ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Jun 30; 7(12):1891-1895.
https://doi.org/10.3889/oamjms.2019.456
eISSN: 1857-9655
***Basic Science***

# Variation of *Resistin* Gene Is Correlated with Insulin Resistance in Obese People of Indonesia

Pramudji Hastuti[1*], Tasmini Tasmini[2], Rizki Fajar Utami[3], Meirlin Rambu Kaita Riwa[1], Steven Steven[1], Ahmad Hamim Sadewa[4]

*[1]Universitas Gadjah Mada, Yogyakarta, Indonesia; [2]Department of Biochemistry, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia; [3]Faculty of Medicine, Universitas Islam Indonesia, Yogyakarta, Indonesia; [4]Department of Biochemistry, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia*

**Citation:** Hastuti P, Tasmini T, Utami RF, Riwa MRK, Steven S, Sadewa AH. Variation of *Resistin* Gene Is Correlated with Insulin Resistance in Obese People of Indonesia. Open Access Maced J Med Sci. 2019 Jun 30; 7(12):1891-1895.
https://doi.org/10.3889/oamjms.2019.456

**Keywords:** Blood glucose; Insulin resistance; Obesity; Polymorphism; Resistin gene

***Correspondence:** Pramudji Hastuti. Universitas Gadjah Mada, Yogyakarta, Indonesia. E-mail: pramudji.has@ugm.ac.id

**Received:** 20-Mar-2019; **Revised:** 20-May-2019; **Accepted:** 21-May-2019; **Online first:** 19-Jun-2019

**Copyright:** © 2019 Pramudji Hastuti, Tasmini Tasmini, Rizki Fajar Utami, Meirlin Rambu Kaita Riwa, Steven Steven, Ahmad Hamim Sadewa. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

**Funding:** This research was financially supported by the Faculty of Medicine, Public Health and Nursing through Universitas Gadjah Mada through the funding of the Community Fund with number UPPM/241/M/04/05.15, Indonesia

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

**Abstract**

**BACKGROUND:** Obesity is considered associated with an increase of resistin levels that plays a role in the regulation of energy and maintaining fasting blood glucose. Polymorphism of resistin is thought to be correlated with the levels of resistin and insulin resistance.

**AIM:** This study aimed to examine the association of +299G > A and -420C > G resistin (RETN) gene with resistin level and insulin resistance in obese people of Indonesia.

**METHODS:** We examined 142 healthy unrelated subjects consisting of 71 obese and 71 controls. Fasting blood glucose was measured by the enzymatic method while the resistin and insulin levels were measured by Elisa method. Insulin resistance was calculated by HOMA-IR index. Polymorphisms of RETN genes were examined by the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method, and the data was tested. The data were correlated with Kruskal Wallis continue logistic regression and simple linear regression

**RESULTS:** In the obese group, there was an increased level of insulin (17.74 vs 11.27 mU/L) and insulin resistance (HOMA-IR 3.9 vs 1.46) compared to the control group. Polymorphism of +299G > A was associated with insulin resistance (GA and GA + AA genotype significantly different compare GG genotype with $P < 0.001$). Resistin level was negatively correlated with insulin level ($P = 0.017$).

**CONCLUSION:** In this study, polymorphism of +299G > A was identified as a risk factor for insulin resistance, and there was a significant association of serum resistin level with insulin level in the population of Indonesia.

## Introduction

Obesity is a chronic inflammatory state characterised by elevated pro-inflammatory adipokines [1]. Adipose tissue in obese subjects is characterised by increased macrophages infiltration, which is thought to play a role in the pathogenesis of pro-inflammatory events [2]. Resistin is one of the pro-inflammatory adipokines and has been associated with obesity. The expression of resistin increases with adipose differentiation [3]. Resistin plays an important role in regulating energy, glucose and fat homeostasis by modulating hepatic insulin action with single nucleotide polymorphisms (SNPs) [4].

Two-thirds of plasma resistin variations are influenced by genetics [5]. Resistin gene is on the 19p3.2 chromosome, consists of 4 exons and 3 introns. This gene is polymorphic in SNPs of promoters, introns and 3'UTR. The SNP promoter -420C/G (rs1862513) is associated with obesity [6], insulin sensitivity and resistance, elevated blood sugar

levels and lipid profile [7], [8]. However, results from some studies in various populations did not show any association of lipid profiles [5] and obesity [3]. The effect of SNP -420C > G on its expression was estimated due to its effect on the binding of a transcription factor on RETN promoter [8]. Research on the +299 *RETN* gene polymorphism showed +299G > A polymorphism was significantly associated with elevated plasma resistin [9]. Polymorphism of +299G > A *RETN* gene in a cohort study showed that A allele is associated with increased risk of Metabolic Syndrome [10], but in a study of Iranian patients with Gestational Diabetes Mellitus, this allele was not significantly different with controls [11].

This study aimed to correlate the polymorphisms of +299G > A (rs3745367) and -420C > G (rs1862513) *RETN* gene in obese Indonesian population with levels of resistin and insulin resistance.

## Methods

This research was a case-control study involving 142 healthy participants 18-40 years old, consisting of 71 subjects with Body Mass Index (BMI) ≥ 25 kg/m$^2$ as the obese group and 71 subjects with BMI 18.50 to 23 Kg/m$^2$ as the control group. Samples were taken with consecutive sampling.

All subjects had normal blood glucose concentrations. Subjects who consumed anti-diabetes and anti-inflammatory drugs were excluded from this research. All subjects agreed to participate and signed an informed consent form. This study was by the Helsinki Declaration and had approval from the Medical and Health Research Ethics Committee of the Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia, number: KE/FK/569/EC/2015.

### *Samples*

Blood was drawn from subjects after fasting 8 hours, and the plasma was separated from buffy coat. Fasting blood glucose, insulin and resistin levels were measured from plasma. DNA was isolated from buffy coat for determination of *RETN* polymorphism. Blood glucose was determined by the enzymatic method with the Dyasis kit. Resistin and insulin levels were measured with Elisa kit (Elabscience catalogue number: E-EL-H1213). Determination of insulin resistance was calculated by the following formula:

HOMA-IR = {insulin (μU/mL) X glucose (mmol/L)}/22.5

HOMA-IR > 2.0 was grouped as glucose intolerance [12].

Isolation of DNA was done with Promega kit, and genotyping of resistin genes was examined with the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) methods.

### *Genotyping of +299G > A RETN gene*

DNA was amplified with forwarding primer 5'GAGAGGATCCAGGAGGTCG 3' and reverse primer 5'GTGAGACCAAACGGTCCCT 3'. The PCR was done as follows:

Denaturation at 95°C for 5 min, pre-annealing at 59°C 1 min, elongation at 72°C for 2 min, then 35 cycles of 30 s at 95°C, 30 s at 59°C and 1 min 15 s at 72°C, and elongation at 72°C for 10 min. Product of PCR was 373 base pair (bp) then digested by AluI enzymes (MBI-Fermentas, United Kingdom) and separated by electrophoresis with 2% agarose gel and stained with ethidium bromide [13].

### *Genotyping of -420G > C RETN gene*

DNA was amplified with forwarding primer 5'-TGTCATTCTCACCCAGAGACA-3' and reversed primer 5'-TGGGCTCAGCTAACCAAATC-3'. The PCR method was as follows: denaturation at 95°C for 7 min, pre-annealing at 61°C for 1 min, elongation at 72°C for 2 min followed by 35 cycles of 30 s at 95°C, 30 s at 64°C and for 1 min 15 s at 72 °C and final elongation for 10 min at 72°C. The 534 bp PCR products were digested with BbsI enzyme at 37°C for 16 hours. The digestion products were separated by electrophoresis with 2% agarose gel. The G allele was visualised at 327 bp and C allele at 207 bp [14].

### *Statistical Analysis*

All statistical analyses were performed using SPSS 17 software. Variables that were not normally distributed were presented as mean with Interquartile Range (IQR). The associations of +299 G > A and -420 C > G *RETN* with resistin level were analysed with the Kruskal Wallis test. The associations of +299G > A and -420C > G *RETN* with insulin resistance were analysed with logistic regression. Serum resistin level associated with BMI and insulin resistance parameters were analysed with simple linear regression, and the results were considered significantly different when $p < 0.05$.

## Results

### *Characteristics of subjects*

The BMI, insulin level and HOMA-IR in the obese group were higher and significantly different

compared with the control group ($p < 0.05$) (Table 1).

**Table 1: Characteristic of subjects**

| Variable | Obese (n = 71) | Control (n = 71) | *P* |
|---|---|---|---|
| Sex | | | |
| Male | 30 (42.3%) | 25 (35.2%) | 0.389 |
| Female | 41 (57.7%) | 46 (64.8%) | |
| Age (Year) | 25.8 ± 5.9 | 25.4 ± 5.7 | 0.545 |
| BMI (kg/m$^2$) | 31.9 ± 4.02 | 20.86 ± 1.66 | < 0.0001 |
| FBG (mg/dL) | 89.10 ± 1.23 | 88.55 ± 1.15 | 0.830 |
| Insulin (mU/L) | 17.74 ± 1.88 | 11.27 ± 1.88 | < 0.0001 |
| HOMA-IR | 3.90 ± 2.04 | 1.46 ± 1.26 | < 0.0001 |
| Resistin (pg/mL) | 310.1 (1.07-1645.89) | 313.35 (1.10-1835.99) | 0.375 |

BMI: Body Mass Index; FBG: Fasting Blood Glucose; HOMA-IR: Homeostatic Model Assessment-Insulin Resistance. Data were presented as the mean ± standard deviation for normally distributed data. Data were presented as median (minimum-maximum) for the data not normally distributed.

The polymorphism of +299G > A *RETN* gene, frequency of GG, GA and AA genotypes were not significantly different ($p = 0.429$) and G allele compared to A allele between obese and control groups were not significantly different ($p = 0.719$). The frequency of CC genotype compared with other types (CG and GG) of -420 C > G *RETN* gene were not significantly different ($p = 0.583$) and C allele compared to G allele in obese, and control groups were not significantly different ($p = 0.905$) (Figure 1).

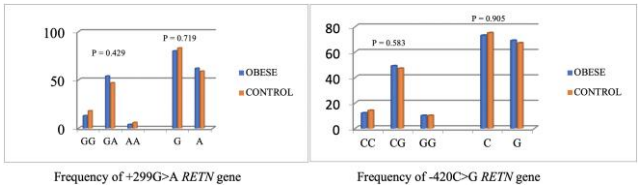



*Figure 1: Frequency of +299G > A and -420C > G genotypes and allele RETN gene in obese and control groups*

The association of resistin level with +299G > A and -420C > G *RETN* gene showed there were no significant differences in the obese and control groups (Table 2).

**Table 2: Level of Resistin in +299G>A and -420C>G *RETN* genotype in obese and control groups**

| | Resistin level (pg/mL) | | | *P* |
|---|---|---|---|---|
| +299G > A | GG | GA | AA | |
| Obese | 239.53 | 337.52 | 522.59 | 0.390 |
| (n = 71) | (390.47) | (555.75) | (1079.84) | |
| Control | 438.51 | 310.10 | 165.52 | 0.339 |
| (n = 71) | (292.55) | (562.05) | (370.21) | |
| -420C > G | CC | CG | GG | |
| Obese | 168.42 | 310.10 | 310.45 | 0.722 |
| (n = 71) | (708.32) | (2508.71) | (224.52) | |
| Control | 376.27 | 336.62 | 82.89 | |
| (n = 71) | (373.33) | (498.96) | (149.44) | 0.224 |

Data presented as median (IQR) because the data was not normally distributed.

The association between polymorphism of +299G > A and -420C > G *RETN* gene with insulin resistance showed GA genotypes compared with GG genotype were correlated with insulin resistance ($p < 0.001$), as well as GA+AA genotype, was correlated with insulin resistance ($p < 0.001$). Polymorphism of -420C > G *RETN* gene, however, was not correlated with insulin resistance ($p > 0.05$) (Table 3).

**Table 3: Association of +299G>A and -420C/G polymorphism of the *RETN* gene with insulin resistance**

| +299 *RETN gene* (GG compared with GA, AA and GA+AA) | Coefficient | Odds Ratio | 95% CI for OR | *P* |
|---|---|---|---|---|
| GA | 1.68 | 5.375 | (2.20 - 13.13) | < 0.001 |
| AA | 2.13 | 8.437 | (0.95 - 74.85) | 0.055 |
| GA + AA | 1.72 | 5.566 | (2.31 - 13.44) | < 0.001 |
| -420 *RETN* gene (CC compared with CG, GG and CG+GG) | | | | |
| CG | -0.43 | 0.649 | (0.20, 2.09) | 0.470 |
| GG | -0.86 | 0.424 | (0.10, 1.78) | 0.240 |
| CG + GG | -0.51 | 0.599 | (0.19, 1.89) | 0.383 |

Serum resistin levels were significantly associated with insulin level with simple linear regression and had negative coefficient ($p = 0.017$), which indicated there were increases of resistin levels that were correlated with decreases of insulin level (Tables 4).

**Table 4: Simple linear regression of the variables with resistin level**

| Variable | Coefficient | 95% CI | *P* | $R^2$ |
|---|---|---|---|---|
| BMI | 2.24 | (-8.84) – (13.32) | 0.721 | 0.001 |
| Blood glucose | 2.05 | (-2.24) – (6.33) | 0.342 | 0.007 |
| INSULIN | -6.33 | (-11.44) – (-1.16) | 0.017 | 0.040 |
| HOMA-IR | -19.13 | (-39.94) - (1.69) | 0.075 | 0.023 |

## Discussion

The results of this study show there were significant differences in the levels of insulin and HOMA-IR between obese compared with the controls. The polymorphism of +299G > A *RETN* gene was correlated with insulin resistance but not for the -420C > G *RETN* gene. In the association of resistin level to insulin level and insulin resistance, we found serum resistin levels were significantly correlated with insulin level but for HOMA-IR index.

HOMA-IR index in the obese group was higher than the control group like findings in some studies [5,15]. Resistin level between obese and control groups in this study did not differ significantly. This is in line with a study by Savage et al., found no relationship between adiposity and resistin mRNA expression in freshly adipocytes subjects with ranging BMI from 22 – 59 kg/m$^2$ and insulin resistance [16]. Resistin gene in humans is on chromosome 19p13.3 in an area that is not related to the vulnerability of obesity and insulin resistance [17].

