala E, Jarzab B. Rearrangement of the RET gene in papillary thyroid carcinoma. Wiad Lek. 2001;54(Suppl 1):64-71. PMid:12182064

33. Nikiforov YE. RET/PTC rearrangement in thyroid tumors. Endocr Pathol. 2002;13(1):3-16. http://dx.doi.org/10.1385/EP:13:1:03

34. Xing M. BRAF mutation in thyroid cancer. Endocr Relat Cancer. 2005;12(2):245-62. http://dx.doi.org/10.1677/erc.1.0978 PMid:15947100

35. Nozhat Z, Hedayati M. PI3K/AKT Pathway and Its Mediators in Thyroid Carcinomas. Mol Diagn Ther. 2016;20(1):13-26. http://dx.doi.org/10.1007/s40291-015-0175-y PMid:26597586

36. Chien W, Koeffler HP. Molecular Biology of Thyroid Cancer. G.D. Braunstein (ed.). Thyroid Cancer, Endocrine Updates 30, 35. Springer Science+Business Media, LLC 2012.

37. Brehar AC, Brehar FM, Dumitrache C, Parhon CI, Arseni B, Davila C. Genetic and epigenetic alterations in differentiated thyroid carcinoma. J Med Life. 2013;6(4):403-8. PMid:24868250 PMCid:PMC4034295

38. Orton RJ, Sturm OE, Vyshemirsky V, Calder M, Gilbert DR, Kolch W. Computational modelling of the receptor-tyrosine-kinase-activated MAPK pathway. Biochem J. 2005;392(Pt 2):249-61. http://dx.doi.org/10.1042/BJ20050908 PMid:16293107 PMCid:PMC1316260

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):523-525.
http://dx.doi.org/10.3889/oamjms.2016.087
eISSN: 1857-9655
Review Article

Venous Thromboembolism – Current Diagnostic and Treatment Modalities

Marijan Bosevski*, Elizabeta Srbinovska-Kostovska

University Cardiology Clinic, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia

Citation: Bosevski M, Srbinovska-Kostovska E. Venous Thromboembolism – Current Diagnostic and Treatment Modalities. Open Access Maced J Med Sci. 2016 Sep 15; 4(3):523-525. http://dx.doi.org/10.3889/oamjms.2016.087

Keywords: venous thromboembolism; pulmonary embolism; deep venous thrombosis treatment; novel oral anticoagulants.

***Correspondence:** Marijan Bosevski, MD, PhD. University Cardiology Clinic, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia. E- mail: marijanbosevski@yahoo.com

Received: 21-Jun-2016; **Revised:** 15-Jul-2016; **Accepted:** 22-Jul-2016; **Online first:** 24-Aug-2016

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: Pulmonary embolism and deep venous thrombosis, known as venous thromboembolism (VTE), are associated with a high proportion of morbidity and mortality.

AIM: Aim of this review is to emphasise current diagnostic and therapeutic modalities for VTE.

RESULTS: No differences have been noticed in European and American guidelines in diagnostic approach of this disorder. Today there is enough clinical information for the use of heparin (either, unfractionated or low molecular) and vitamin K antagonists in the treatment of acute and chronic phases of VTE. Novel oral anticoagulants seem to have some advantages in the treatment of this disorder. Rivaroxaban has been approved widespread, for use as a single-drug approach of VTE.

CONCLUSION: Both guidelines are almost similar and good basis for evidence-based treatment of this disorder.

Introduction

Pulmonary thromboembolism (PE) is a disorder of the pulmonary circulation because of the presence of thrombi. Thrombi are usually formed in the venous circulation (deep vein thrombosis or DVT). These two entities are known by the common term: venous thromboembolism.

The importance of VTE comes from its association with a high proportion of morbidity and mortality [1]. This disorder is often asymptomatic, misdiagnosed, unrecognised and untreated.

Diagnosis

The diagnosis is established on the basis of clinical presentation and risk factors present. Clinical signs and symptoms are often nonspecific, for which everyVTE is hardly familiar. The most common symptoms are loss of consciousness (due to a relative lack of oxygen supply to the brain), dyspnea or tachypnea (because of respiratory decline in vital capacity, atelectasis and vasoconstriction), chest pain (RH=right heart ischemia, pleural effusion), hemoptysis, (alveolar hemorrhage), and high temperature and cough (additional infection). Symptoms are present with the obstruction of more than 50 % of pulmonary circulation. Sudden cardiac death occurs in obstruction of the pulmonary arteries. This, in turn, produces large pulmonary peripheral resistance, triggers right heart failure and systemic hypotension, while isolated segmental and supsegmental VTE do not initiate these dramatic clinical symptoms [2].

The clinical probability for PE is based on the existence of risk factors and their significance. There

is not a universal cutpoint for D-dimer, it is assayed dependent D-dimer test is used as a test to exclude PE.

Doppler ultrasound of deep veins of the legs is a diagnostic test for the presence of VTE. Ventilation-perfusion lung scan is a proven diagnostic test for suspected PE with low sensitivity.

Echocardiography has a limited role in PE. However, it is used, where transfer for definitive imaging is not possible and thrombolysis is being considered. CT angiography (multislice) is a method of choice for the diagnosis of PE by direct evidence of a clot, breakdown charge pulmonary arterial branch. Pulmonary angiography by Seldinger is the gold standard, although is used less often. All mentioned diagnostic tools are part of the diagnostic algorithm for PE. Echocardiography is sufficient to diagnose PE in high-risk patients. CT angiography is completed when the patient is stabilised, or when it is available.

Differential diagnoses are noted as acute myocardial infarction, myocarditis or pneumothorax or specific conditions of right heart failure. Diagnosis of deep vein thrombosis is based on to the vascular ultrasound examinations. Vein ultrasound detects noncompresibiily of affected vein, loss of respiratory phasic signal and visualisation of thrombus [3].

Treatment

Rapid initiation of treatment is most important even when there is a suspicion of VTE [4, 5].

Acute treatment of patients with PE (without high risk) consists of the use of heparin (unfractionated or low-molecular) or fondaparinux. During this phase,a laboratory with blood tests is required, along with the values of activated thromboplastin or kaolin cefalin's time. Both heparins are known to be safe and as effective. In comparison with low-molecular-weight heparin, initial therapy with unfractionated heparin was associated with higher mortality and a higher rate of fatal pulmonary embolism in patients**.** The treatment continues with oral vitamin K anticoagulants (Acenocoumarol) in accordance with appropriate scheme value of INR (International proportion value to be between 2 and 3). Alternatively, treatment with Dabigatran or Rivaroxaban is equivalent in the long term phase of treatment.

High-risk PE is an indication, however,for quick treatment with fibrinolytic therapy.

Novel oral anticoagulants seem to have some advantages in the treatment of this disorder. Current Cohen's meta-analysis indicates that the NOACs have a clinical benefit over conventional therapy while compared to their relative differences in bleeding profile.

Rivaroxaban has been approved for widespread use as a single-drug approach of VTE. This drug is not inferior in the treatment of PE (not high risk) in its acute phase. In patients who had acute symptomatic proximal DVT, without symptomatic PE, Rivaroxaban showed: Non-inferiority for efficacy when compared with treatment regimen LMWH/VKA, same safety outcomes (irrespective of age, gender and body weight, renal function or presence of cancer) and no evidence of hepatic toxicity.

But, until today, there is no statement on which NOACs might be more beneficial (or have greater evidence) versus standard coagulation in the acute phase of VTE [6]. Rivaroxaban is approved as a medication for all indications in the treatment and prevention of venous thromboembolism, the prevention of VTE in patients undergoing major orthopaedic surgery, and for nonvalvular atrial fibrillation in order to prevent systemic peripheral embolism, including stroke.These recommendations are contained in the current guidelines of the European Society of Cardiology and the American Lung Physicians' Association. These noted here are based on EINSTEIN studies' results.