One study by Kuminski [18] found expression of mRNA was not directly associated with expression of the protein, which, when modified both in the post-transcriptional and post-translational process can influence resistin levels. The relationship between the expression and secretion of resistin and other inflammatory markers, including IL-6, C- reactive protein (CRP) were reported in patients with severe inflammatory diseases. Mild inflammation due to obesity will interfere with the level of resistin [19]. The

differences of this result with others are probably due to differences in obesity grouping [20]. Additionally, differences in the environmental factors may be affecting the HOMA-IR index. Polymorphism of +299G > A was associated with insulin resistance showing GA and GA+AA genotypes were risk factors for insulin resistance compared with GG genotype, but the polymorphism of -420C > G genotype was not a risk factor for insulin resistance.

This finding was different from studies in Korea [21] and Thailand which showed no association between polymorphisms of +299G > A and -420C > G RETN gene with the parameters of the metabolic syndrome [8]. A Lebanon population showed +299G > A polymorphism was not involved in the pathogenesis of obesity and diabetes mellitus [17]. Research in Japan and Egypt indicated polymorphism of -420C > G *RETN* gene was associated with obesity [22], [23]. The differences in some of these studies may be because there were differences in ethnicity that cause unequal frequency in each population.

Polymorphism of +299G > A and -420C > G *RETN* gene in this study was not associated with levels of resistin and is in line with research in China [24]. This result is in contrast with other studies showing this polymorphism is associated with increased levels of resistin in some East Asian populations, including Japanese, Korean, and Chinese, although data from the Caucasian population shows that this polymorphism is not associated with higher levels of resistin [1]. *RETN* polymorphism is correlated to increased BMI, blood pressure, fasting blood glucose, HOMA-IR, triglycerides, total cholesterol, resistin level, and decreased in HDL-cholesterol [5].

The results of research in non-obese type 2 diabetes mellitus patients showed the polymorphism of AA/AG genotypes of +299G > A *RETN* gene is considered as a risk factor for increased insulin resistance, based on fasting blood glucose and HOMA-IR index [13]. These results demonstrate ethnicity as a factor underlying the differences of SNPs in the influencing of human resistin expression [19]. In this study, polymorphism of +299G > A *RETN* gene was associated with insulin resistance. This result is similar to research in Iraq which showed that polymorphism *RETN* +299G > A gene plays a role in insulin resistance [25] and this polymorphism was found to increase the risk of diabetes mellitus in a population in Thailand [8]. Polymorphism of -420C > G *RETN* gene in this study was not associated with insulin resistance. This result is similar to a study in the Han population of China showing polymorphism of the *RETN* gene is not a risk factor for diabetes mellitus [26]. A study by Wen [27] in China reported the opposite results, finding this polymorphism increases the risk of diabetes mellitus.

Obesity is a polygenic effect that is influenced by a combination of several genes and environmental factors. Frequency of *RETN* gene varies with ethnicity and environment. Exposure to different environments such as dietary habits and genetic background other than differences in study design may cause different effects on obesity [5]. These differences probably cause the different results in studies concerning the influence of the polymorphism of the *RETN* gene in the pathogenesis of obesity along with the small number of samples that might contribute to differences in results. Additional research is needed to identify factors associated with inflammation due to obesity and the correlation of resistin levels with polymorphisms of genes should be further investigated.

In conclusion, polymorphism of +299G > A of *RETN* gene may be considered as a risk factor for insulin resistance but not the -420C > T of *RETN* gene. The level of resistin is correlated with the insulin level in Indonesia.

## Acknowledgement

We would like to thank the Faculty of Medicine, Public Health and Nursing through Universitas Gadjah Mada which support this research through funding of the Community Fund with number UPPM/241/M/04/05.15, Indonesia.

## References

1. Zhu ZL, Yang QM, Li C, Chen J, Xiang M, Chen MM, et al. Association between the resistin gene -420C>G polymorphism and obesity: an updated meta-analysis. Eur Rev Med Pharmacol Sci. 2016; 20(23):4922-4929.

2. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante Jr. AW. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest. 2003; 112(12):1796-1808. https://doi.org/10.1172/JCI200319246 PMid:14679176 PMCid:PMC296995

3. Beckers S, Zegers D, Van Camp JK, Boudin E, Nielsen TL, Brixen K. Resistin polymorphisms show associations with obesity, but not with bone parameters in men: results from the Odense Androgen Study. Mol Biol Reports. 2013; 40(3):2467-2471. https://doi.org/10.1007/s11033-012-2327-z PMid:23203410

4. Osawa, H, Yamada K, Onuma H, Murakami A, Ochi M, Kawata H, et al. The G/G genotype of a resistin single-nucleotide polymorphism at−420 increases type 2 diabetes mellitus susceptibility by inducing promoter activity through specific binding of Sp1/3. Am J Hum Genet. 2004; 75(4):678-686. https://doi.org/10.1086/424761 PMid:15338456 PMCid:PMC1182055

5. Menzaghi C, Coco A, Salvemini L, Thompson R, Cosmo S De, Doria A, et al. Heritability of serum resistin and its genetic correlation with insulin resistance-related features in nondiabetic Caucasians. J Clin Endocrinol Metab. 2006; 91(7):2792-5. https://doi.org/10.1210/jc.2005-2715 PMid:16670163

6. Hivert MF, Manning AK, McAteer JB, Dupuis J, Fox CS, Cupples

LA, et al. Association of variants in RETN with plasma levels and diabetes-related traits in the Framingham Offspring Study. Diabetes. 2009; 58(3):750-6. https://doi.org/10.2337/db08-1339 PMid:19074981 PMCid:PMC2646076

7. Wang H, Chu WS, Hemphill C, Elbein SC. Human resistin gene: molecular scanning and evaluation of association with insulin sensitivity and type 2 diabetes in Caucasians. J Clin Endocr Metab. 2002; 87(6):2520-4. https://doi.org/10.1210/jcem.87.6.8528 PMid:12050208

8. Suriyaprom K, Tungtrongchitr R, Namjuntra P. Association of resistin levels with resistin gene polymorphism and metabolic syndrome in Thais. J Med Biochem. 2015; 34(2):170-178. https://doi.org/10.2478/jomb-2014-0034 PMid:28356829 PMCid:PMC4922327

9. Asano H, Izawa H, Nagata K, Nakatochi M, Kobayashi M, Hirashiki A, et al. Plasma resistin concentration determined by common variants in the resistin gene and associated with metabolic traits in an aged Japanese population. Diabetologia. 2010; 53(2):234-46. https://doi.org/10.1007/s00125-009-1517-2 PMid:19727657

10. Tsukahara T, Nakashima E, Watarai A, Hamada Y, Naruse K, Kamiya H. Polymorphism in resistin promoter region at-420 determines the serum resistin levels and may be a risk marker of stroke in Japanese type 2 diabetic patients. Diabetes Res Clin Pr. 2009; 84(2):179-186. https://doi.org/10.1016/j.diabres.2008.10.021 PMid:19269054

11. Takhshid MA, Zare Z, Resistin - 420 C/G polymorphism and serum resistin level in Iranian patients with gestational diabetes mellitus, J Diabetes & Met Disorders. 2015; 14:37. https://doi.org/10.1186/s40200-015-0165-y PMid:25945322 PMCid:PMC4419403

12. Khalil O, Alnahal A, Ghonium M, Fawzy S, Ibrahem M, Raafat N. Does resistin gene polymorphisms + 299 (G>A) participate in insulin resistance in Egyptian non-obese Type 2 Diabetes? Int J Genomic Med. 2014; 2(1):1-7.

13. Wongwananuruk T, Rattanachaiyanont M, Leerasiri P, Indhavivadhana S, Techatraisak K, Angsuwathana S. The usefulness of homeostatic measurement assessment -insulin resistance (HOMA-IR) for detection of glucose intolerance in Thai women of reproductive age with polycystic ovary syndrome. Int J Endocrinol. 2012; 2012:571035. https://doi.org/10.1155/2012/571035 PMid:22737168 PMCid:PMC3378956

14. Kunnari A, Ukkola O, Kesäniemi YA. Resistin polymorphisms are associated with cerebrovascular disease in Finnish Type 2 diabetic patients. Diabet Med. 2005; 22(5):583-9. https://doi.org/10.1111/j.1464-5491.2005.01480.x PMid:15842513

15. Lim SM, Choi DP, Rhee Y, Kim HC. Association between obesity indices and insulin resistance among healthy Korean adolescents: The JS High School Study. PloS One. 2015; 10(5):e0125238. https://doi.org/10.1371/journal.pone.0125238 PMid:25970186 PMCid:PMC4429969

16. Savage D, Sewter C, Klenk E, Segal D, Vidal-Puig A, Considine R et al. Resistin/Fizz3 expression in relation to obesity and peroxisome proliferator-activated receptor gamma action in humans. Diabetes. 2001; 50(10):2199-2202. https://doi.org/10.2337/diabetes.50.10.2199 PMid:11574398

17. Fakhoury R. The study of the association of the resistin gene RETN polymorphism with obesity and T2DM in Lebanese diabetic subjects. J Mol Biomark Diagn. 2013; 4:3.

18. Kusminski CM, Ternan PG, Kumar S. Role of resistin in obesity, insulin resistance and Type II diabetes. Clin Sci (Lond). 2005 Sep; 109(3):243-56. https://doi.org/10.1042/CS20050078 PMid:16104844

19. Huang X, Yang Z. Resistin's, obesity and insulin resistance: the continuing disconnect between rodents and humans. J Endocrinol Invest. 2016; 39(6):607-615. https://doi.org/10.1007/s40618-015-0408-2 PMid:26662574

20. Piestrzeniewicz K, Łuczak K, Komorowski J, Maciejewski M, Wika JJ, Goch JH, Resistin increases with obesity and atherosclerotic risk factors in patients with myocardial infarction , Metabolism Clin and experiment. 2008, (57): 488–493 https://doi.org/10.1016/j.metabol.2007.11.009 PMid:18328349

21. Cho YM, Youn BS, Chung SS, Kim KW, Lee HK, Yu KY. Common genetic polymorphisms in the promoter of resistin gene are major determinants of plasma resistin concentrations in humans. Diabetologia. 2004 Mar; 47(3):559-65. https://doi.org/10.1007/s00125-003-1319-x PMid:14740159

22. Miyamoto Y, Morisaki H, Kokubo Y, Yamanaka I, Tomoike H,Okayama A, Yoshimasa Y, Morisaki T. Resistin gene variations are associated with the metabolic syndrome in Japanese men, Obes Res Clin Pract. 2009; 3(2):65-74. https://doi.org/10.1016/j.orcp.2008.11.003 PMid:24345560

23. El-Sayed Moustafa JS, Froguel P. From obesity genetics to the future of personalized obesity therapy. Nat Rev Endocrinol. 2013; 9(7):402-13. https://doi.org/10.1038/nrendo.2013.57 PMid:23529041

24. Chen BH, Song Y, Ding EL, Manson JE, Roberts CK, Rifai N. Association of resistin promoter polymorphisms with plasma resistin levels and type 2 diabetes in women and men. Int J Mol Epidemiol Genet. 2010; 1(3):167-174.

25. Al-hilali HA, Abduljaleel AK. The role of TNF and Resistin Gene + 299 (G/A) polymorphism in the development of insulin resistance in non-obese Type 2 Diabetes Mellitus Iraqi patients. Int J Curr Microbiol App Sci. 2015; 4(10):475-486.

26. Jiang B, Liu Y, Liu Y, Fang F, Wang X, Li B. Association of four insulin resistance genes with type 2 diabetes mellitus and hypertension in the Chinese Han population. Mol Biol Rep. 2014; 41(2):925-33. https://doi.org/10.1007/s11033-013-2937-0 PMid:24414038 PMCid:PMC3929032

27. Wen Y, Lu P, Dai L. Association between resistin gene -420 C / G polymorphism and the risk of type 2 diabetes mellitus: a meta-analysis. Acta Diabetol. 2013; 50(2):267-72. https://doi.org/10.1007/s00592-010-0247-8 PMid:21190046

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Jun 30; 7(12):1896-1899.
https://doi.org/10.3889/oamjms.2019.482
eISSN: 1857-9655
***Basic Science***

# Distribution of *Clostridium Difficile* Ribotypes in Macedonian Patients and their Antimicrobial Susceptibility

Kiril Mihajlov*, Aneta Andreska, Nadica Ristovska, Tatjana Grdanoska, Elena Trajkovska-Dokic

*Institute of Microbiology and Parasitology, Medical Faculty, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia*

**Citation:** Mihajlov K, Andreska A, Ristovska N, Grdanoska T, Trajkovska-Dokic E. Distribution of *Clostridium Difficile* Ribotypes in Macedonian Patients and their Antimicrobial Susceptibility. Open Access Maced J Med Sci. 2019 Jun 30; 7(12):1896-1899. https://doi.org/10.3889/oamjms.2019.482

**Keywords:** C. difficile; Toxigenicity; Ribotype; Antimicrobial susceptibility

***Correspondence:** Kiril Mihajlov. Institute of Microbiology and Parasitology, Medical Faculty, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia. E-mail: ki_mi_81@yahoo.com

**Received:** 16-Apr-2019; **Revised:** 22-May-2019; **Accepted:** 23-May-2019; **Online first:** 30-Jun-2019

**Copyright:** © 2019 Kiril Mihajlov, Aneta Andreska, Nadica Ristovska, Tatjana Grdanoska, Elena Trajkovska-Dokic. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

**Funding:** This research did financially supported by a grant from the Federation of European Microbiological Societies (FEMS), ID#: FEMS-RG-2016-0100

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

**Abstract**

**BACKGROUND:** *Clostridium difficile* is a major nosocomial pathogen. In Europe, this bacterium is mostly characterised by PCR ribotyping. Most of the *Clostridium difficile* infections (CDI) are treated with vancomycin or metronidazole, although prolonged antibiotic use is considered as one of the main risk factors for CDI.

**AIM:** This study aimed to detect the presence of various *C. difficile* ribotypes in hospitalised patients and to investigate their toxigenicity and antibiotic susceptibility.

**MATERIAL AND METHODS:** All stool samples obtained from each patient were inoculated on Columbia blood agar and cycloserine cefoxitine fructose agar (CCFA) for isolation of *C. difficile*. Glutamate dehydrogenase and toxins A and B were investigated by immunochromatographic tests. Final confirmation of the isolates was performed by Vitek 2 and MALDI-TOF. A total of 21 isolates were collected for further investigation. PCR ribotyping was performed as described by Janezic and Rupnik. PCR ribotype profiles were analysed using software (Bionumerics, Applied Maths). Antibiotic susceptibility was determined by E-tests for metronidazole, vancomycin, tetracycline, clindamycin, erythromycin, imipenem, ciprofloxacin and moxifloxacin.

**RESULTS:** About 48% of *C. difficile* isolates belonged to ribotype 001/072. So, this ribotype was the most common ribotype in this study. The remaining 52% of *C. difficile* isolates consisted of 10 different ribotypes: 017, SLO 160, SLO 187, SLO 120, 255/258, 014/020, 046, 002, 070 and 027. Furthermore, 20 (95.2 %) out of 21 isolates of *C. difficile* were toxigenic. Toxins A and B were detected simultaneously in 90.5 % of *C. difficile* isolates. Two isolates from the ribotype 017 were toxin B positive only. Treatments with any of the following antimicrobials: clindamycin, erythromycin, ciprofloxacin and moxifloxacin (as well as many other antibiotics), could be a risk factor for CDI due to the high resistance of the strains in this study. About 90% of the strains from the most common ribotype 001/072 have MICs for clindamycin and erythromycin > 256 μg/ml.

**CONCLUSION:** All strains isolated are highly resistant to ciprofloxacin. All strains were susceptible to vancomycin (median MIC was 0.63 μg/ml) and metronidazole (median MIC was 0.084 μg/ml), so these two antimicrobials remain optimal treatment option for CDI.

## Introduction

*Clostridium difficile* is one of the most common causes of infections in hospitalised patients, especially in those with long term hospital stay [1], [2]. Although in many underdeveloped countries, *Clostridium difficile* infection (CDI) was under-diagnosed for a very long time, during the last decade, a significant increase in its prevalence was detected [3]. *C.difficile* infection (CDI) can turn even into hospital outbreak very frequently [4].

Many typing methods are involved in the investigation of the mode of spreading of CDI, but molecular methods are used almost exclusively, due to their higher discriminative power [5]. PCR ribotyping is the most widely used typing method for *C. difficile* in Europe, although some other sequencing-based molecular methods are used in many other countries worldwide [6]. PCR ribotyping was also used for characterisation of the hypervirulent *C. difficile* strains that have caused a large outbreak in Canada in 2002 [6]. Since 2005, the most frequent hypervirulent *C. difficile* strain (027/NAP1/BI) has

been identified in many patients from the USA and Europe [7]. Ribotype 027 is known as a strain which produces toxins A, B and binary toxin, as well as a strain with the increased ability for sporulation and high antimicrobial resistance [9]. In recent years some other hypervirulent strains, like ribotypes 017 and 078, have also emerged. Those two ribotypes have been involved in many serious outbreaks recently, which were quite rare in the past [7], [8], [9], [10].

Metronidazole and vancomycin are the most effective antimicrobial agents for the effective treatment of CDI so far. In the last decade, the emergence of reduced susceptibility to both of these drugs has been reported [11]. It is crucial to monitor the susceptibility of *C. difficile* isolates to antimicrobial agents, not only for selecting the optimal treatment option but also for risk assessment of acquiring CDI in the future. It is well known that using many of the broad-spectrum antimicrobials is a major risk factor for acquiring CDI like antibiotic-associated diarrhoea or pseudomembranous colitis [1].

This study aimed to detect the presence of *C. difficile* ribotypes in hospitalised patients and to investigate their antibiotic susceptibility and their toxigenicity.

## Material and Methods

A group of 21 strains of *C. difficile* were isolated from stool samples obtained from patients with symptoms of CDI, hospitalised in different clinics within the Mother Theresa Clinical Centre in Skopje, Macedonia, in the period from 2016 to 2018. All stool samples were tested for glutamate dehydrogenase (GDH) and both *C.difficile* toxins: A and B, with immunochromatographic tests (Mascia Brunelli). Both of these quick tests were performed according to the manufacturer's instructions. The stool samples were also inoculated on selective CCFA agar (Oxoid) and incubated for 48 hours in an anaerobic atmosphere. At the same time, an alcohol shock test was performed with subsequent inoculation on Columbia agar (Oxoid) and incubation under the same conditions. *Clostridium difficile* colonies were identified by their typical appearance and smell. They were further microscopically confirmed by Gram staining. Final confirmation was performed by Vitek 2 (Biomerieux) and MALDI-TOF (Bruker). The 21 *Clostridium difficile* isolates were collected for their further typing and antimicrobial susceptibility testing.

### *PCR Ribotyping*

As a molecular method, PCR ribotyping is based on the amplification of intergenic spacer region (ITS) between 16S and 23S rDNA and was performed as described by Janezic and Rupnik [12], [13[. PCR ribotype profiles of *C. difficile* isolates were analysed by software (Bionumerics, Applied Maths).

### *Antimicrobial Susceptibility Testing*

Antibiotic susceptibility of all *C. difficile* isolates was investigated through the determination of the minimal inhibitory concentrations (MICs) obtained by performing the E test (Biomerieux). One McFarland turbidity standard bacteria suspension was prepared and inoculated on Mueller-Hinton agar supplemented with 5% sheep blood. Antimicrobial strips (Biomerieux) were applied at every single plate, and they were incubated at the same conditions as for the primary isolation. For that purpose, the following antimicrobial agents were used: metronidazole 0.016 - 256 µg/ml, vancomycin 0.016 - 256 µg/ml, tetracycline 0.016 - 256 µg/ml, clindamycin 0.016 – 256 µg/ml, erythromycin 0.016 - 256 µg/ml, imipenem 0.002 - 32 µg/ml, ciprofloxacin 0.002-32 µg/ml and moxifloxacin 0.002-32 µg/ml. The susceptibility of the isolates was analysed according to CLSI M100-S25 and EUCAST v. 8.0, 2018.

**Table 1: Interpretation criteria for antimicrobial susceptibility of *C. difficile* isolates**

| | VAN** | MTZ** | TC* | EM* | CM* | CI* | MX* | IP* |
|---|---|---|---|---|---|---|---|---|
| S (µg/ml) | ≤ 2 | ≤ 2 | ≤ 4 | - | ≤ 2 | ≤ 2 | ≤ 2 | ≤ 4 |
| I (µg/ml) | - | - | 8 | - | 4 | 4 | 4 | 8 |
| R (µg/ml) | > 2 | > 2 | ≥ 16 | ≥8 | ≥ 8 | ≥8 | ≥ 8 | ≥ 16 |

*Interpretation based on CLSI M100-S25; **Interpretation based on The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 8.0, 2018.

Chi-square and Fisher's exact tests were used for testing the differences between proportions, and p-value less than 0.05 was considered statistically significant.

Isolation of the strains, toxins determination, Vitek 2 confirmations and antimicrobial susceptibility tests were performed at the Institute of Microbiology and Parasitology, Medical Faculty Skopje, Macedonia. MALDI-TOF confirmations and PCR ribotyping were performed at the National Laboratory of Health, Environment and Food in Maribor, Slovenia.

## Results

During the study period, 21 *C. difficile* isolates were obtained from hospitalised patients from 7 to 80 years of age. 16 out of 21 *C. difficile* isolates were detected in old patients at the age above 60. 13 (62%) of the patients were female. All isolates were distributed in four different clinics. The total number of *C. difficile* isolates belonged to 11 PCR ribotypes (Table 2 and Figure 1).

**Table 2: Distribution of *Clostridium difficile* ribotypes**

| Clinic | 11 different *C. difficile* ribotypes | Number of particular *C. difficile* ribotype | Toxins in *C. difficile* ribotypes |
|---|---|---|---|
| Clinic for pediatric diseases | 001/072 | 1 | A and B |
| | 002 | 1 | A and B |
| | SLO 120 | 1 | A and B |
| | 001/072 | 3 | A and B |
| Clinic for internal diseases | 014/020 | 1 | A and B |
| | SLO160 | 1 | A and B |
| | 255/258 | 1 | A and B |
| Surgery clinic with ICU | 001/072 | 5 | A and B |
| | 017 | 1 | B only |
| | 001/072 | 1 | A and B |
| | 017 | 1 | B only |
| Clinic for infectious diseases | 027 | 1 | A and B |
| | 046 | 1 | None |
| | 070 | 1 | A and B |
| | SLO 187 | 1 | A and B |

ICU- Intensive care unit.

The relatedness of *C. difficile* isolates is shown on the dendrogram in Figure 1. It presents the similarity of the band's distribution after the electrophoresis.

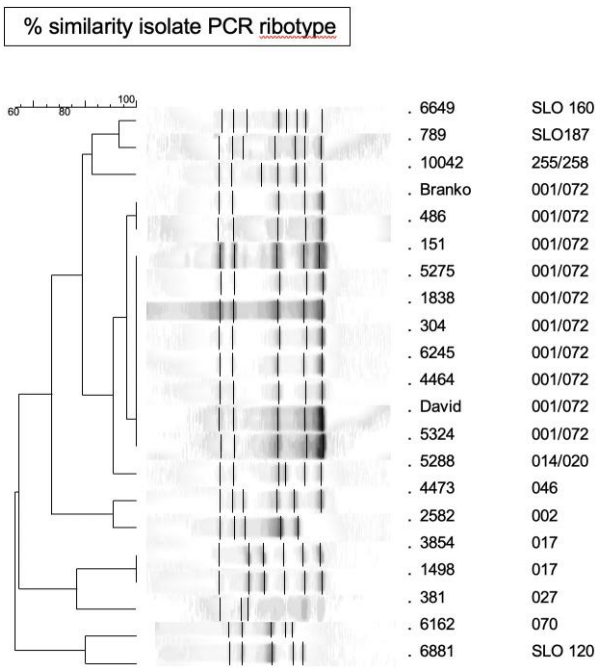



*Figure 1: Dendrogramic representation of the twenty-one isolate of Clostridium difficile characterised by PCR ribotyping*

Antimicrobial susceptibility of the isolates can be observed by comparing the MICs from Table 3 to the interpretation criteria shown in Table 1.

**Table 3: Minimal inhibitory concentrations (µg/ml) of the eight antimicrobials towards the isolates of Clostridium difficile**

| *C. difficile* Ribotype | Mtz | Van | Tc | Cm | Em | Ip | Ci | Mx |
|---|---|---|---|---|---|---|---|---|
| 001/072 | 0.047 | 0.75 | 1.5 | > 256 | > 256 | > 32 | > 32 | > 32 |
| 001/072 | < 0.016 | 1 | 2 | > 256 | > 256 | > 32 | > 32 | > 32 |
| 001/072 | < 0.016 | 0.38 | 2 | > 256 | > 256 | > 32 | > 32 | > 32 |
| 001/072 | 0.094 | 0.50 | 0.047 | 3 | 1 | 4 | > 32 | 0.25 |
| 001/072 | 0.047 | 0.50 | 2 | > 256 | > 256 | 6 | > 32 | > 32 |
| 001/072 | 0.023 | 0.38 | 1.5 | > 256 | > 256 | 4 | > 32 | > 32 |
| 001/072 | 0.094 | 0.75 | 1 | > 256 | > 256 | 4 | > 32 | > 32 |
| 001/072 | 0.094 | 1 | 3 | > 256 | > 256 | 6 | > 32 | > 32 |
| 001/072 | 0.032 | 0.50 | 2 | > 256 | > 256 | 4 | > 32 | > 32 |
| 001/072 | < 0.016 | 0.75 | 2 | > 256 | > 256 | > 32 | > 32 | > 32 |
| 002 | 0.047 | 0.38 | 0.094 | 6 | 1.5 | > 32 | > 32 | 0.5 |
| 014/020 | 0.094 | 0.38 | 0.094 | 4 | 0.75 | 3 | >32 | 0.5 |
| 017 | 0.032 | 0.38 | 8 | >256 | >256 | >32 | >32 | 0.5 |
| 017 | 0.023 | 0.75 | 8 | >256 | >256 | >32 | >32 | >32 |
| 027 | 0.047 | 0.50 | 0.064 | 3 | >256 | >32 | >32 | >32 |
| 046 | 0.047 | 0.38 | 0.064 | >256 | >256 | 4 | >32 | 0.38 |
| 070 | 0.25 | 0.75 | 0.094 | 6 | 1 | >32 | >32 | 0.5 |
| SLO120 | 0.19 | 0.50 | 0.125 | 6 | 1 | 4 | >32 | 0.5 |
| SLO160 | 0.19 | 1.5 | 0.125 | 12 | >256 | 4 | >32 | 0.5 |
| SLO187 | 0.023 | 0.50 | 0.064 | 4 | 1 | 4 | >32 | 0.5 |
| 255/258 | 0.064 | 0.75 | 0.094 | 4 | 1 | 3 | >32 | 0.75 |

Mtz-Metronidazole; Van-Vancomycin; Tc-Tetracycline; Cm-Clindamycin; Em-Erythromycin; Ip-Imipenem; Ci-Ciprofloxacin, Mx-Moxifloxacin.

## Discussion

About 76.2% of *C. difficile* isolates were detected in patients above 60 years of age. Older age in patients is a statistically significant risk factor for *C. difficile* infection ($p < 0.05$). The isolation rate of *Clostridium difficile* was not statistically different between male and female patients ($p > 0.05$), which is consistent with many other studies [14]. *Clostridium difficile* infection was identified at all clinics included in the study. This finding is not unusual since most of the patients in these clinics are constantly receiving broad-spectrum antibiotics, which is considered as a major risk factor for acquiring CDI.