A comparison of VKA antagonist, NOAC and direct thrombin inhibitors is given in Table 1.

Table 1: Characteristics of VKA antagonist, NOAC and direct thrombin inhibitors [7]

	Vitamin K Antagonists	FXa Inhibitors			Direct Thrombin Inhibitors	
	Warfarin	Rivaroxaban	Apixaban	Edoxaban	Dabigatran	Ximelagatran
Mode of action	Inhibition of hepatic synthesis of vitamin K-dependent coagulation factors	Direct inhibition of FXa	Direct inhibition of FXa	Direct inhibition of FXa	Direct inhibition of clot-bound and free thrombin (FIIa)	Direct inhibition of thrombin (FII)
Time to peak effect (hours)	72–96	0.5–3	3	1.5	2–3	1.6–1.9
Half-life hours	20–60	5–9 (9–13 in elderly)	8–13	9–11	14–17	4–5
Bioavailability %	100	80	66	50	6.5	20
Recommended therapeutic dose and frequency	Adjusted-dose based on INR; once daily	20 mg; once daily	5 mg; twice daily	30 mg or 60 mg; once daily	150 mg; twice daily	Not available in the U.S.
Monitoring	Required using INR	Not required In case of hemorrhage or renal impairment, FXa-dependent assays may be used[27]	Not required due to predictable pharmacokinetics In hemorrhage or renal impairment, FXa-dependent assays may be used[27]	Not required due to predictable pharmacokinetics	Not required except in subgroups such as patients with renal impairment [28] Ecarin clotting time can be used if needed[29]	Not required
Renal excretion[30]	1% excreted unchanged in the urine	66% renal elimination	50% renal elimination	45% renal elimination	80% renal elimination	Main route of elimination
Interactions	CYP2C9, CYP1A2, CYP3A4 inhibitors Dietary vitamin K[31]	Potent CYP3A4 inhibitors and P-glycoprotein inhibitors[30]	Potent CYP3A4 inhibitors[32]	P-glycoprotein inhibitors[33]	P-glycoprotein inhibitors Proton pump inhibitors[34]	NA
Drug reversal	Vitamin K, fresh frozen plasma, prothrombin complex concentrate, recombinant FVIIa[35]	FVIIa partially reverses rivaroxaban anticoagulant effect[27] Prothrombin complex concentrate completely reverses its anticoagulant effect[27]	No available antidote	No available antidote	It is partially dialyzable[34]	NA
Precautions	Severe active bleeding, pregnancy, breast feeding, documented hypersensitivity[35] Severe renal impairment (glomerular filtration rate <30 mL/min/1.73m²)[36]	Severe active bleeding; severe renal impairment[30]	Severe active bleeding; severe renal impairment	Severe active bleeding; severe renal impairment	Severe active bleeding, severe renal impairment[30]	NA

VKA have some disadvantages as narrow therapeutic window, regular coagulation monitoring and dose adjustment, lack of staying in therapeutic range (INR 2-3) for all pts and consequent increase in the risk of thromboembolic events or side effects – bleeding, significant inter - and intra - individual variations in the response due to: numerous interactions with food and medicine and complex pharmacokinetics and pharmacodynamics.

Because of the foreseeable pharmacology and broad therapeutic window, Rivaroxaban is the drug of choice and replaced other anticoagulants.

The minimum duration of anticoagulation for VTE is 3 months (m); Till today, there is no evidence to suggest 6 m is more effective than 3 m. Duration of

treatment of patients with transient risk factor is up to 6 months. Long-term anticoagulant therapy is common in patients without apparent risk factors and those with proven thrombophilia (hypercoagulability). It is aimed for prevention of fatal and non- fatal recurrent VTE events taking into account the risk of bleeding.

Due to CHEST Guidelines, for VTE long-term anticoagulant therapy, rivaroxaban and another NOAC is suggested one, (Grade 2B), over vitamin K antagonist (VKA) therapy, and VKA therapy instead of low-molecular-weight heparin (LMWH; Grade 2C). For VTE and cancer, we suggest LMWH over VKA (Grade 2B), Rivaroxaban and other NOACS (Grade 2C) [8]. European Guidelines on pulmonary embolism is an evidence-based document for VTE with equivalent evidence for its treatment. A Macedonian recommendation for treatment of PE is an evidence-based document, coming from European Guideline.

Prognosis of venous thromboembolism depends on of disease severity, aetiology and timing from diagnosis to initiation of therapy. VTE pts with appropriate clot lysis have a better survival rate. Rivaroxaban, as a novel anticoagulant is approved for the acute and chronic phase of treatment of VTE. Patients' adherence according to CHEST guidelines was high and resulted in low rates of recurrent VTE and bleeding. The risk of VTE recurrence after discontinuation of therapy is primarily determined by two factors: whether the acute episode was treated effectively and internal risks of a new episode of VTE. All patients with present pulmonary hypertension have impaired quality of life and worse survival [9].

In conclusion, pulmonary embolism and deep venous thrombosis, also known as venous thromboembolism (VTE), are associated with the high proportion of morbidity and mortality. Current guidelines propose Computer Tomography angiography, Echocardiography, Venous ultrasound and D-dimmers test for diagnosis of VTE.

Early initiation of treatment of VTE is a crucial one. Rivaroxaban and other novel oral anticoagulant are effective in a treatment of venous thromboembolism, as traditional anticoagulants are. In that way, both guidelines are almost similar and good basis for evidence-based treatment of this disorder.

References

1. Monreal M, Mahé I, Bura-Riviere A, Prandoni P, Verhamme P, Brenner B, Wells PS, Di Micco P, Bertoletti L. Pulmonary embolism: Epidemiology and registries. Presse Med. 2015;44(12 Pt 2):e377-83. http://dx.doi.org/10.1016/j.lpm.2015.10.006 PMid:26547675

2. Skhiri M, Hunt SA, Denault AY, Haddad F. Evidence-Based Management of Right Heart Failure: a Systematic Review of an Empiric Field. Rev Esp Cardiol. 2010;63(4):451-71. http://dx.doi.org/10.1016/S0300-8932(10)70066-X

3. Jiménez D, de Miguel-Díez J, Guijarro R, Trujillo-Santos J, Otero R, Barba R, Muriel A, Meyer G, Yusen RD, Monreal M; RIETE Investigators.Trends in the Management and Outcomes of Acute Pulmonary Embolism: Analysis From the RIETE Registry. J Am Coll Cardiol. 2016;67(2):162-70. http://dx.doi.org/10.1016/j.jacc.2015.10.060 PMid:26791063

4. Kearon C, Akl EA, Ornelas J, Blaivas A, Jimenez D, Bounameaux H, Huisman M, King CS, Morris TA, Sood N, Stevens SM, Vintch JR, Wells P, Woller SC, Moores L. Antithrombotic Therapy for VTE Disease: CHEST Guideline and Expert Panel Report. Chest. 2016;149(2):315-52. http://dx.doi.org/10.1016/j.chest.2015.11.026 PMid:26867832

5. SEC Working Group for the ESC 2014 Guidelines on the Diagnosis and Management of Acute Pulmonary Embolism; Expert Reviewers for the ESC 2014 Guidelines on the Diagnosis and Management of Acute Pulmonary Embolism; SEC Clinical Practice Guidelines Committee. Comments on the 2014 ESC Guidelines on the diagnosis and management of acute pulmonary embolism. Rev Esp Cardiol (Engl Ed). 2015;68(1):10-6.

6. Moliterno DJ, Kristensen SD, De Caterina R. Therapeutic advances in thrombosis. Second edition. Wiley-Blackwel, 2013.

7. DeLoughery G. Hemostasis and Thrombosis. 3rd Edition. Spinger, 2015. http://dx.doi.org/10.1007/978-3-319-09312-3

8. Toth PP. Considerations for long-term anticoagulant therapy in patients with venous thromboembolism in the novel oral anticoagulant era. Vasc Health Risk Manag. 2016;12:23-34. http://dx.doi.org/10.2147/VHRM.S88088 PMid:26929637 PMCid:PMC4754098

9. Martinez K, Kosirog E, Billups SJ, Clark NP, Delate T, Witt DM. Clinical outcomes and adherence to guideline recommendations during the initial treatment of acute venous thromboembolism. Ann Pharmacother. 2015;49(8):869-75. http://dx.doi.org/10.1177/1060028015583892 PMid:25931575

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):526-532.
http://dx.doi.org/10.3889/oamjms.2016.103
eISSN: 1857-9655
Review Article

An Updated Mini Review of Vitamin D and Obesity: Adipogenesis and Inflammation State

Zujaja-Tul-Noor Hamid Mehmood, Dimitrios Papandreou*

College of Natural and Health Sciences, Zayed University, Abu Dhabi, United Arab Emirates

Citation: Hamid Mehmood Z-T-N, Papandreou D. An Updated Mini Review of Vitamin D and Obesity: Adipogenesis and Inflammation State. Open Access Maced J Med Sci. 2016 Sep 15; 4(3):526-532. http://dx.doi.org/10.3889/oamjms.2016.103

Keywords: 1,25(OH)2D3; obesity; adipocyte; inflammation.

***Correspondence:** Dimitrios Papandreou, PhD., Ed.M, M.S., RD. Associate Professor of Dietetics, CNHS, Zayed University, Abu Dhabi, UAE. Tel.: 00971 2 5993677. E-mail: Dimitrios.papandreou@zu.ac.ae

Received: 16-Aug-2016; **Revised:** 04-Aug-2016; **Accepted:** 05-Aug-2016; **Online first:** 06-Sep-2016

Competing Interests: The authors have declared that no competing interests exist.

Abstract

Vitamin D related research continues to expand and theorise regarding its involvement in obesity, as both hypovitaminosis D and obesity strike in pandemic proportions. Vitamin D plays an important role in immune system through Vitamin D Receptors (VDR), which are transcription factors located abundantly in the body. Due to this characteristic, it is potentially linked to obesity, which is a state of inflammation involving the release of cytokines from adipose tissue, and exerting stress on other organs in a state of positive energy balance. Research trials in the past couple of years and systematic reviews from SCOPUS and MEDLINE will be discussed. The role of Vitamin D throughout the lifespan (from fetal imprinting until older age), and in various other obesity mediated chronic conditions shall be highlighted. Various mechanisms attributed to the inverse relationship of Vitamin D and obesity are discussed with research gaps identified, particularly the role of adipokines, epigenetics, calcium and type of adipose tissue.

Introduction

This study reviews articles available on PUBMED, Scopus and Google using the following search keywords: (Vitamin D OR Ergocalciferol OR Cholecalciferol) AND (Leptin/ adiponectin/ VDR/ inflammation/ adiposity/ body fat/ weight). The search was limited to articles in the English language until June 2016. Selected articles were also used to identify further relevant studies. Most relevant research articles were included; usually, review articles and meta-analysis to present an analysed picture of clinical trials regarding relevant information.

Vitamin D is a micronutrient that is categorically non-essential due to the endogenous production in the body with the aid of specific ultraviolet rays, but it has now become an essential component of diet as Vitamin D deficiency now engulfs the world as a pandemic [1]. This idea was established many years ago as Vitamin D inadequacy was observed even among people living closer the equator [2]. Hence, regulations directing fortification of food is either implemented or are under consideration [3]. In the 2011 conference of Institute of Medicine (IOM), the Recommended Dietary Allowance (RDA) (average daily level of intake sufficient to meet the nutrient requirements of nearly all (97%-98%) healthy people) for Vitamin D was set at 600 IU/d for ages 1-70 years and 800 IU/d for ages 71 years and older [4]. According to the Institute of Medicine (IOM) Committee, the scientific evidence supports the key role of Vitamin D in skeletal health and extra-skeletal health; however, the extra-skeletal health outcomes are not yet consistent to establish a cause-and-effect relationship [4]. Doses to treat and/or maintain Vitamin D status are still subjective and research studying the needs across different life spans and conditions continue. Vitamin D status now conjoins many other health conditions after the discovery of Vitamin D binding proteins and their receptors in many tissues [5]. Vitamin D binding receptors (VDR) are transcription factor responsible for extensive biological responses. It is shown to play a role in cell

proliferation inhibition, cell maturation, immune system, and possibly colonic, breast and prostate cancer [6]. Vitamin D status is influenced by sun exposure, adiposity, body composition, race/ethnicity, and genetic factors, but these need elucidations through research, as suggested by IOM [6].

Vitamin D deficiency has been historically defined and recently recommended by the Institute of Medicine (IOM) as a 25(OH)D of less than 20 ng/ml. Vitamin D insufficiency has been defined as a 25(OH)D of 21–29 ng/ml. Vitamin D deficiency results in abnormalities in calcium, phosphorus, and bone metabolism. Specifically, vitamin D deficiency causes a decrease in the efficiency of intestinal calcium and phosphorus absorption of dietary calcium and phosphorus, resulting in an increase in PTH levels. Secondary hyperparathyroidism maintains serum calcium in the normal range at the expense of mobilising calcium from the skeleton and increasing phosphorus wasting in the kidneys. The PTH-mediated increase in osteoclastic activity creates local foci of bone weakness and causes a generalised decrease in bone mineral density (BMD), resulting in osteopenia and osteoporosis. Phosphaturia caused by secondary hyperparathyroidism results in a low normal or low serum phosphorus level. This results in an inadequate calcium-phosphorus product, causing a mineralisation defect in the skeleton. This results in an inadequate calcium-phosphorus product, causing a mineralisation defect in the skeleton [7].

A major health issue linked with Vitamin D is the growing obesity rate. World Health Organization states that in 2014 more than 1.9 billion adults were overweight, of which 600 million were obese. According to the Global Burden of Disease report of USA, the potentially avoidable risk factors to rising disease burden included high BMI and physical inactivity for the healthy years lost [8]. Healthy years measure the expected number of years a person of a certain age could live without disability or be free of any activity limitation. Despite increased levels of sufficient physical activity in male and female, only 9 countries experienced a decline in obesity rate in the United States (with statistically insignificant results) compared to increment in the rest of the counties in obesity during 2001 and 2009 [8]. Systematic analysis to study Global, regional and national prevalence of overweight and obesity in children and adults 1980-2013 did not report any significant decline in rate over the past 33 years, but only a slowdown in increase rate of overweight and obesity was observed just in developed countries [9]. This is not satisfactory, as it indicates that an increasing obesity trend endures in other countries. This demands special attention from the public health sector to search and address socio-economic implications in maintaining Vitamin D. To explain the deficiency of this fat-soluble vitamin in people with excessive adipocytes, the following possible mechanisms have been suggested: lower dietary intake; altered behavior that reduces cutaneous synthesis, reduced synthetic capacity, reduced intestinal absorption, altered metabolism, and sequestration in adipose tissue [10,11]. However, extensive research is needed to establish a cause-effect relationship and explain these factors under various conditions, because Vanlint concluded in his review that the evidence for vitamin D affecting fat mass and distribution is not yet compelling, and it is difficult to determine which effects are due to vitamin D itself and which are mediated via calcium when based on evidence from in vitro studies [11].