*C. difficile* ribotype 001/072 is the most frequent PCR ribotype in our patients ($p < 0.05$). This finding is similar to the results obtained in the previous study [15]. 50% of *C.difficile* isolates with this ribotype were detected at the Surgery clinic and the Intensive care unit related to it. This high percentage of 001/072 ribotype suggests that it may be an intrahospital ribotype of *Clostridium difficile* at the Surgery clinic. But due to the low number of isolates, this suggestion should be confirmed with the larger number of isolates and by performing additional typing methods in the future. Unlike the results obtained in one of our neighbouring countries based on the same type of investigation [15], [16], where 027 [15] was revealed as the most common *C. difficile* ribotype, in our study this ribotype was detected in one patient only.

All isolates except the one in this study were toxigenic. Non-toxigenic strains are not capable of causing a symptomatic disease [17]. Both *C. difficile* toxins (A and B) were detected in 90% of isolates. Only two isolates belonging to the ribotype 017 were toxin B positive, but toxin A negative. Some authors have emphasised that toxin B can not only act like cytotoxin but also like enterotoxin [18]. So, patients infected with *C. difficile* strains toxin B positive, toxin A negative, could be able to develop identical clinical symptoms as patients infected with *C. difficile* toxin A and B positive strains [19]. *C. difficile* strains positive for toxin A only has not been confirmed so far [20].

Treatments with any of the following antimicrobials: clindamycin, erythromycin, ciprofloxacin and moxifloxacin, could be a risk factor for CDI due to the high resistance of the strains in this study. Ninety per cent of the strains from the most common ribotype 001/072 have MICs for clindamycin and erythromycin > 256 µg/ml. All strains isolated are highly resistant to ciprofloxacin. Resistance to moxifloxacin is somewhat lower, but it is still very common in the most dominant ribotype 001/072. Nine out of ten strains are highly resistant to this antibiotic. All *C. difficile* isolates were susceptible to tetracycline except two of them belonging to the ribotype 017 which revealed intermediate susceptibility.

All strains were susceptible to vancomycin

(median MIC was 0.63 μg/ml) and metronidazole (median MIC was 0.084 μg/ml), so these two antimicrobials remain an optimal treatment option for CDI (Table 2). There is an emergence of resistance of *C. difficile* strains to these two antibiotics globally [11], so it should be necessary to monitor the susceptibility of all the isolates continuously in the future.

## Acknowledgements

This work was supported by a grant from the Federation of European Microbiological Societies (FEMS), ID#: FEMS-RG-2016-0100. We would also like to thank Maja Rupnik and her team from the National Laboratory for Health, Environment and Food from Maribor, Slovenia for their support.

## References

1. Kelly CP, LaMont JT. Clostridium difficile - more difficult than ever. N Engl J Med. 2008; 359:1932-40. https://doi.org/10.1056/NEJMra0707500 PMid:18971494

2. Laffan AM, Bellantoni MF, Greenough WB, et al. Burden of Clostridium difficile-associated diarrhea in a long-term care facility. J Am Geriatr Soc. 2006; 54:1068-73. https://doi.org/10.1111/j.1532-5415.2006.00768.x PMid:16866677

3. European Centre for Disease Prevention and Control. Point prevalence survey of healthcare associated infections and antimicrobial use in European acute care hospitals. Stockholm: ECDC; 2013.

4. Birgand G, Blankaert K, Carbonne A, et al. Investigation of a large outbreak of Clostridium difficile PCR-ribotype 027 infections in northern France, 2006-2007 and associated clusters in 2008-2009. Euro Surveill. 2010; 15(25):19597. https://doi.org/10.2807/ese.15.25.19597-en PMid:20587362

5. Killgore G, Thompson A, Johnson S, et al. Comparison of seven techniques for typing international epidemic strains of Clostridium difficile: restriction endonuclease analysis, pulsed-field gel electrophoresis, PCR-ribotyping, multilocus sequence typing, multilocus variable number tandem-repeat analysis, amplified fragment length polymorphism, and surface layer protein A gene sequence typing. J Clin Microbiol. 2008; 46(2):431-7. https://doi.org/10.1128/JCM.01484-07 PMid:18039796 PMCid:PMC2238077

6. Van Steenbergen, J., Debast, S., Van Kregten, et al. Isolation of Clostridium difficile ribotype 027, toxinotype III in the Netherlands after increase in C. difficile-associated diarrhoea. Euro Surveill. 2005; 10(28):2745. https://doi.org/10.2807/esw.10.28.02745-en

7. Hensgens MP, Goorhuis A, Notermans D, et al. Decrease of hypervirulent Clostridium difficile PCR ribotype 027 in the Netherlands. Euro Surveill. 2009; 14. https://doi.org/10.2807/ese.14.45.19402-en PMid:19941791

8. Akerlund T, Persson I, Unemo M, et al. Increased sporulation rate of epidemic Clostridium difficile Type 027/NAP1. J Clin Microbiol. 2008; 46:1530-3. https://doi.org/10.1128/JCM.01964-07 PMid:18287318 PMCid:PMC2292905

9. McDonald LC, Killgore GE, Thompson A, et al. An epidemic, toxin gene-variant strain of Clostridium difficile. N Engl J Med. 2005; 353: 2433-41. https://doi.org/10.1056/NEJMoa051590 PMid:16322603

10. Dawson LF, Valiente E, Wren BW. Clostridium difficile--a continually evolving and problematic pathogen. Infect Genet Evol. 2009; 9:1410-7. https://doi.org/10.1016/j.meegid.2009.06.005 PMid:19539054

11. Barkin JA, Sussman DA, Fifadara N, et al. Clostridium difficile Infection and Patient-Specific Antimicrobial Resistance Testing Reveals a High Metronidazole Resistance Rate. Dig Dis Sci. 2017; 62:1035. https://doi.org/10.1007/s10620-017-4462-9 PMid:28116592

12. Janezic S, M Rupnik. Molecular typing methods for Clostridium difficile: pulsed-field gel electrophoresis and PCR ribotyping. Methods Mol Biol. 2010; 646:55-65. https://doi.org/10.1007/978-1-60327-365-7_4 PMid:20597002

13. Janežič S, Štrumbelj I, Rupnik M. Use of modified PCR ribotyping for direct detection of Clostridium difficile ribotypes in stool samples. Journal of Clinical Microbiology. 2011; 49(8):3024-5. https://doi.org/10.1128/JCM.01013-11 PMid:21632902 PMCid:PMC3147761

14. Esteban-Vasallo MD, Pellicer SN, Domínguez-Berjón MF, Caballero MC, Asensio Á, Saravia G, Astray-Mochales J. Age and gender differences in Clostridium difficile-related hospitalization trends in Madrid (Spain) over a 12-year period. European Journal of Clinical Microbiology & Infectious Diseases. 2016; 35(6):1037-44. https://doi.org/10.1007/s10096-016-2635-7 PMid:27056555

15. Rupnik M, Tambic Andrasevic A, Trajkovska Dokic E, et al. Distribution of Clostridium difficile PCR ribotypes and high proportion of 027 and 176 in some hospitals in four South Eastern European countries. Anaerobe. 2016; 42:142-144. https://doi.org/10.1016/j.anaerobe.2016.10.005 PMid:27751937

16. Kuijper EJ, Barbut F, Brazier JS, Kleinkauf N, Eckmanns T, Lambert ML, Drudy D, Fitzpatrick F, Wiuff C, Brown DJ, Coia JE. Update of Clostridium difficile infection due to PCR ribotype 027 in Europe, 2008. Eurosurveillance. 2008; 13(31):18942.

17. Poxton IR, McCoubrey J, Blair G. The pathogenicity of Clostridium difficile. Clin Microbiol Infect. 2001; 7:421-7. https://doi.org/10.1046/j.1198-743x.2001.00287.x PMid:11591205

18. Pothoulakis C, Lamont JT. Microbes and microbial toxins: paradigms for microbial-mucosal interactions II. The integrated response of the intestine to Clostridium difficile toxins. American Journal of Physiology-Gastrointestinal and Liver Physiology. 2001; 280(2):G178-83. https://doi.org/10.1152/ajpgi.2001.280.2.G178 PMid:11208538

19. Drudy D, Harnedy N, Fanning S, Hannan M, Kyne L. Emergence and control of fluoroquinolone-resistant, toxin A-negative, toxin B-positive Clostridium difficile. Infect Control Hosp Epidemiol. 2007; 28: 932-40. https://doi.org/10.1086/519181 PMid:17620240

20. Lyras D, O'Connor JR, Howarth P, et al. Toxin B is essential for virulence of Clostridium difficile. Nature. 2009; 458:1176-9. https://doi.org/10.1038/nature07822 PMid:19252482 PMCid:PMC2679968

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Jun 30; 7(12):1900-1904.
https://doi.org/10.3889/oamjms.2019.331
eISSN: 1857-9655
***Basic Science***

# Influence of Flavonoids on the Cytotoxic Activity of Mononuclear Blood Cells in Model Tests

Liudmila Ivanovna[1], Tatiana Mikhailovna[1], Dmitrii Vladimirovich[1*], Mikhail Valentinovich[2], Olga Vladimirovna[1], Elizaveta Aleksandrovna[1]

*[1]Sechenov First Moscow State Medical University, 8-2 Trubetskaya Street, Moscow, Russian Federation; [2]N. N. Blokhin National Medical Research Center of Oncology, Ministry of Health of Russia, 24 Kashirskoe Highway, Moscow, Russian Federation*

**Citation:** Ivanovna L, Mikhailovna T, Vladimirovich D, Valentinovich M, Vladimirovna O, Aleksandrovna E. Influence of Flavonoids on the Cytotoxic Activity of Mononuclear Blood Cells in Model Tests. Open Access Maced J Med Sci. 2019 Jun 30; 7(12):1900-1904. https://doi.org/10.3889/oamjms.2019.331

**Keywords:** Flavonoids; Cytotoxic activity; Plant extract; Cytotoxic test; Mononuclear blood cells

***Correspondence:** Dmitrii Vladimirovich Babaskin. Sechenov First Moscow State Medical University, Moscow, Russian Federation. E-mail: babaskind@yandex.ru

**Received:** 11-Mar-2019; **Revised:** 05-May-2019; **Accepted:** 06-May-2019; **Online first:** 30-Jun-2019

**Copyright:** ©, 2019 Liudmila Ivanovna, Tatiana Mikhailovna, Dmitrii Vladimirovich, Mikhail Valentinovich, Olga Vladimirovna, Elizaveta Aleksandrovna. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

**Funding:** The study was supported by the "Russian Academic Excellence Project 5-100"

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

**Abstract**

**BACKGROUND:** The spread of phytocomplex application and justification of its selective effects on tumour cells (mainly due to the presence of flavonoids) require research of its cytotoxic and immunomodulatory activity.

**AIM:** The goal wa`s to study the direct cytotoxic effect of the phytocomplex and its modulating effect on the cytotoxic activity of the donor's mononuclear blood cells in in vitro experiments.

**METHODS:** The phytocomplex was a dry extract from marsh cinquefoil, creeping alfalfa and common hop; its main active ingredients were flavonoids. Transplantable monolayer cultures of lung adenocarcinoma, colorectal cancer, erythroblastic leukaemia, and fibroblasts were used as target cells. The cytotoxic activity was assessed using a cytotoxic test based on the selective ability to live cells to reduce MTT (3-[4, 5-dimethyltriazol-2-yl]-2, 5 diphenyltetrazolium bromide) to formazan in mitochondria. Quantitative determination of formazan was performed using spectrophotometry.

**RESULTS:** A direct cytotoxic effect of the phytocomplex in concentrations of at least 2.5 mg/ml on tumour cells has been established. Its modulating effect on the cytotoxic activity of mononuclear blood cells at a concentration of 0.05 mg/ml was shown. The phytocomplex in doses of 0.25 and 0.5 mg/ml increased the killer activity of the mononuclear cells in a diseased person's blood, but did not affect these blood cells in a healthy donor. Incubation of lymphocytes with a phytocomplex for 24 hours increased the cytotoxic activity of mononuclear cells by 20-25%.

**CONCLUSION:** The direct cytotoxic effect of the phytocomplex and its modulating effect on the cytotoxic activity of mononuclear blood cells in model experiments in vitro have been established.

## Introduction

Natural products in dietary sources including fruits, vegetables, and spices, which consist of biologically active components such as phytosterols, flavonoids, saponins, lycopene, triperpenoids, and many others, are assumed to have anticancer properties [1]. Interest in flavonoids (polyphenols of plant origin) is currently largely due to their anti-inflammatory, antioxidant, antitumor, immunomodulatory, antimicrobial and other types of activity [2], [3], [4] [5].

The most relevant, for purposes of the practical application of flavonoids, are their immunomodulating and antitumor properties [6], [7], [8], [9], [10], [11]. The phytocomplex under study was a dry extract from marsh cinquefoil, creeping alfalfa and common hop. It contained a variety of biologically active substances, including flavonoids, coumestans, polysaccharides, steroids, essential oils, tannins, hydroxycinnamic and phenol carboxylic acids, essential amino acids, vitamins, and mineral components, all of which makes it useful in medicine. The main active ingredients of the phytocomplex were flavonoids [12]. Recent studies have shown the promise and the need for further study of the cytotoxic and immunomodulatory activity of the phytocomplex for use together with physiotherapeutic methods [13].

The goal of this work was to investigate the direct cytotoxic effect of the phytocomplex and its modulating effect on the cytotoxic activity of the donor's mononuclear blood cells (MNCs) in *in vitro* experiments.

## Material and Methods

The phytocomplex that was used in the study was a dry extract from leaves and roots of marsh cinquefoil, leaves of creeping alfalfa and multiple fruits (or cones) of common hop (TS 9375-021-00003938-11 “Dry extract of marsh cinquefoil, creeping alfalfa and common hop (phytocomplex)” [14]. The main flavonoids present in the phytocomplex are shown in Figure 1. The flavonoid composition of the raw materials of the used medicinal plants was compatible and balanced in terms of the content of flavones, flavonols and their glycosides, isoflavones, which should have a positive impact on the cytotoxic and immunomodulatory activity of the phytocomplex. The predominant flavonoids were quercetin, rutin and isoflavones: biochanin A, genistein, daidzein.