Finally, this article highlights how these public health issues of Vitamin D with its immune-related properties are associated with obesity, which is a state of low-grade inflammation. Various gaps identified by IOM committee is briefly reviewed, such as, non-skeletal health outcomes, epigenetic role, physiology and pathways of Vitamin D [4]. Researchers have previously reviewed data on this topic, [12, 14, 15], but due to constant addition of knowledge in this area we considered updating. Unlike some previous articles, we have concentrated on discussing Vitamin D and inflammatory properties in obesity, and further supplemented suggested mechanisms with clinical studies.

Obesity, a state of low-grade inflammation

Ectopic fat storage, due to overflow from adipose tissues upon fat overloading leads to the formation of foam cells from macrophages that engulf fat droplets during transportation or storage. Macrophages phagocytize lipid droplets in weight loss from adipocytes that could describe basic inflammatory nature of adipocytes. Hyperplasia and hypertrophy of adipocytes can cause mitochondrial and endoplasmic stress, which during fat overloading releases additional inflammatory cytokines other than adipokines, attracting more macrophages. During adipocytes hypertrophy TNF-α (Tumor Necrosis Factor- alpha), IL-6 (Interleukin-6), IL-1β (Interleukin-1 Beta), PG-E2 (Prostaglandin E2) expression is induced, adipocytes die and neutrophils, monocytes and T-Cells are persistently activated. CRP is also enhanced in the liver to respond to inflammatory cytokines, amplifying the cytokines pro-inflammatory effect. Hyperplastic adipocytes also induce genes TNF-α, interleukins (IL)-1, IL-6, monocyte chemoattractant protein-1 (MCP-1), and plasminogen activator inhibitor-1 (PAI-1) due to hypoxia from clustered formation, which are distant from the vasculature. Macrophages embedded in adipocytes phagocytize lipid droplets and engulf dead adipocytes that burst from high lipid accumulation, releasing reactive oxygen species and inducing further cellular stress [16]. The unfolded protein response (UPR) to

cope with Endoplasmic Reticulum (ER) stress promotes NF-κB (nuclear factor kappa-B) and JNK (c-Jun N-terminal kinases) inflammatory pathways due to increased protein demand such as in hyperglycemia, or during accumulation of misfolded protein. Excessive nutrient influx increases superoxide production and reactive oxygen species by mitochondria. The down-regulation of autophagy in the liver of lipid droplets in hepatocytes associated with obesity leads to accumulation of triglyceride, ER stress and Insulin resistance [17].

Visceral or subcutaneous fat in obesity-related inflammation is still questionable due to variability in results. A study investigating concentrations of pro-inflammatory enzymes presented higher concentration of IL-6 and IL-15 (Interleukin-15) in Subcutaneous Adipose Tissue (SAT) synthesis compared to Visceral Adipose Tissue (VAT). However, obesity was associated with VAT, since IL-6 and IL-15 were significantly more in obese individuals compared to normal-weight ones, whereas, the cytokines difference was not significant between two groups in SAT related cytokines expression [18]. On the other hand, some studies do suggest increased pro-inflammatory cytokines expression in SAT compared to VAT proposing its contribution to meta-inflammation [18, 19]. In conclusion, visceral fat compared to subcutaneous fat may cause metabolic abnormalities by secreting inflammatory adipokines, such as interleukin, tumour necrosis factor-α, macrophage chemoattractant protein-1, and resistin, which induce insulin resistance and diabetes and Vitamin D metabolic abnormalities.

Role of Vitamin D in obesity and suggested mechanisms

Research to explore the relationship between Vitamin D and obesity gains interest, because studies investigating obesity (a state of low-grade inflammation) and Vitamin D (with its role in immunity) indicate potential links. Many studies report changes in Vitamin D status with BMI changes. A change in serum Vitamin D levels as a function of adiposity/weight loss was noted over 1-2 years [20, 21]. An inverse relationship between Vitamin D and BMI was recognised in Mendelian randomization analysis, [22] and a link with abdominal visceral or subcutaneous adipose tissue was also recognised [23, 24]. In a meta-Analysis of observational studies up to April 2014 in PubMed/Medline, Vitamin D deficiency was prevalent in obese subjects irrespective of age, latitude and cut-offs defining vitamin D deficiency [14].

Another meta-analysis of literature focusing on the last 5 years proposed various mechanisms to discern body weight and Vitamin D relationship, which include: Vitamin D Receptor (VDR) polymorphism shown in transgenic mice and its overexpression in adipocytes that led to fatty acid β-oxidation, lipolysis and reduced energy metabolism; increased parathyroid hormone levels in Vitamin D deficiency that can increase adiposity by influx of calcium into adipocytes promoting lipogenesis; Vitamin D as “essential factor” in leptin depletion which may contribute to increased appetite and obesity in Vitamin D deficient conditions; and outdoor activity, food intake and exercise which can also influence Vitamin D levels as confounding factors [25].

Adipokines relationship with Vitamin D is studied due to their role in obesity. In vitro leptin, secretion by adipose tissue is powerfully inhibited by Vitamin D deficiency [12]. Although clinical trials showed an increase in serum leptin with Vitamin D supplementation, [26, 27] the clinical significance remains to be asserted [12, 28]. A significant effect of Vitamin D supplementation on adiponectin and leptin was not observed in a meta-analysis of 9 Randomized Controlled Trials (RCT) [29]. Serum changes in Vitamin D were significantly associated with plasma leptin levels, independent of plasma adiponectin concentrations. Further larger clinical trials and meta-analysis to effectively review these adipokines, especially focusing on obesity, are needed, as more meta-analysis could not be found.

The dose-response relationship between serum Vitamin D levels have changed and BMI showed a quadratic curve in a research involving various Vitamin D3 doses that suggested rate-limiting mechanism to avoid excessive formation of 1,25-(OH)2D3 (the active metabolite) [30]. The dose-response curves, although parallel, were noted for their difference between the curves, which was approximately 17.5 nmol/L lower for obese subjects compared to normal ones, and approximately 12.5nmol/L lower levels in overweight compared to normal-weight subjects. Extracellular pool size was suggested as the potential factor in this discrepancy rather than fat [29].

Vitamin D-metabolizing enzymes are expressed differently in Adipose tissue as well. There was decreased expression of the 25-hydroxylase CYP2J2 and the 1α-hydroxylase CYP27B1 (which converts $25(OH)D_3$ to the active $1,25(OH)_2D_3$) in Subcutaneous adipose tissue, and increased expression of CYP24A1 (which inactivates calcitriol binding and activating VDR) after weight loss [30].

Calcium-sensing receptors (CaSR) gene and protein expression were found similar in white adipose tissue of obese and control mice group. Obese group had lower serum vitamin D and amino acid concentrations, and significantly higher serum triglyceride (TG), total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), TNF-α, IL-6 and PTH levels, which suggests that Calcium-sensing receptors

function through allosteric regulation [31]. CaSR elevates pro-inflammatory cytokines in adipose tissue and decreases cyclic AMP, protein kinase An activity, hormone-sensitive lipase and adipose triglyceride lipase that are key players in the lipolytic pathway [32]. Low Calcium-induced 1,25-di$(OH)_2D_3$ secretion upregulates CaSR expression in adipose tissue, which is followed by an increase in [Ca2+] and reduced lipolysis, and possibly lipogenesis finally yielding fat accumulation in adipocytes. It was shown that higher BMI shows a greater increase in CaSR protein and thus more pro-inflammatory cytokines secreted from obese tissue [33].