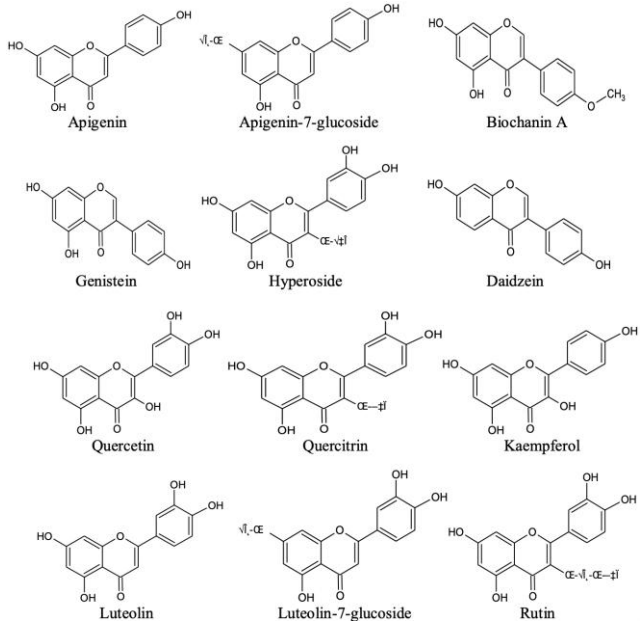



*Figure 1: Structural formulas of the main flavonoids of the phytocomplex*

A cytotoxic test, based on the selective ability to live cells to reduce MTT (3-[4, 5-dimethyltriazol-2-yl]-2, 5 diphenyltetrazolium bromide, Sigma, USA) to formazan in mitochondria, was used to study the direct cytotoxic effect of the phytocomplex. Quantitative determination of formazan was performed using spectrophotometry. Based on the optical density data, the number of dead target cells (i.e. cytotoxic activity) was calculated.

The following human cell lines were used: lung adenocarcinoma (LAC, A-549) and colorectal cancer (Colo), a model of solid tumours, erythroblastic leukaemia (K-562), a model of hemoblastosis and normal fibroblasts (obtained from the laboratory of tumour cells genetics of the Blokhin Federal Medical Research Center for Oncology) were used as target cells. Target cells were applied in 96-well flat-bottomed microplates in quantity of 2 x $10^6$ cells per well in 100 µl of the working culture medium (WCM): RPMI-1640 (No. FSR 2007/00859, Chumakov Institute of Poliomyelitis and Viral Encephalitis, Russia) with 5% fetal calf serum. In the experimental series, working solutions of the phytocomplex were added to the target cells. The final concentrations of the phytocomplex in the wells were 5 mg/ml, 2.5 mg/ml, 0.5 mg/ml, 0.05 mg/ml, 0.005 mg/ml. In the control series, a saline solution was added to the target cells. The total volume of the solution in the wells was 200 µl.

Target cells and effectors were placed in a carbon-oxygen incubator (Binder C 150 $CO_2$ incubator, Germany) at 37°C for 18 hours. At the end of the experiment, 10 µl of a sterile MTT dye solution was added to each well. Plates protected with aluminium foil were placed in a dark place at 37°C for four hours, then centrifuged for five minutes. The supernatant was removed with a pipette without disturbing the cell pellet. Formazan crystals were dissolved in dimethyl sulfoxide, which was added into the well to bring the volume up to 200 µl. The complete dissolution of the formazan crystals was achieved with stirring on a nutator for 5-15 minutes using an invertoscope to control the process.

The optical density was measured immediately after the complete dissolution of the formazan crystals using a spectrophotometer (Jasco V-730, Japan) at a wavelength of 540 nm.

To study the modulating effect of the phytocomplex on the cytotoxic activity of MNCs, extract concentrations that did not have a direct cytotoxic effect on the target cells were used. MNCs were isolated from the blood samples of six donors, obtained from the blood transfusion department of the Blokhin Federal Medical Research Center for Oncology. Six primary patients with stage II lung cancer (three) and colon cancer (three) without signs of metastasis were included in the study.

Heparinised blood (15 units/ml) was diluted twice with medium 199 (No. ФСР 2011/10969, Chumakov Institute of Poliomyelitis and Viral Encephalitis, Russia), layered on ficoll-verografin (density 1.077 g/$cm^3$) in the 2:1 ratio and centrifuged for 30 minutes. MNCs were sampled using a Pasteur pipette and washed three times with medium 199 for ten minutes. The concentration of the suspension in WCM was adjusted to 5 x $10^6$ cells/ml. In the experiment with donors, MNCs, LAC, Colo, K-562 cells and fibroblasts were used as target cells. For cancer patients, MNCs investigated only NK activity on the NK-sensitive K-562 cells. The ratio of target cells and effectors was 1:5.

Statistical processing of the results was carried out using the SPSS. Statistics. v17. Multilingual-EQUiNOX Software (SPSS Inc.).

## Results

The results of the experiment for the study of direct cytotoxic action of the phytocomplex on target cells are presented in Figure 2.

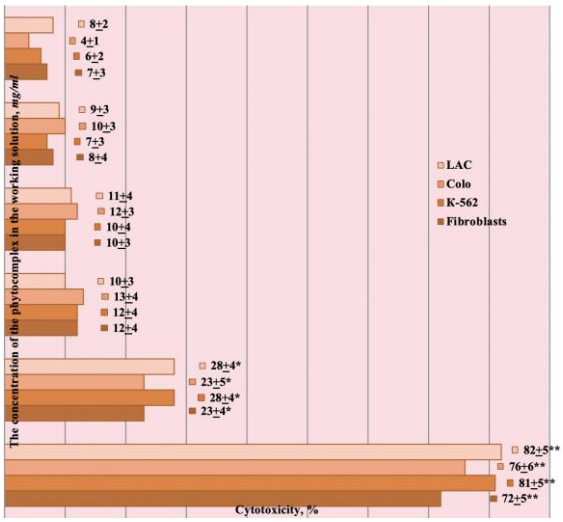



*Figure 2: Cytotoxic activity of the phytocomplex (* P < 0.05, ** P < 0.01)*

It was found that the addition of working solutions of the phytocomplex at a concentration of 5 mg/ml to the target cells resulted in the death of a significant proportion of tumour cells (72-82%). A two-fold decrease in the effective concentration (2.5 mg/ml) caused lysis of 23-28% of tumour cells. At lower concentrations from 0.5 to 0.005 mg/ml, the cytotoxicity was 4-12%, which was not significantly different from the data for the control series.

Extract concentrations that did not have a direct cytotoxic effect on target cells were used for further studies of the modulating effect of the phytocomplex on the cytotoxic activity of MNCs. The results of the experiment are presented in Figure 3.

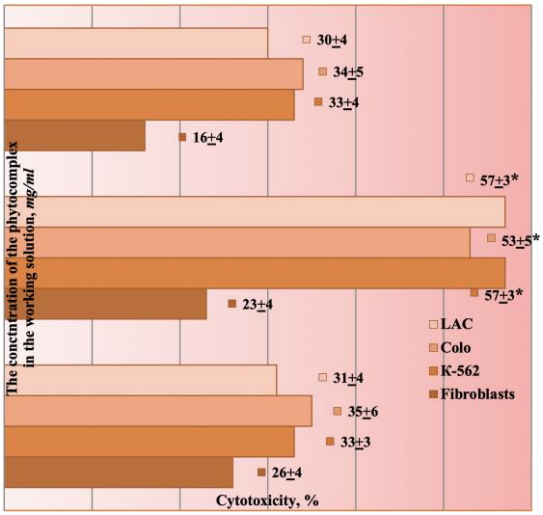



*Figure 3: The modulating influence of the phytocomplex on the cytotoxic activity of MNCs (* P < 0.05)*

It has been shown that out of the working solutions of the phytocomplex with concentrations ranging from 0.001 to 0.5 mg/ml only the dose of 0.05 mg/ml had a significant stimulating effect on the antitumor cytotoxic activity of MNCs (53-57%, LAC, Colo and K-562 cells were used). Increase of the concentration to 0.5 mg/ml did not lead to an increase in the spontaneous activity of MNCs, and a further increase of the dose could have a direct cytotoxic effect on tumour cells. The obtained data corresponded to the data on the effect of other well-known immunomodulators, such as Interleukin-1 and Interleukin-2, although the latter do not have a dose-dependent effect. No effect of the phytocomplex on the cytotoxic activity of MNCs was shown when using fibroblasts as target cells.

Additionally, the modulating effect of the phytocomplex on the killer activity of MNCs of a healthy donor and a cancer patient was investigated (Figure 4). Cells of K-562 cell culture, which are sensitive to natural killers, were used as target cells. Working solutions with phytocomplex concentrations of 0.5 and 0.25 mg/ml did not have a direct cytotoxic effect on K-562 cells. It was established that the phytocomplex in concentrations of 0.5 and 0.25 mg/ml does not have a significant modulating effect on the cytotoxic activity of MNCs of a healthy donor, but increases the killer activity of MNCs of a cancer patient by 15-18% compared to the control.

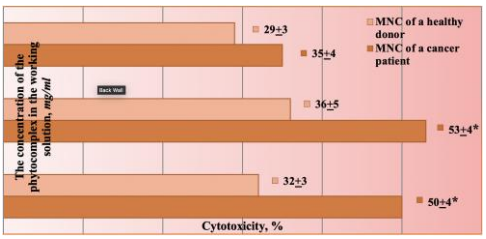



*Figure 4: The modulating influence of the phytocomplex on the cytotoxic activity of MNCs of a healthy donor and a diseased person when tested using K-562 cells (* P < 0.05)*

It is known that the maximum effect of classical immunomodulators is achieved after 24-48 hours. Therefore, we incubated lymphocytes with the phytocomplex for 24 hours at 37°C (Figure 5).

Experiments have shown that incubation increases the cytotoxic activity of donor MNCs by 20-25% compared with the control.

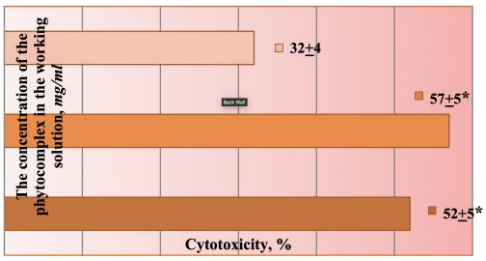



*Figure 5: The modulating influence of the phytocomplex on the cytotoxic activity of MNCs of a healthy donor when tested using K-562 cells after incubation for 24 hours (* P < 0.05)*

In a special series of experiments, the modulating effect of the phytocomplex on the cytotoxic activity of MNCs was studied using different ratios of target cells and MNCs: 1:1, 1:2 and 1:5 (Figure 6). In the control series, a solution of the phytocomplex was not added.

It was established that the phytocomplex increases the cytotoxic activity of MNCs by 20-29% compared with the control, regardless of the target cells to MNCs ratio.

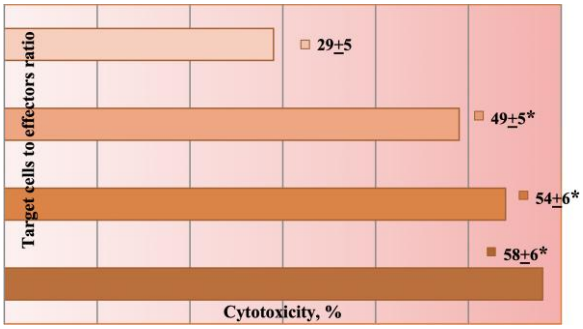



*Figure 6: The modulating effect of the phytocomplex at a concentration of 0.05 mg/ml on the cytotoxic activity of MNCs of a healthy donor when tested using K-562 cells with different MNCs to target cells ratios (* P < 0.05)*

## Discussion

For the first time, the cytotoxic activity of the phytocomplex was studied using transplantable monolayer cultures of lung adenocarcinoma, colorectal cancer, erythroblastic leukaemia and fibroblasts in *vitro* experiments. It has been established that the phytocomplex has a direct cytotoxic effect on tumour cells in concentrations of 5.0 and 2.5 mg/ml.

The modulating effect of the phytocomplex on the cytotoxic activity of MNCs at a concentration of 0.05 mg/ml (53-57%) was registered when using cell cultures of lung adenocarcinoma, colorectal cancer and erythroblastic leukaemia. An increase in the concentration of the phytocomplex did not lead to an increase in the spontaneous activity of blood mononuclear cells.

It has been established that the phytocomplex in concentrations of 0.25 and 0.5 mg/ml increases the killer activity of the MNCs of a diseased person by 15-18%, but does not affect the cytotoxic activity of the blood mononuclear cells of a healthy donor.

It has been shown that incubation of lymphocytes with phytocomplex for 24 hours increases the cytotoxic activity of mononuclear cells by 20-25%. Variations in the ratio of target cells and blood mononuclear cells did not significantly affect the immunomodulating effect of the phytocomplex.

In conclusion, the direct cytotoxic effect of the phytocomplex and its modulating effect on the cytotoxic activity of mononuclear blood cells in model experiments in vitro have been established.