Studies in relationship to Vitamin D and obesity in general population

Obesity in adults is not only of concern for their reduced productivity in life but it may also affect new lives. Observational studies and animal studies now propose and explore the mechanism about how maternal BMI and offspring adiposity from an early age are independently associated, and how in-utero environmental exposures increase susceptibility to obesity and are related to cardiometabolic disorders in later life [34, 35]. An increased risk of prenatal and early postnatal overweight in offspring (1st year of life) was found, which was attenuated by 4 years of age [36]. Vitamin D status during pregnancy could have an epigenetic role since it is not only pivotal in maternal skeletal maintenance and fetal skeletal development, but it could influence fetal “imprinting”, which can affect chronic disease susceptibility soon after birth [37]. Decreased placental expression of VDR in the placenta may be a contributing factor to the pathology of idiopathic FGR (Fetal Growth Restricted)-affected pregnancies [38]. Maternal vitamin D deficiency during pregnancy was associated with impaired lung development in 6-year-old offspring; neurocognitive difficulties at the age of 10, an increased risk of eating disorders in adolescence, and lower peak bone mass at the age of 20 after relevant covariates were adjusted. Randomised controlled trials with long-term follow-up of offspring are required to examine beneficence for offspring and to determine the optimal level of maternal serum 25(OH)D for fetal development [39].

Optimal Vitamin D level is also essential from the adolescent years until the old age that is needed for health benefits. Serum Vitamin D levels decline with puberty onset, and holds a higher risk for obesity, and much greater for Insulin Resistance in pre-pubertal children with suboptimal Vitamin D serum levels [40, 41]. Hypovitaminosis D in overweight or obese adults is registered in many studies, usually accompanied with other health conditions.[13] Mice on high-fat diet and low-fat diet was treated with calcitriol to demonstrate its effectiveness in reducing obesity-associated renal abnormality. Suggested mechanism was through reduction of cytokines, such as Toll-like Receptors (TLR) that are down-regulated by Vitamin D, hence reducing Interleukin-6 (IL-6) or by preventing abnormal growth of parathyroid hormone (PTH). The lipid droplets were found to be in a degenerative stage in mice fed High-Fat-Diet (HFD) with calcitriol treatment, which showed a causal relationship between calcitriol intake in renal tubules causing structural changes under HFD conditions [42]. A study that included people above 65 years of age suggested an increased risk of vitamin D deficiency in overweight and higher body fat percentages [43]. As previously mentioned, studies also support an inverse relationship between weight loss and Vitamin D serum changes. This is shown to be effective in eliminating obesity-related inflammation since significant reductions in levels of IL-6 were noted with intervention combining Vitamin D3 supplementation and weight-loss program [44]. Low serum 25(OH)D was found to be significantly associated with high serum IL-6 in overweight/obese children and with increased hs-CRP in obese children [45]. 1,25$(OH)_2D_3$ is also found to have a strong inhibitory effect on NFκB signalling in human adipocytes [46]. On the contrary, a meta-analysis conclusive of 13 RCT suggests that Vitamin-D supplementation does not affect inflammatory markers: CRP, TNF-a, IL-6 in overweight or obese subjects [47]. Some studies do not support any link between Vitamin D supplementation and obesity. Supplementation with vitamin D showed no effect on adiposity measures in adults [48]. An increase in serum levels of 25OHD or other inflammatory markers was not observed in overweight and obese youths with 150,000IU supplemented every 3 months, which demands investigation regarding potential dosage and frequency, [49] since another trial with dosage as low as 400IU up till 4800IU daily yielded serum changes when administered for 12 months [50].

Role of Vitamin D in other obesity mediated diseases

Chronic diseases are usually linked with obesity, which has been further explored in relation to Vitamin D status or to investigate the effectiveness of supplementation in attenuating related symptoms. Visceral obesity has been also found to be related with low levels of Vitamin D [51]. Visceral obesity is also highly correlated with Non-Alcoholic Fatty Liver disease (NAFLD) thus it is expected that Vitamin D is also related with NAFLD. A recent study in adults demonstrated the strong link between vitamin D and NAFLD [52]. The authors examined a total of 1081 adults and concluded that low vitamin D levels were

highly associated with NAFLD independent of visceral obesity in subjects with Diabetes or insulin resistance [52]. BMI was also strongly associated with plasma 25- hydroxy Vitamin D, [25(OH)D] and PTH concentrations with possible influence of plasma 25(OH)D in the pathogenesis of hypertriglyceridemia and atherogenic dyslipidemia through inflammation, because the association disappeared when uCRP (ultrasensitive C-Reactive Protein) was introduced as covariable [53]. 25(OH)D low levels and unfavourable lipid patterns have been also found in children [54]. No effect on β-cell function or insulin action in obese non-diabetic adolescents was observed upon administration of Vitamin D3 supplementation [55]. A systematic review provides evidence of the insignificant effect of supplementation with vitamin D on glucose and insulin metabolism in overweight and obese individuals but a positive influence on the serum concentration of 25(OH)D [47]. Serum 25(OH)D level in diabetic patients (Type 2) was found to be inversely correlated with monocyte adhesion to endothelial cells. $1,25(OH)_2D_3$ suppressed ER stress, [56] and promoted M1-predominant phenotype with lower endothelial adhesion. Vitamin D suppresses both subsets of monocytes, with M1 predominant, however, M1 is involved in advanced plaques compared to M2 in early stages in simple terms [57]. Real paradigms might be more complex and needs further research. A decrease in systolic blood pressure and adiposity in middle-aged subjects after a weight-loss intervention was observed with an increase in plasma 25(OH)D level [58]. Lower inflammatory profile, better insulin sensitivity, higher Vitamin D levels and IGF-1 (Insulin-like Growth Factor-1) in lean mass of obese patients recorded in the study suggest physical activity programs potential to create a better metabolic profile [59]. Hence, special programs to support a lifestyle that incorporate dietary changes and physical activity programs can be used to attain better Vitamin D levels with a range of other health benefits.

In addition, visfatin has been recently found to be associated with Vitamin D levels. Visfatin is a new adipokine involved in several processes. Visfatin plays an important role in inflammatory processes [60]. In a very recent study [61], the authors examined 50 patients with chronic hepatitis with elevated visfatin levels. After administration of vitamin D3 (15,000 IU)/weekly, the patients' visfatin levels were significantly reduced after a 12, 14, and 48-week period compared with the baseline data. The researchers concluded that Vitamin D supplementation may offer beneficial effects in reducing inflammation in these patients. More studies though are needed to elucidate these optimal effects.

In conclusion, the latest research on Vitamin D deficiency and obesity pandemic supports the role for Vitamin D in prevention and occurrence of obesity. Hence, public health sector needs to address the implying socio-economic aspects influencing Vitamin D status in order to prevent the burden of the disease which the possibly an outcome of Vitamin D deficiency. The adipokines secretion and inflammatory cytokines expression are importantly linked to Vitamin D metabolism. However, the mechanisms need further elucidation, as research is both equivocal and inadequate to establish a direct relationship in some cases. The role of visceral fat is stronger compared to subcutaneous fat in inflammation, related to obesity. The clinical trials identified in this paper usually involve Vitamin D supplementation in attaining sufficient Vitamin D levels, which indicates the need for trials to determine if same effects of Vitamin D can be observed with dietary sources too, as it is imperative in deciding on fortification of food across different socio-economic groups.

References

1. Kohlmeier M. Introduction. In: Kohlmeier M, Ed. Nutrient Metabolism: Structures, Functions, and Genes. 2nd ed. Academic Press, 2015: Pp. xxvii. http://dx.doi.org/10.1016/B978-0-12-387784-0.00016-X

2. Holick MF. The vitamin D deficiency pandemic and consequences for nonskeletal health: mechanisms of action. Mol Aspects Med 2008; 29(6): 361-8. http://dx.doi.org/10.1016/j.mam.2008.08.008 PMid:18801384 PMCid:PMC2629072

3. National Institute of Health 2014. Vitamin D Fact Sheet for Health Professionals. From: https://ods.od.nih.gov/pdf/factsheets/VitaminD-HealthProfessional.pdf. Accessed: January 2016.

4. Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium. Dietary Reference Intakes for Calcium and Vitamin D. Washington (DC): National Academic Press (US), 2011.

5. Ryan JW, Anderson PH & Morris HA. Pleiotropic Activities of Vitamin D Receptors - Adequate Activation for Multiple Health Outcomes. Clin Biochem Rev. 2015; 36(2): 53-61. PMid:26224895 PMCid:PMC4504155

6. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab. 2011; 96(1): 53-8. http://dx.doi.org/10.1210/jc.2010-2704 PMid:21118827 PMCid:PMC3046611

7. Hollick MF. Vitamin D Deficiency. N Engl J Med 2007;357:266-281. http://dx.doi.org/10.1056/NEJMra070553 PMid:17634462

8. Institute for Health Metrics and Evaluation. The State of US Health: Innovations, Insights, and Recommendations from the Global Burden of Disease Study. Seattle, WA: Institute for Health Metrics and Evaluation, 2013.

9. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2014; 384(9945):766-781. http://dx.doi.org/10.1016/S0140-6736(14)60460-8

10. Vanlint S. Vitamin D and obesity. Nutrients. 2013; 5(3): 949-56. http://dx.doi.org/10.3390/nu5030949 PMid:23519290 PMCid:PMC3705328

11. Hawkins R. Total 25-OH vitamin D concentrations in Chinese, Malays and Indians. Ann Lab Med. 2013; 33(2): 156-8.

http://dx.doi.org/10.3343/alm.2013.33.2.156 PMid:23479565 PMCid:PMC3589645

12. Koszowska A, Nowak J, Dittfeld A, Brończyk-Puzoń A, Kulpok A, Zubelewicz-Szkodzińska B. Obesity, adipose tissue function and the role of vitamin D. Cent Eur J Immunol. 2014; 2:260-264. http://dx.doi.org/10.5114/ceji.2014.43732 PMid:26155133 PMCid:PMC4440015

13. Pourshahidi L. Vitamin D and obesity: current perspectives and future directions. Proc Nutr Soc. 2014; 74(02):115-124. http://dx.doi.org/10.1017/S0029665114001578 PMid:25359323

14. Pereira-Santos M, Costa P, Assis A, Santos C, Santos D. Obesity and vitamin D deficiency: a systematic review and meta-analysis. Obes Rev. 2015; 16(4):341-349. http://dx.doi.org/10.1111/obr.12239 PMid:25688659

15. Zuk A, Fitzpatrick T, Rosella L. Effect of Vitamin D3 Supplementation on Inflammatory Markers and Glycemic Measures among Overweight or Obese Adults: A Systematic Review of Randomized Controlled Trials. PLoS One. 2016; 11(4):e0154215. http://dx.doi.org/10.1371/journal.pone.0154215 PMid:27116227 PMCid:PMC4846157

16. Tripathi YB, Pandey V. Obesity and endoplasmic reticulum (ER) stresses. Front Immunol. 2012; 3240. http://dx.doi.org/10.3389/fimmu.2012.00240

17. Chang YC, Hee SW, Hsieh ML, Jeng YM & Chuang LM. The Role of Organelle Stresses in Diabetes Mellitus and Obesity: Implication for Treatment. Anal Cell Pathol (Amst). 2015; 2015:972891. http://dx.doi.org/10.1155/2015/972891

18. Jonas MI, Kurylowicz A, Bartoszewicz Z, Lisik W, Jonas M, Wierzbicki Z et al. Interleukins 6 and 15 Levels Are Higher in Subcutaneous Adipose Tissue, but Obesity Is Associated with Their Increased Content in Visceral Fat Depots. Int J Mol Sci. 2015; 16(10): 25817-30. http://dx.doi.org/10.3390/ijms161025817 PMid:26516848 PMCid:PMC4632828

19. Spoto B, Di Betta E, Mattace-Raso F, Sijbrands E, Vilardi A, Parlongo RM et al. Pro- and anti-inflammatory cytokine gene expression in subcutaneous and visceral fat in severe obesity. Nutr Metab Cardiovasc Dis. 2014; 24(10): 1137-43. http://dx.doi.org/10.1016/j.numecd.2014.04.017 PMid:24984824

20. Gangloff A, Bergeron J, Pelletier-Beaumont E, Nazare JA, Smith J, Borel AL et al. Effect of adipose tissue volume loss on circulating 25-hydroxyvitamin D levels: results from a 1-year lifestyle intervention in viscerally obese men. Int J Obes (Lond). 2015; 39(11): 1638-43. http://dx.doi.org/10.1038/ijo.2015.118 PMid:26095245

21. Ceglia L, Nelson J, Ware J, Alysandratos KD, Bray GA, Garganta C et al. Association between body weight and composition and plasma 25-hydroxyvitamin D level in the Diabetes Prevention Program. Eur J Nutr. 2015 [Epub ahead of print]. http://dx.doi.org/10.1007/s00394-015-1066-z PMid:26525562

22. Vimaleswaran KS, Berry DJ, Lu C, Tikkanen E, Pilz S, Hiraki LT et al. Causal relationship between obesity and vitamin D status: bi-directional Mendelian randomization analysis of multiple cohorts. PLoS Med. 2013; 10(2): e1001383. http://dx.doi.org/10.1371/journal.pmed.1001383 PMid:23393431 PMCid:PMC3564800

23. Hannemann A, Thuesen BH, Friedrich N, Volzke H, Steveling A, Ittermann T et al. Adiposity measures and vitamin D concentrations in Northeast Germany and Denmark. Nutr Metab (Lond). 2015; 12(24). http://dx.doi.org/10.1186/s12986-015-0019-0

24. Kremer R, Campbell PP, Reinhardt T & Gilsanz V. Vitamin D status and its relationship to body fat, final height, and peak bone mass in young women. J Clin Endocrinol Metab. 2009; 94(1): 67-73. http://dx.doi.org/10.1210/jc.2008-1575 PMid:18984659 PMCid:PMC2630864

25. Yao Y, Zhu L, He L, Duan Y, Liang W, Nie Z et al. A meta-analysis of the relationship between vitamin D deficiency and obesity. Int J Clin Exp Med. 2015; 8(9): 14977-84. PMid:26628980 PMCid:PMC4658869

26. Ghavamzadeh S, Mobasseri M, Mahdavi R. The Effect of Vitamin D Supplementation on Adiposity, Blood Glycated Hemoglobin, Serum Leptin and Tumor Necrosis Factor-α in Type 2 Diabetic Patients. Int J Prev Med. 2014; 5(9):1091-8. PMid:25317290 PMCid:PMC4192769

27. Maggi S, Siviero P, Brocco E, Albertin M, Romanato G, Crepaldi G. Vitamin D deficiency, serum leptin and osteoprotegerin levels in older diabetic patients: an input to new research avenues. Acta Diabetol. 2013; 51(3):461-469. http://dx.doi.org/10.1007/s00592-013-0540-4 PMid:24356952