## References

1. Rawat P, Kumar A, Singh TD, Pal M. Chemical composition and cytotoxic activity of methanol extract and its fractions of strobilus asper leaves on human cancer cell lines. Pharmacognosy magazine. 2018; 14(54):141-44. https://doi.org/10.4103/pm.pm_391_17 PMid:29720821 PMCid:PMC5909305

2. Fu YF, Jiang LH, Zhao WD, Xi-Nan M, Huang SQ, Yang J, Hu TJ, Chen HL. Immunomodulatory and antioxidant effects of total flavonoids of Spatholobus suberectus Dunn on PCV2 infected mice. Scientific reports. 2017; 7(1):8676-8679. https://doi.org/10.1038/s41598-017-09340-9 PMid:28819143 PMCid:PMC5561176

3. Guo Z, Xu HY, Xu L, Wang SS, Zhang XM. In vivo and in vitro immunomodulatory and anti-inflammatory effects of total flavonoids of Astragalus. African Journal of Traditional, Complementary and Alternative Medicines. 2016; 13(4):60-73. https://doi.org/10.21010/ajtcam.v13i4.10 PMid:28852721 PMCid:PMC5566160

4. Kilani-Jaziri S, Mustapha N, Mokdad-Bzeouich I, El Gueder D, Ghedira K, Ghedira-Chekir L. Flavones induce immunomodulatory and anti-inflammatory effects by activating cellular anti-oxidant activity: a structure-activity relationship study. Tumour Biology. 2016; 37(5):6571-9. https://doi.org/10.1007/s13277-015-4541-5 PMid:26638168

5. Zeinali M, Rezaee SA, Hosseinzadeh H. An overview of immunoregulatory and anti-inflammatory properties of chrysin and flavonoids substances. Biomedicine & Pharmacotherapy. 2017; 92:998-1009. https://doi.org/10.1016/j.biopha.2017.06.003 PMid:28609844

6. Chen L, Wei Y, Zhao S, Zhang M, Yan X, Gao X, Li J, Gao Y, Zhang A, Gao Y. Antitumor and immunomodulatory activities of total flavonoids extract from persimmon leaves in H22 liver tumour-bearing mice. Sci Rep. 2018; 8(1):10523-10528. https://doi.org/10.1038/s41598-018-28440-8 PMid:30002398 PMCid:PMC6043534

7. Gandhi GR, Neta MT, Sathiyabama RG, Quintans JD, e Silva AM, de Souza Araújo AA, Narain N, Júnior LJ, Gurgel RQ. Flavonoids as Th1/Th2 cytokines immunomodulators: a systematic review of studies on animal models. Phytomedicine. 2018; 44:74-84. https://doi.org/10.1016/j.phymed.2018.03.057 PMid:29895495

8. Karimi G, Hassanzadeh-Josan S, Memar B, Esmaeili SA, Riahi-Zanjani B. Immunomodulatory effects of silymarin after subacute exposure to mice: A tiered approach immunotoxicity screening. Journal of pharmacopuncture. 2018; 21(2):90-97.

9. Li J, Xu L, Sang R, Yu Y, Ge B, Zhang X. Immunomodulatory and anti-inflammatory effects of total flavonoids of Astragalus by regulating NF-KB and MAPK signalling pathways in RAW 264.7 macrophages. Die Pharmazie-An International Journal of Pharmaceutical Sciences. 2018; 73(10):589-93.

10. Nani BD, Franchin M, Lazarini JG, Freires IA, da Cunha MG, Bueno-Silva B, de Alencar SM, Murata RM, Rosalen PL. Isoflavonoids from Brazilian red propolis down-regulate the expression of cancer-related target proteins: A pharmacogenomic analysis. Phytotherapy research. 2018; 32(4):750-4. https://doi.org/10.1002/ptr.6016 PMid:29377427 PMCid:PMC5893349

11. Ribeiro D, Proenca C, Rocha S, Lima JL, Carvalho F,

Fernandes E, Freitas M. Immunomodulatory effects of flavonoids in the prophylaxis and treatment of inflammatory bowel diseases: a comprehensive review. Current medicinal chemistry. 2018; 25(28):3374-412. https://doi.org/10.2174/0929867325666180214121734 PMid:29446723

12. Babaskina LI, Litvinova TM, Babaskin DV, Winter EA, Krylova OV. Improving the Chemical Status of Peloids by Adding the Phytocomplex in the Rehabilitation of Patients with Osteoarthritis: Experimental Study. Journal of Pharmaceutical Sciences and Research. 2018; 10(5):1238-42.

13. Babaskin DV, Litvinova TM, Babaskina LI. Transdermal Delivery of Biologically Active Substances During Electrophoresis of the Phytocomplex in Model Experiments. Journal of Pharmaceutical Sciences and Research. 2018; 10(9):2125-8.

14. Babaskina LI, Litvinova TM, Babaskin DV. Key Points in the Development of Medicinal Products for Electro-and Phonophoresis Based on a Phytocomplex in the Rehabilitation of Patients with Osteoarthritis. Journal of Pharmaceutical Sciences and Research. 2018; 10(8):1991-

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Jun 30; 7(12):1905-1910.
https://doi.org/10.3889/oamjms.2019.548
eISSN: 1857-9655
***Basic Science***

# The Expression of Transforming Growth Factor Beta-1 and Interleukin-6 on Human Prostate: Prostate Hyperplasia and Prostate Cancer

Afdal Afdal[1*], Eryati Darwin[2], Yanwirasti Yanwirasti[3], Rizal Hamid[4]

*[1]Postgraduate Biomedical Science, Faculty of Medicine, Andalas University, Padang, Indonesia; [2]Department of Histology, Faculty of Medicine, Andalas University, Padang, Indonesia; [3]Department of Anatomy, Faculty of Medicine, Andalas University, Padang, Indonesia; [4]Department of Surgery, Faculty of Medicine, Indonesia University, Jakarta, Indonesia*

**Citation:** Afdal A, Darwin E, Yanwirasti Y, Hamid R. The Expression of Transforming Growth Factor Beta-1 and Interleukin-6 on Human Prostate: Prostate Hyperplasia and Prostate Cancer. Open Access Maced J Med Sci. 2019 Jun 30; 7(12):1905-1910. https://doi.org/10.3889/oamjms.2019.548

**Keywords:** Prostate Hyperplasia; Prostate Cancer; IL-6; TGFβ-1; Gleason Index

***Correspondence:** Afdal Afdal. Doctoral Student of Postgraduate Biomedical Science, Faculty of Medicine, Andalas University, Padang, Indonesia. E-mail: afdalurologi@gmail.com

**Received:** 27-May-2019; **Revised:** 12-Jun-2019; **Accepted:** 13-Jun-2019; **Online first:** 30-Jun-2019

**Copyright:** © 2019 Afdal Afdal, Eryati Darwin, Yanwirasti Yanwirasti, Rizal Hamid. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

**Funding:** This research did not receive any financial support

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

**Abstract**

**BACKGROUND:** Prostate hyperplasia and prostate cancer are two of the most common pathological condition of the prostate to be found on male. Both of these diseases share common pathogenesis involving inflammation of prostatic tissues. Chronic inflammation will induce the release of cytokines, followed by cells injury and tissues damage. One of the cytokines that play a role in prostate pathology is IL-6. The inflammation will also induce the releases of anti-inflammatory cytokines such as TGFβ-1.

**AIM:** This study aims to analyse the expression of IL-6 and TGFβ-1, in prostate hyperplasia and prostate cancer.

**MATERIAL AND METHODS:** This is an observational study, using paraffin-embedded tissue samples of prostate hyperplasia and prostate cancer. Samples were obtained from the laboratory of Pathological Anatomy, Faculty of Medicine, Andalas University, Padang, Indonesia. Immunohistochemistry was performed to detect the cytokine expression, and a semiqunatitaves measurement according to Immunoreactive score (IRS) was performed for evaluation. For the TGFβ-1, the stromal expression was also analysed by measurement of the stromal stained area. The correlation of cytokine expression to Gleason index score was also analysed in prostate cancer.

**RESULTS:** The result showed that this study found that TGFβ-1 was detected both in the stromal component as well as epithelial. With the stromal being the dominant site of expression. The stromal TGFβ-1 expression was of significantly higher in prostate hyperplasia compares to prostate cancer ($p < 0.05$), while the epithelial expression of TGFβ-1 was not found to be significantly different. IL-6 was mostly expressed intracytoplasmic in epithelia. The IL-6 expression was significantly higher in prostate cancer compared to hyperplasia. However, there was no significant correlation to found between IL-6 expression to the Gleason Score among prostate cancers.

**CONCLUSION:** This study concluded that there were differences in expression of both TGFβ-1 and IL-6 between prostate hyperplasia and prostate cancer tissue by immunohistochemistry.

## Introduction

The benign prostate hyperplasia and prostate cancer are the most common pathological condition to be found on the male's urothelial system. An epidemiological study showed that the incidence of this disease was increasing annually, worldwide [1]. Prostate hyperplasia is the most common benign neoplasm on elderly male. Histopathological study on autopsy reports showed that the prevalence of prostate hyperplasia was found up to 50% on males between 50-60 years of age, and it increases to over 80% in the 70 years group of ages [1], [2], [3], [4], [5]. In the other hand, prostate cancer is the most common non-skin cancer to be found on male, worldwide. This disease serves as the second most killer cancer among males in the United States and Europe [2], [6].

Prostate hyperplasia and prostate cancer share a similar pathogenesis, in which both are related to hormones and inflammation [7], [8], [9]. Despite having different predilection, both diseases are known as a chronic disease that has an early initiation, followed by a slow progression course, until it shows clinical symptom [2], [7]. In the recent five years, it has been revealed that chronic inflammation of prostate tissue is one of the risk factors for both prostate hyperplasia and prostate cancer [2], [3], [7], [10], [11], [12]. Although the pathogenesis is still unclear, nowadays there are many studies showed the relations of chronic inflammation to both prostate hyperplasia and prostate cancer [3], [4], [9], [13]. Recently the treatment of BPH and prostate cancer

also started to incorporate the prevention and treatment of chronic inflammation as an integrated treatment [10], [14], [15], [16].

Chronic inflammation will induce the release of cytokine and other inflammatory factors, both from inflammatory cells, as well as the hypoxic prostate epithelium [1], [17]. These cytokines will interact with stromal cells and cause further tissue damage [2], [17]. The inflammation will induce infiltrations of T cells, B cells and macrophages [8], [18]. These inflammatory cells will induce the release of the proinflammatory IL-2, IL-8 and IL-6 from epithelial as well as stromal cells [19]. In return, the increasing of proinflammatory cytokines will induce the production and secretion of an anti-inflammatory cytokine such as TGF β and FGF. The complex interaction of those cytokines will eventually affect the function of prostate gland [18], [19], [20], [21], [22]. TGF-β is known as a controlling factor of tumour progressiveness. This cytokine has a biphasic role in carcinogenesis. On the early phase of cancer, it serves as a tumour suppressor agent by inhibiting cell proliferation [23], [24]. However, on the later stage, it functions as a tumour promoter, in which, it will induce the cellular changes related to tumour cells invasion [23], [24], [25].

IL-6 is a cytokine that involves in the malignancies process and could serve as a factor that inhibits the apoptosis of tumour cells as well as inducing angiogenesis [24], [26], [27], [28]. Pace *et al*. (2011) showed that the IL-6 was found to be significantly higher on a patient with prostate cancer compared to prostate hyperplasia [27]. The present study aims to analyse the differential expression of cytokines that involves in the pathogenesis of BPH and prostate cancer, especially TGFβ-1 and IL-6 in prostate tissues.

## Materials and Methods

### *Experimental Design*

The present study used formalin fixed paraffin embedded tissues from prostate lesion obtained from a surgical procedure. Forty samples of previously diagnosed as histologically, (29), as well as 40 samples of prostate cancers, were used in this study. The tissues were obtained from the laboratory of pathological anatomy, Faculty of Medicine, Andalas University, Padang, Indonesia.

### *Immunohistochemistry*

Immunohistochemistry was used to investigate the expression of cytokines using the avidin-biotin complex method. Following primary antibody were used; Rabbit polyclonal anti-human TGFβ-1, Bioss, 0086R with a dilution of 1:200, and Rabbit polyclonal anti-human IL-6. Bioss, 07-82R, with dilution 1:100. The goat anti-rabbit Igg, Vector laboratories, of dilution 1:200 was used as secondary antibody and the 3-3' diaminobenzidine (DAB), Dojindo Laboratories, was applied as the chromogen. The immunohistochemistry was performed as suggested in the primary antibody datasheet accordingly. The cellular expression of the cytokines was performed by a semi-quantitative system according to Immunoreactive score (IRS). Both the proportion and intensity of cellular staining were measured. The final IRS score is the multiplication of proportion score to intensity score, which ranges between 0 to 12. In this present study, the IRS score of 0 to 4 was considered as "low IRS score", and the score above 4 is treated as "high IRS score". The stromal staining of TGFβ-1were also analysed by measuring the proportion of stained area using the Image J software, (Image J 1.49v software, National Institute of Health, Bethesda, MD, USA). For the samples of prostate cancer, the correlation of cytokine expression to the Gleason score was also analysed. The prostate cancers samples were grouped either as "high Gleason score" (score 8-10) or "low Gleason score" (score 7 or below).

### *Statistical analysis*

Chi-square was used for statistical analysis. And the Shapiro-Wilk test was performed as a test of normality.

## Results

Before data analysis, the distribution of data was assessed for normality test. Since each of the groups contains less than 50 samples, the normality test was performed with the Shapiro-Wilk test, as shown in Table 1.

**Table 1: Normality Test for Independent Variables**

| Variables | Group | Shapiro-Wilk | | |
|---|---|---|---|---|
| | | Statistic | df | Sig. |
| TGFβ-1 | Prostate hyperplasia | 0.852 | 40 | 0.000 |
| | Prostate cancer | 0.743 | 40 | 0.000 |
| IL-6 | Prostate hyperplasia | 0.955 | 40 | 0.109 |
| | Prostate cancer | 0.914 | 40 | 0.005 |

Table 1 shows the distribution of variables. None of the variables shows a normal distribution ($p < 0.05$). Therefore, the test was continued by transforming the data, and a repeating normality test was performed, as shown in Table 2.

**Table 2: Post-Transformation Normality Test**

| Variables (Log) | Group | Shapiro-Wilk | | |
|---|---|---|---|---|
| | | Statistic | df | Sig. |
| LOG TGFβ-1 | Prostate hyperplasia | 0.899 | 23 | 0.024 |
| | Prostate cancer | 0.946 | 24 | 0.223 |
| LOG IL-6 | Prostate hyperplasia | 0.799 | 23 | 0.000 |
| | Prostate cancer | 0.868 | 24 | 0.005 |

Post-transformation normality test showed that only TGFβ-1 on prostate cancer data shows normal distribution. Therefore, to investigate the correlation between independent and dependent variables, the statistical analysis will be done using the non-parametric Mann Whitney test. The value of each variable will be presented by the average value and standard of deviation.

### *The Epithelial Expression of TGFβ-1 on Prostate Lesions*

Immunohistochemistry shows that TGFβ-1 was expressed both in the epithelial component as well as the stromal component (Figure 1). However, the majority of the staining with strong signal intensities were to be found in the stromal area. The epithelial expression of TGFβ-1 can be found in prostatic epithelial both in prostate hyperplasia as well as prostate cancer (Figure 1A and B). Most of the staining of the TGFβ-1 in prostate hyperplasia showing of low expression in IRS score (97.5%). Prostate cancer also mostly showed low TGFβ-1 epithelial expression (87.5%) (Table 3). The average IRS values of TGFβ-1 epithelial expression is slightly lower in prostate Hyperplasia (IRS score 0.7) compared to the prostate cancer group (IRS score 1.3). However, the differences are statistically non-significant. Interestingly there are 5 samples of prostate cancers that showed high epithelial TGFβ-1 expression as can be seen in Table 3 and Figure 1B.

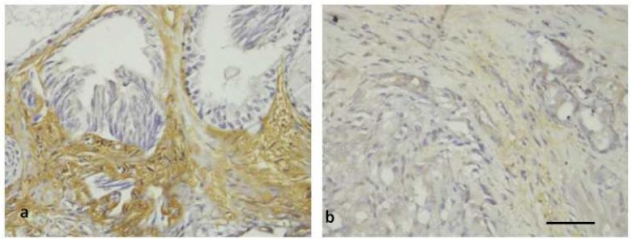



*Figure 1: The Expression of TGFβ-1 by Immunohistochemistry on Human Prostate Tissue. Prostate Hyperplasia (A) and Prostate Cancer (B). TGFβ-1 are mostly Expressed on Stromal Component Prostate Hyperplasia (A); The Stromal Staining is Greatly Reduced in Prostate Cancer, However Cancer Epithelial can Show TGFβ-1 Staining in Some Cases (B) Immunoperoxidase of Rabbit Anti Human TGFβ-1. Scale Bar 100 μm*

Table 3 shows the expression of epithelial TGFβ-1 on prostate hyperplasia and prostate cancer. Both groups show mostly low IRS scores. The carcinoma groups contain slightly higher IRS score. However, there are no significant differences to be found between groups (p = 0.21).

**Table 3: The Epithelial Expression of TGFβ-1 on Prostate Hyperplasia and Prostate Cancer Tissue**

| Group | n | Epithelial TGFβ-1 (IRS Score) | | |
|---|---|---|---|---|
| | | Low (%) | High (%) | p |
| Prostate hyperplasia | 40 | 39 (97.5%) | 1 (2.5%) | 0.201 |
| Prostate carsinoma | 40 | 35 (87.5%) | 5 (12.5%) | |

### *The Stromal Expression of TGFβ-1 on Prostate Lesions*

The immunohistochemistry staining of TGFβ-1 showed a distinct pattern between hyperplasia and prostatic cancer. Most of the prostate hyperplasia showed a high stromal staining intensity surrounding the gland (Figure 1A and 2A). In the other hand, prostate cancer shows low expression of TGFβ-1 in the stromal area (Figure 1B and 2C). The measurement of stromal staining was done by Image J software by selecting the brown staining area, converting the image into a black and white image and measured the stained area.

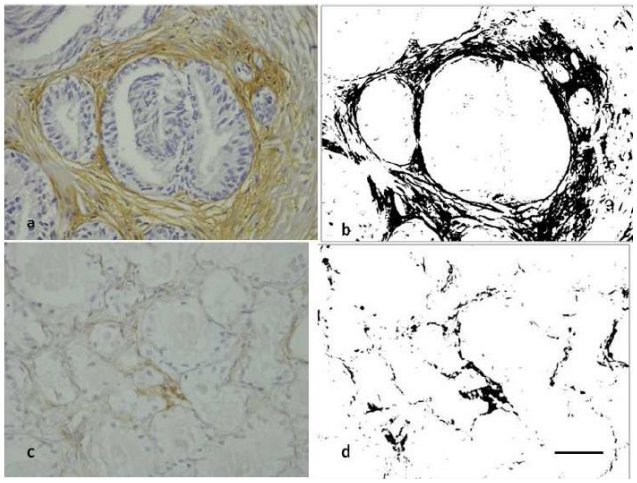



*Figure 2: The Expression of TGFβ-1 by Immunohistochemistry on Human Prostate Tissue. Prostate Hyperplasia (A) and Prostate Cancer (B); The Stomal Area is Measured by Image J Software by Isolate the Brown Stained Area and Measuring the Proportion of Stained Area. There is a Higher Proportion of Stained Area in Prostate Hyperplasia (B) Compared to Prostate Cancer (D). Immunoperoxidase of Rabbit Anti Human TGFβ-1. Scale Bar 100 μm*

The average stromal area of TGFβ-1 in prostate hyperplasia is 15.3% and is significantly higher compares to that of the prostate cancer 4.5%. The stromal expression of TGFβ-1 can be seen in Table 4.

**Table 4: The Stromal Expression of TGF-β1 on Prostate Hyperplasia and Prostate Cancer Tissue**

| Group | n | TGFβ-1 (% Area) | | |
|---|---|---|---|---|
| | | Average Value | St-Deviation | p |
| Prostate hyperplasia | 40 | 15.32 | 8.74 | 0.00 |
| Prostate carcinoma | 40 | 4.47 | 4.84 | |

Table 4 shows the expression of stromal TGFβ-1 on prostate hyperplasia and prostate cancer. There is a significantly higher expression of TGFβ-1

on hyperplasia prostate, compared to prostate cancers ($p < 0.05$).

### *The Expression of IL-6 on Prostate Lesions and its Correlation to the Gleason Score in Prostate Cancer*

Immunohistochemistry shows that IL- 6 was expressed in the epithelial component both in hyperplasia prostate and prostate cancer. The staining can also be detected in the stromal area but a faintly low intensity (Figure 3). The majority (62.5%) of prostate hyperplasia showed low expression of IL-6 in IRS score, in contrast, the majority (85%) of samples in carcinoma group showed a stronger expression, as can be seen in Table 5.

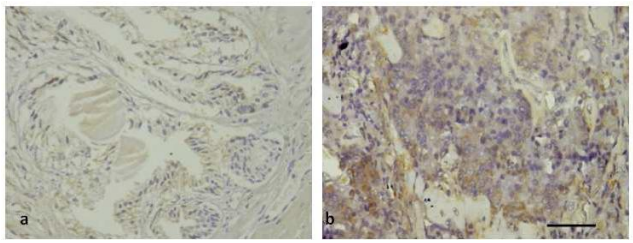



*Figure 3: The Expression of IL-6 by Immunohistochemistry on Human Prostate Tissue. Prostate Hyperplasia (A) and Prostate Cancer (B). IL-6 is Expressed on Low Intensity on Prostate Hyperplasia (A). The Expression is Higher in Prostate Cancer Cells (B) Immunoperoxidase of Rabbit Anty Human IL-6. Scale Bar; 100µm*

Table 5 shows the expression of IL-6 on prostate hyperplasia and prostate cancer. There is a significantly higher expression of IL-6 on prostate cancers compared to hyperplasia prostate ($p < 0.05$).

**Table 5: The Expression of IL-6 on Prostate Hyperplasia and Prostate Cancer Tissue**

| Group | n | IL-6 (IRS Score) | | |
|---|---|---|---|---|
| | | Low | High | p |
| Prostate hyperplasia | 40 | 25 (62.5%) | 15 (37.5%) | 0.001 |
| Prostate carsinoma | 40 | 6 (15%) | 34 (85%) | |

When the prostate cancer was grouped into “high” and “low” Gleason score index group, both groups showed high IL-6 IRS score, there is no significant correlation between IL-6 expression to the Gleason index score in prostate cancer, as can be seen in Table 6.

**Table 6: The Correlation of Gleason Score to the Expression of IL-6 on Prostate Carcinoma**

| Gleason Score | n | IL-6 IRS score (%) | | |
|---|---|---|---|---|
| | | Low | High | p |
| Low | 40 | 1 (7.1%) | 13 (92.9%) | 0.399 |
| High | 40 | 5 (19.2%) | 21 (80.8%) | |

## Discussion

### *The Expression of TGFβ-1 on Prostate Lesions: Prostate Hyperplasia and Prostate Cancer*

Chronic inflammation plays a significant role in the initiation and progression to the wide spectrum of pathogenesis in prostate lesions. The inflammation will attract the infiltrations of B cells, T Cells, as well as macrophages. These immune cells will increase the secretion of proinflammatory cytokine IL-2, IL-6 and IL-8 both on the epithelial tissue or from storms. In response to the increases of proinflammatory cytokines, the epithelial and stromal cells will produce anti-inflammatory cytokines such as TGFβ-1 and FGF, which in turn will affect the normal function of prostate glands.

In this present study, we found that TGFβ-1 was highly expressed in hyperplasia prostate tissue in the extracellular matrix surrounding the glands. The expression of TGFβ-1 was significantly different compared to prostate cancer. This result is suggesting that TGFβ-1 play an important role in prostate hyperplasia pathogenesis. It has been known that TGFβ-1, acts as a modulator of other protein such as bFGF 2, synthesis of extracellular matrix and angiogenesis via VEGF. This interaction plays roles in the pathogenesis of hyperplasia prostate.

TGFβ-1 is a potent mitotic factor for fibroblast as well as other mesenchymal cells [22], [30]. It also regulates the synthesis of the extracellular matrix and induces the secretion of fibrogenic bFGF-2. TGFβ-1 expression act as chemoattractant towards fibroblast, which played roles in the early process of fibrosis on early phase and was instrumental in the process of fibrosis [31]. TGFβ-1 stimulates fibroblast transformation into myofibroblast and smooth muscle cells through induction of extracellular matrixes. Other studies showed the role of TGFβ-1 in fibrosis. Zuhirman (2014) shows the increases of TGFβ-1 in chronic ureteral obstruction [32]. Untergasser *et al*., (2005) showed the increases of TGFβ-1 stimulates the collagen synthesis by stromal cells on prostate hyperplasia, as well as the transformation of fibroblast into myofibroblast [6].

In Contrast to prostatic hyperplasia, our present study shows that there are decreases in stromal TGFβ-1 expression in prostate cancer. Interestingly we also found the increases of cytoplasmic expression of TGFβ-1 in some samples. There were variations of the stromal TGFβ-1 expression level among prostate cancer in our study, and the average values of stained area proportion were of 4%. Most prostate cancers in the present study show negative cytoplasmic staining of TGFβ-1, 19 samples showed positive staining but only on a low level (IRS score 1). TGFβ-1 was known to have a biphasic role in carcinogenesis and has been well

known as a factor of tumour progression in the early phase TGFβ-1 act as a tumour suppressor by inhibiting cell proliferation, and induces cells differentiation as well as apoptosis. However, in the late stage, TGFβ-1 loses its tumour suppressing-function and act as a tumour promoter. In the present study, most of our samples showed a low expression of TGFβ-1 in cancer cells, suggesting a decreased synthesis or secretion of this protein in prostate cancer. The low expression might have roles in the pathogenesis of prostate cancers. Loss of TGFβ-1 function with low expression was also reported in some cancers such as breast, ovarium, oesophagus, and head and neck cancer.

However, in the late stages of carcinogenesis, TGFβ-1 can play an opposite function as a tumour growth promoter. It is suggested that some tumour is actively secreted TGFβ-1 in the late stages of carcinogenesis. The TGFβ-1 in these tumour plays a complex role related to angiogenesis, immune cells suppression, cellular transformation related to invasiveness and metastatic ability, as well as mediating interactions of tumour cells and extracellular matrix. Interestingly in our present study, we found 5 samples with higher expression of TGFβ-1 (IRS score 4). The high TGFβ-1 expression on some cancer cells raises the question of whether these differences related to the biological behaviour of tumour cells, the tendencies to metastasise or other clinical outcomes. Further study is required to answers these questions in prostate cancers.

### *The Expression of IL-6 on Prostate Lesions; Prostate Hyperplasia and Prostate Cancer*

Chronic inflammation plays a significant role in the initiation and progression of the prostate lesion. Inflammation was believed to have a strong correlation to prostatitis, prostatic hyperplasia and prostate cancer. Inflammation will invite T cells, B cells and macrophages to the prostate glandular structures and stroma. After the initiation process, the dendritic cells will be activated and maintained the T cells responses within the prostate gland; this will cause a chronic and progressive pathological process that will eventually facilitate the progression of prostate hyperplasia or prostate cancer.

Immune cells infiltration will increase the secretion of a pro-inflammatory cytokine such as IL-2, IL-6, and IL-8. The activation of various cytokines will disrupt the balance of cell proliferation a d apoptosis. IL-6 is produced by various type of cells, including macrophages endothelial, and lymphocytes. IL-6 expression and can be detected both intracellular within cells cytoplasm as well on extracellular matrix. Higher expression of IL-6 was detected on a prostate cancer group with strong intensities. In the other hand, most of the prostate hyperplasia showed weak expression of IL-6.

Our present study showed a similar result with some other study. Engelhard *et al*. (2014) found that the expression of IL-6 in prostate cancer is significantly higher compared to prostate hyperplasia [20]. We also observed a variation of IL-6 expression among prostate cancer; 7 of the samples exhibit a high IL-6 expression of IRS score 9 or above. The strong expression in some of prostate cancer samples raises the question of whether this finding has any relation to the biological behaviour of cancer cells. Duscharla *et al*. (2017) reported that a high serum level of IL-6 is related to the bone metastasis of prostate cancer [33].

IL-6 secreted by immune cells infiltrate will be captured by IL-6-R, this will activate JAK, STAT3 and MAPK pathway, these, in turn, will induce cell proliferation through androgen receptor inductionangiogenesis and facilitates metastasis. IL-6 are also known to induce intraprostatic testosterone through activation of steroidogenic enzymes. Iliopoulos *et al*. (2009) suggested the correlation between inflammation, IL-6 activation, STAT1, PI3K, and NFkB in the pathogenesis of prostate cancer. Another study also confirmed that the increase of IL-6 was correlated to the prognosis and showed a negative relation to the survival rate. Based on the above results, the present study supports the theory that IL-6 plays a role significant role in the pathogenesis and progression of prostate cancer (34).

However, in the present study, the expression of IL-6 was not correlated to the Gleason score significantly. Our study employed immunohistochemistry to detect the expression of IL-6 in prostate tissues. The nature of IL-6 as a soluble cytokine could sometimes be difficult to be measured quantitatively by immunohistochemistry. Further study is required to investigate the relation of IL-6 to the Gleason score quantitatively, using a different and more sensitives method.