28. Dinca M, Serban M, Sahebkar A, Mikhailidis D, Toth P, Martin S et al. Does vitamin D supplementation alter plasma adipokines concentrations? A systematic review and meta-analysis of randomized controlled trials. Pharmacol Res. 2016; 107:360-371. http://dx.doi.org/10.1016/j.phrs.2016.03.035 PMid:27038530

29. Gallagher JC, Sai A, Templin I,Thomas & Smith L. Dose Response to Vitamin D Supplementation in Postmenopausal WomenA Randomized Trial. Ann Intern Med. 2012; 156(6): 425-37. http://dx.doi.org/10.7326/0003-4819-156-6-201203200-00005 PMid:22431675

30. Wamberg L, Christiansen T, Paulsen SK, Fisker S, Rask P, Rejnmark L et al. Expression of vitamin D-metabolizing enzymes in human adipose tissue -- the effect of obesity and diet-induced weight loss. Int J Obes (Lond). 2013; 37(5): 651-7. http://dx.doi.org/10.1038/ijo.2012.112 PMid:22828938

31. He Y, Perry B, Bi M, Sun H, Zhao T, Li Y et al. Allosteric regulation of the calcium-sensing receptor in obese individuals. Int J Mol Med. 2013; 32(2): 511-8. PMid:23740560

32. Cifuentes M, Fuentes C, Tobar N, Acevedo I, Villalobos E, Hugo E et al. Calcium sensing receptor activation elevates proinflammatory factor expression in human adipose cells and adipose tissue. Mol Cell Endocrinol. 2012; 361(1-2): 24-30. http://dx.doi.org/10.1016/j.mce.2012.03.006 PMid:22449852 PMCid:PMC3761973

33. He YH, Song Y, Liao XL, Wang L, Li G, Alima et al. The calcium-sensing receptor affects fat accumulation via effects on antilipolytic pathways in adipose tissue of rats fed low-calcium diets. J Nutr. 2011; 141(11): 1938-46. http://dx.doi.org/10.3945/jn.111.141762 PMid:21940515

34. Patel N, Pasupathy D & Poston L. Determining the consequences of maternal obesity on offspring health. Exp Physiol. 2015; 100(12); 1421-8. http://dx.doi.org/10.1113/EP085132 PMid:26464224

35. Penfold NC & Ozanne SE. Developmental programming by maternal obesity in 2015: Outcomes, mechanisms, and potential interventions. Horm Behav. 2015; 76; 143-52. http://dx.doi.org/10.1016/j.yhbeh.2015.06.015 PMid:26145566

36. Morales E, Rodriguez A, Valvi D, Iniguez C, Esplugues A, Vioque J et al. Deficit of vitamin D in pregnancy and growth and overweight in the offspring. Int J Obes (Lond). 2015; 39(1): 61-8. http://dx.doi.org/10.1038/ijo.2014.165 PMid:25189178

37. Hossein-nezhad A, Holick MF. Vitamin D for Health: A Global Perspective. Mayo Clin Proc. 2013; 88(7): 720-55. http://dx.doi.org/10.1016/j.mayocp.2013.05.011 PMid:23790560 PMCid:PMC3761874

38. Murthi P, Yong H, Ngyuen T, Ellery S, Singh H, Rahman R et al. Role of the Placental Vitamin D Receptor in Modulating Feto-Placental Growth in Fetal Growth Restriction and Preeclampsia-Affected Pregnancies. Front Physiol. 2016; 7:43. http://dx.doi.org/10.3389/fphys.2016.00043 PMid:26924988 PMCid:PMC4757640

39. Hart P, Lucas R, Walsh J, Zosky G, Whitehouse A, Zhu K et al. Vitamin D in Fetal Development: Findings From a Birth Cohort Study. Pediatrics. 2014; 135(1):e167-e173. http://dx.doi.org/10.1542/peds.2014-1860 PMid:25511121

40. Cediel G, Corvalán C, Aguirre C, de Roma-a D, Uauy R. Serum 25-Hydroxyvitamin D associated with indicators of body fat and insulin resistance in prepubertal chilean children. Int J Obes (Lond). 2015; 40(1):147-152. http://dx.doi.org/10.1038/ijo.2015.148 PMid:26264757

41. Cediel G, Corvalan C, Lopez de Romana D, Mericq V, Uauy R. Prepubertal Adiposity, Vitamin D Status, and Insulin Resistance. Pediatrics. 2016;138(1):e20160076-e20160076. http://dx.doi.org/10.1542/peds.2016-0076 PMid:27335379

42. Alkharfy KM, Ahmed M, Yakout SM & Al-Daghri NM. Effects of calcitriol on structural changes of kidney in C57BL/6J mouse model. Int J Clin Exp Med. 2015; 8(8): 12390-6. PMid:26550149 PMCid:PMC4612834

43. Oliai AS, van Dijk SC, Ham AC, Brouwer-Brolsma EM, Enneman AW, Sohl E et al. BMI and Body Fat Mass Is Inversely Associated with Vitamin D Levels in Older Individuals. J Nutr Health Aging. 2015; 19(10): 980-5. http://dx.doi.org/10.1007/s12603-015-0657-y

44. Duggan C, de Dieu Tapsoba J, Mason C, Imayama I, Korde L, Wang CY et al. Effect of Vitamin D3 Supplementation in Combination with Weight Loss on Inflammatory Biomarkers in Postmenopausal Women: A Randomized Controlled Trial. Cancer Prev Res (Phila). 2015; 8(7): 628-35. http://dx.doi.org/10.1158/1940-6207.CAPR-14-0449 PMid:25908506 PMCid:PMC4491001

45. Rodriguez-Rodriguez E, Aparicio A, Andres P & Ortega RM. Moderate vitamin D deficiency and inflammation related markers in overweight/obese schoolchildren. Int J Vitam Nutr Res. 2014; 84(1-2): 98-107. http://dx.doi.org/10.1024/0300-9831/a000197 PMid:25835240

46. Ding C, Wilding JP & Bing C. 1,25-dihydroxyvitamin D3 protects against macrophage-induced activation of NFkappaB and MAPK signalling and chemokine release in human adipocytes. PLoS One. 2013; 8(4): e61707. http://dx.doi.org/10.1371/journal.pone.0061707 PMid:23637889 PMCid:PMC3634852

47. Jamka M, Wozniewicz M, Jeszka J, Mardas M, Bogdanski P & Stelmach-Mardas M. The effect of vitamin D supplementation on insulin and glucose metabolism in overweight and obese individuals: systematic review with meta-analysis. Sci Rep. 2015; 516142. http://dx.doi.org/10.1038/srep16142

48. Chandler PD, Wang L, Zhang X, Sesso HD, Moorthy MV, Obi O et al. Effect of vitamin D supplementation alone or with calcium on adiposity measures: a systematic review and meta-analysis of randomized controlled trials. Nutr Rev. 2015; 73(9): 577-93. http://dx.doi.org/10.1093/nutrit/nuv012 PMid:26180255 PMCid:PMC4537389

49. Shah S, Wilson DM & Bachrach LK. Large Doses of Vitamin D Fail to Increase 25-Hydroxyvitamin D Levels or to Alter Cardiovascular Risk Factors in Obese Adolescents: A Pilot Study. J Adolesc Health. 2015; 57(1): 19-23. http://dx.doi.org/10.1016/j.jadohealth.2015.02.006 PMid:25873553

50. Gallagher JC, Peacock M, Yalamanchili V & Smith LM. Effects of vitamin D supplementation in older African American women. J Clin Endocrinol Metab. 2013; 98(3): 1137-46. http://dx.doi.org/10.1210/jc.2012-3106 PMid:23386641 PMCid:PMC3590472

51. Cheng S, Massaro JM, Fox CS, Larson MG, Keyes MJ, et al. Adiposity, cardiometabolic risk, and vitamin D status: the Framingham Heart Study. Diabetes. 2010; 59: 242–248. http://dx.doi.org/10.2337/db09-1011 PMid:19833894 PMCid:PMC2797928

52. Seo JiA, Eun CR, Cho H, Lee SK, You HG, Kim SG et al. Low vitamin D status is associated with nonalcoholic Fatty liver disease independent of visceral obesity in Korean adults. PLoS One. 2013; 9: 8(10):e75197.

53. Guasch A, Bullo M, Rabassa A, Bonada A, Del Castillo D, Sabench F et al. Plasma vitamin D and parathormone are associated with obesity and atherogenic dyslipidemia: a cross-sectional study. Cardiovasc Diabetol. 2012; 11:149. http://dx.doi.org/10.1186/1475-2840-11-149 PMid:23228198 PMCid:PMC3541071

54. Rusconi RE, De Cosmi V, Gianluca G, Giavoli C & Agostoni C. Vitamin D insufficiency in obese children and relation with lipid profile. Int J Food Sci Nutr. 2015; 66(2): 132-4. http://dx.doi.org/10.3109/09637486.2014.959902 PMid:25657099

55. Javed A, Vella A, Balagopal PB, Fischer PR, Weaver AL, Piccinini F et al. Cholecalciferol supplementation does not influence beta-cell function and insulin action in obese adolescents: a prospective double-blind randomized trial. J Nutr. 2015; 145(2): 284-90. http://dx.doi.org/10.3945/jn.114.202010 PMid:25644349

56. Riek AE, Oh J, Darwech I, Moynihan CE, Bruchas RR & Bernal-Mizrachi C. 25(OH) vitamin D suppresses macrophage adhesion and migration by downregulation of ER stress and scavenger receptor A1 in type 2 diabetes. J Steroid Biochem Mol Biol. 2014; 144 (Pt A):172-9.

57. Riek AE, Oh J, Sprague JE, Timpson A, de las Fuentes L, Bernal-Mizrachi L et al. Vitamin D suppression of endoplasmic reticulum stress promotes an antiatherogenic monocyte/macrophage phenotype in type 2 diabetic patients. J Biol Chem. 2012; 287(46): 38482-94. http://dx.doi.org/10.1074/jbc.M112.386912 PMid:23012375 PMCid:PMC3493893

58. Ibero-Baraibar I, Navas-Carretero S, Abete I, Martinez JA & Zulet MA. Increases in plasma 25(OH)D levels are related to improvements in body composition and blood pressure in middle-aged subjects after a weight loss intervention: Longitudinal study. Clin Nutr. 2015; 34(5): 1010-7. http://dx.doi.org/10.1016/j.clnu.2014.11.004 PMid:25434574

59. Fornari R, Francomano D, Greco EA, Marocco C, Lubrano C, Wannenes F et al. Lean mass in obese adult subjects correlates with higher levels of vitamin D, insulin sensitivity and lower inflammation. J Endocrinol Invest. 2015; 38(3): 367-72. http://dx.doi.org/10.1007/s40618-014-0189-z PMid:25352234

60. AL-Suhaimi EA, Shehzad A. Leptin, resistin and visfatin: the missing link between endocrine metabolic disorders and immunity. Eur J Med Res. 2013;18(12):1–13. http://dx.doi.org/10.1186/2047-783x-18-12

61. Sabry D, Al-Ghussein M, Hamdy G, Abul-Fotouh A, Motawi T, El Kazaz A et al. Effect of vitamin D therapy on interleukin-6, visfatin, and hyaluronic acid levels in chronic hepatitis C Egyptian patients. Clin Risk Manag. 2015; 11: 279–288. http://dx.doi.org/10.2147/TCRM.S66763 PMid:25737638 PMCid:PMC4344182

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2016 Sep 15; 4(3):533.
http://dx.doi.org/10.3889/oamjms.2016.070
eISSN: 1857-9655
Letter to the Editor

How to Verify and Manage the Translational Plagiarism?

Viroj Wiwanitkit*

Suvannhabhumi Clinical Training, Research and Development Center, Institute of Natural Medicine Science Development and Establishment Project, Surindra Rajabhat University, Surin Province, Thailand

Citation: Wiwanitkit V. How to Verify and Manage the Translational Plagiarism? Open Access Maced J Med Sci. 2016 Sep 15; 4(3):533. http://dx.doi.org/10.3889/oamjms.2016.070

Keywords: translational plagiarism; Google Translate tool; non-English reference; non-English article; publication ethics.

***Correspondence:** Professor Viroj Wiwanitkit. Wiwanitkit House, Bangkhae, Bangkok Thailand. Email: wviroj@yahoo.com

Received: 21-Jun-2016; **Revised:** 22-Jun-2016; **Accepted:** 23-Jun-2016; **Online first:** 24-Jun-2016

Competing Interests: The authors have declared that no competing interests exist.

Abstract

The use of Google translator as a tool for determining translational plagiarism is a big challenge. As noted, plagiarism of the original papers written in Macedonian and translated into other languages can be verified after computerised translation in other languages. Attempts to screen the translational plagiarism should be supported. The use of Google Translate tool might be helpful. Special focus should be on any non-English reference that might be the source of plagiarised material and non-English article that might translate from an original English article, which cannot be detected by simple plagiarism screening tool. It is a hard job for any journal to detect the complex translational plagiarism but the harder job might be how to effectively manage the case.

Dear Editor,

The recent article “How to Verify Plagiarism of the Paper Written in Macedonian and Translated in Foreign Language?” is quite interesting [1]. The use of Google translator as a tool for determining translational plagiarism is a big challenge. As noted, “Plagiarism of the original papers written in Macedonian and translated into other languages can be verified after computerised translation in other languages [1].” In fact, it is no doubt that translational plagiarism is not uncommon but usually forgotten [2]. This can be also in concordant with any form of copyright violation (such as copying of figure or graph or equation or table) [3]. The good example is my discussed case in Account Res, “Singapore Ped Soc. 1978; page 122–141 in English and *Chula Med J.* 1980; the page in Thai language” [4]. Attempts to screen the translational plagiarism should be supported. The use of Google Translate tool might be helpful. Special focus should be on any non-English reference that might be the source of plagiarised material and non-English article that might translate from an original English article, which cannot be detected by simple plagiarism screening tool. It is a hard job for any journal to detect the complex translational plagiarism but the harder job might be how to effectively manage the case. Sometimes the case is reported to the affiliation but there is no action.

References

1. Spiroski M. How to Verify Plagiarism of the Paper Written in Macedonian and Translated in Foreign Language? Open Access Maced J Med Sci. 2016;4(1):1-4. http://dx.doi.org/10.3889/oamjms.2016.035 PMid:27275319 PMCid:PMC4884226

2. Wiwanitkit V. Plagiarism in translated publication [Internet] BMJ. 2009;339:b3813. [cited 9 Sep 2014]. < http://www.bmj.com/rapid-response/2011/11/02/plagiarism-translated-publication>.

3. Wiwanitkit V. Letter to the editor: Plagiarism screening by the journal: is there still any pitfall? Account Res. 2015;22(3):198-9. http://dx.doi.org/10.1080/08989621.2014.922381 PMid:25635850

4. Wiwanitkit V. Plagiarism and copyright violation: two things in common. Prehosp Disaster Med. 2013;28(1):79. http://dx.doi.org/10.1017/S1049023X12001598 PMid:23137629