In summary, the present study reveals the differential expression of TGFβ-1 and IL-6 between prostate hyperplasia and prostate cancer. The TGFβ-1 were highly expressed in the stromal component of prostate hyperplasia, compared to prostate cancer. In the other hand, the IL-6 showed a higher expression in prostate cancer cells compared to prostate hyperplasia. Further study is required to investigate the function of each cytokine in the pathogenesis of prostate lesion.

## References

1. Abedelmalek S, Wong DP, Chtourou H, Souissi N, Tabka Z. Racial Variation of Interleukin-6 in Soccer Players: The Effect of Short-Term Maximal Exercise. International Journal of Medicine and Pharmaceutical Sciences (IJMPS). 2014; 4(1):37-48. https://doi.org/10.1080/09291016.2014.904574

2. Elkahwaji JE. The role of inflammatory mediators in the development of prostatic hyperplasia and prostate cancer. Res Rep Urol. 2012; 5:1-10. https://doi.org/10.2147/RRU.S23386 PMid:24400229 PMCid:PMC3826944

3. De Nunzio C, Kramer G, Marberger M, Montironi R, Nelson W, Schröder F, et al. The controversial relationship between benign prostatic hyperplasia and prostate cancer: The role of inflammation. Eur Urol. 2011; 60(1):106-17. https://doi.org/10.1016/j.eururo.2011.03.055 PMid:21497433

4. Gandaglia G, Briganti A, Gontero P, Mondaini N, Novara G, Salonia A, Sciarra A, Montorsi F. The role of chronic prostatic inflammation in the pathogenesis and progression of benign prostatic hyperplasia (BPH). BJU international. 2013; 112(4):432-41. https://doi.org/10.1111/bju.12118 PMid:23650937

5. Bardan R, Dumache R, Dema A, Cumpanas A, Bucuras V. The role of prostatic inflammation biomarkers in the diagnosis of prostate diseases. Clinical biochemistry. 2014; 47(10-11):909-15. https://doi.org/10.1016/j.clinbiochem.2014.02.008 PMid:24560954

6. Untergasser G, Madersbacher S, Berger P. Benign prostatic hyperplasia: age-related tissue-remodeling. Experimental gerontology. 2005; 40(3):121-8. https://doi.org/10.1016/j.exger.2004.12.008 PMid:15763388

7. Xu H, Hu MB, Bai PD, Zhu WH, Liu SH, Hou JY, Xiong ZQ, Ding Q, Jiang HW. Proinflammatory cytokines in prostate cancer development and progression promoted by high-fat diet. BioMed research international. 2015; 2015. https://doi.org/10.1155/2015/249741 PMid:25722971 PMCid:PMC4334627

8. Sciarra A, Mariotti G, Salciccia S, Gomez AA, Monti S, Toscano V, et al. Prostate growth and inflammation. J Steroid Biochem Mol Biol. 2008; 108(3-5):254-60. https://doi.org/10.1016/j.jsbmb.2007.09.013 PMid:17935971

9. Sfanos KS, De Marzo AM. Prostate cancer and inflammation: the evidence. Histopathology. 2012; 60(1):199-215. https://doi.org/10.1111/j.1365-2559.2011.04033.x PMid:22212087 PMCid:PMC4029103

10. McLaren ID, Jerde TJ, Bushman W. Role of interleukins, IGF and stem cells in BPH. Differentiation. 2011; 82(4-5):237-43. https://doi.org/10.1016/j.diff.2011.06.001 PMid:21864972 PMCid:PMC3873782

11. Hamid AR, Umbas R, Mochtar CA. Recent role of inflammation in prostate diseases: chemoprevention development opportunity. Acta Med Indones. 2011; 43(1):59-65.

12. Bergamini S, Bellei E, Bonetti LR, Monari E, Cuoghi A, Borelli F, Sighinolfi MC, Bianchi G, Ozben T, Tomasi A. Inflammation: an important parameter in the search of prostate cancer biomarkers. Proteome science. 2014; 12(1):32. https://doi.org/10.1186/1477-5956-12-32 PMid:24944525 PMCid:PMC4061775

13. Thapa D, Ghosh R. Chronic inflammatory mediators enhance prostate cancer development and progression. Biochemical pharmacology. 2015; 94(2):53-62. https://doi.org/10.1016/j.bcp.2014.12.023 PMid:25593038

14. Chughtai B, Lee R, Te A, Kaplan S. Inflammation and benign prostatic hyperplasia: clinical implications. Current urology reports. 2011; 12(4):274-7. https://doi.org/10.1007/s11934-011-0191-3 PMid:21519898

15. Tiwari A. Elocalcitol, a vitamin D3 analog for the potential treatment of benign prostatic hyperplasia, overactive bladder and male infertility. IDrugs: the investigational drugs journal. 2009; 12(6):381-93.

16. Adorini L, Penna G, Fibbi B, Maggi M. Vitamin D receptor agonists target static, dynamic, and inflammatory components of benign prostatic hyperplasia. Annals of the New York Academy of Sciences. 2010; 1193(1):146-52. https://doi.org/10.1111/j.1749-6632.2009.05299.x PMid:20398021

17. Manchanda PK, Kibler AJ, Zhang M, Ravi J, Bid HK. Vitamin D receptor as a therapeutic target for benign prostatic hyperplasia. Indian J Urol. 2012; 28(4):377-81. https://doi.org/10.4103/0970-1591.105745 PMid:23450267 PMCid:PMC3579114

18. Porcaro AB, Novella G, Molinari A, Terrin A, Minja A, De Marco V, Martignoni G, Brunelli M, Cerruto MA, Curti P, Cavalleri S. Prostate volume index and chronic inflammation of the prostate type IV with respect to the risk of prostate cancer. Urologia internationalis. 2015; 94(3):270-85. https://doi.org/10.1159/000362176 PMid:25170543

19. Kashyap M, Pore S, Wang Z, Gingrich J, Yoshimura N, Tyagi P. Inflammasomes are important mediators of prostatic inflammation associated with BPH. Journal of inflammation. 2015; 12(1):37. https://doi.org/10.1186/s12950-015-0082-3 PMid:25991911 PMCid:PMC4436794

20. Engelhardt PF, Seklehne S, Brustrmann H, et al. Immmuno Histochemical Espression of Interleukin-2 Receptor and Interleukin -6 in Patients with Prostate Cancer and Benigh Prostatic Hiperplasia: Association with asymptomatic Inflamatory Prostatitis NIH Category IV. Scan J Urol. 2014; 49(2):120-126. https://doi.org/10.3109/21681805.2014.971427 PMid:25363611

21. Basanta D, Strand DW, Lukner RB, et al. The Role of Transforming Growth Factor-β Mediated Tumor-Stroma Interactons in Prostate Cancer Progression: An Integrative Approach. Cancer Res. 2009; 69(17):7111-7120. https://doi.org/10.1158/0008-5472.CAN-08-3957 PMid:19706777 PMCid:PMC2748342

22. Funahashi Y, O'Malley KJ, Kawamorita N, et al. Upregulation of Androgen-Responsive Gen and Transforming Growth Factor β1 Cascade Genes in a Rat Model of Non-Bacterial Prostatic Inflamation. Prostate. 2014; 74(4):337-345. https://doi.org/10.1002/pros.22668 PMid:24446128 PMCid:PMC3898594

23. Lucia MS, Lambret JR. Growth Factor in Benign Prostatic Hyperplasia: Basic Science Implications. Curren Prostate Reports. 2007; 5:78-84. https://doi.org/10.1007/s11918-007-0011-x

24. Cao Z, Kyprianou N. Mechanism Navigating theTGF-β Pathway in Prostat Cancer. Asian Journal of Urology. 2015; 2:11-1. https://doi.org/10.1016/j.ajur.2015.04.011 PMid:29051866 PMCid:PMC5645057

25. Kattan MW, Shariat SF, Andrews B, et al. The addition of Interleukin 6 Soluble Receptor and Transformng Growth Factor Beta, improves a Preoperative Nomogram for Predicting Biochemical Progression in Patients with Clinically Located Prostate Cancer, Journal of Clinical Oncology. 2003; 21(9):3573-3579. https://doi.org/10.1200/JCO.2003.12.037 PMid:12913106

26. Smith PC, Hobisch A, Lin DL, et al. Interleukin 6 and Prostate Cancer Progression. Cytokine Growth Factor. Reviews. 2001; 12:33-40. https://doi.org/10.1016/S1359-6101(00)00021-6

27. Pace G, Di Massimo C, De Amicis D, Vicentini C, Ciancarelli MGT. Inflammation and endothelial activation in benign prostatic hyperplasia and prostate cancer. Int Braz J Urol. 2011; 37(5):617-22. https://doi.org/10.1590/S1677-55382011000500008 PMid:22099274

28. Cung Z, Steiner H, Bartsch, et al. Interleukin-6 Regulation of Prostate Cancer Cell Growth. Journal of Cellular Biochemistry. 2005; 95;497-505. https://doi.org/10.1002/jcb.20477 PMid:15838876

29. Bostanci Y, Kazzazi A, Momtahen S, Laze J, Djavan B. Correlation between benign prostatic hyperplasia and inflammation. Current opinion in urology. 2013; 23(1):5-10. https://doi.org/10.1097/MOU.0b013e32835abd4a PMid:23159991

30. Steiner MS, Zhou ZZ, Tonb DC, et al. Expression of Transforming Growth Factor β1 in Prostat Cancer Endocrinology. 1994; 135(5):2240-2246. https://doi.org/10.1210/endo.135.5.7956947 PMid:7956947

31. Flavel RA, Sanjabi S, Wrzesinski SH, et al. The Polarization of Immune Cells in the Tumor Environment by TGF-β. Nature Reviews Immunology. 2010; 10:554-567. https://doi.org/10.1038/nri2808 PMid:20616810 PMCid:PMC3885992

32. Zuhirman. Hubungan Transforming Growth Factor β1, Tumor Necrosis Factor α, Matrix Metallo Proteinase-1 dan Fibroblast Growth Factor 2 dengan Fibrosis Ureter pada penderita batu ureter. Disertasi; Fakultas Kedokteran Universitas Andalas Padang, 2014:133-139.

33. Duscharla D, Reddy KRK, Dasari C, et al. Interleukin-6 Induced Over expression of valosin-Containing Protein (VCP) / p97 is Associated with Androgen-Independent Prostate Cancer (AIPC) Progession Wiley Cellular Physiology. 2018; 1-17. https://doi.org/10.1002/jcp.26639 PMid:29693262

34. Illiopoulos, D., Hirsch, H.A and Struhl, K. An epigenetic switch involving NF-kB, Lin28, let-7 microRNA, and IL6 links inflammation to cell transformation. Cell. 2009; 139(4):693-706. https://doi.org/10.1016/j.cell.2009.10.014 PMid:19878981 PMCid:PMC2783826

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Jun 30; 7(12):1911-1916.
https://doi.org/10.3889/oamjms.2019.543
eISSN: 1857-9655
***Basic Science***

# The Comparison of *RhoC* and *PI3K* Gene Expression on the Breast Cancer Tissue and Benign Tumour Tissue

Dessy Arisanty[1*], Wirsma Arif Harahap[2], Daan Khambri[2], Rony Rustam[2], Gestina Aliska[3], Affifatul Achyar[4], Juane Plantika Menra[4]

*[1]Department of Biochemistry, Faculty of Medicine, Andalas University, Padang, Indonesia; [2]Division of Surgical Oncology, Medical School of Dr. M. Djamil Hospital, Andalas University, Padang, Indonesia; [3]Department Farmacology, Faculty of Medicine, Andalas University, Padang, Indonesia; [4]Biomedical Laboratory, Molecular Division, Faculty of Medicine, Andalas University, Padang, Indonesia*

**Citation:** Arisanty D, Harahap WA, Khambri D, Rustam R, Aliska G, Achyar A, Menra JP. The Comparison of *RhoC* and *PI3K* Gene Expression on the Breast Cancer Tissue and Benign Tumour Tissue. Open Access Maced J Med Sci. 2019 Jun 30; 7(12):1911-1916. https://doi.org/10.3889/oamjms.2019.543

**Keywords:** RhoC gene; PI3K gene; Real-time PCR (qPCR); Cloning; Vector

***Correspondence:** Dessy Arisanty. Department of Biochemistry, Faculty of Medicine, Andalas University, Padang, Indonesia. E-mail: dessyarisanty@med.unand.ac.id

**Received:** 08-Apr-2019; **Revised:** 07-Jun-2019; **Accepted:** 08-Jun-2019; **Online first:** 30-Jun-2019

**Copyright:** © 2019 Dessy Arisanty, Wirsma Arif Harahap, Daan Khambri, Rony Rustam, Gestina Aliska, Affifatul Achyar, Juane Plantika Menra. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

**Funding:** This research did not receive any financial support

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

**Abstract**

**BACKGROUND:** The expression of a gene is a process that conveys information of genes to synthesise gene product is functional. Alterations of the molecular biology in breast cancer are very complex because of many factors play a role in the tumorigenesis. *RhoC* is a prometastases gene. The *PI3K* gene is crucial in the regulation of multiple cellular functions, including cell growth, proliferation, metabolism and angiogenesis.

**AIM:** This study aims to compare of *RhoC* and *PI3K* gene expression on the breast cancer tissue and benign tumour tissue.

**MATERIAL AND METHODS:** Expression of the *RhoC* and *PI3K* genes was carried out with qPCR. The absolute quantification method was using breast cancer tissue. As a comparison, benign tumours (FATs) tissue was carried out. The standard curves were obtained from cloning target genes, which were inserted into the gGEMT-easy vector from *E. coli*. The gene expression data was carried out by t-test to see the mean difference between the expression of breast cancer tissue and benign tumours (FATs) with a value of $p \leq 0.005$ in *RhoC* and *PI3K* gene expression. And the relationship between expressions was done by Pearson correlation test.

**RESULTS:** The results showed that it was found that *PI3K* gene expression on breast cancer tissue was higher than the number in a benign tumour (fibroadenoma mammae) as an endogenous control. And also, the expression of *RhoC* is much lower on breast cancer tissue compared with a benign tumour.

**CONCLUSION:** This study concluded that expression of *RhoC* affects the expression of *PI3K* so that the thing this is what causes the proliferation and began to provide support aggressive cancer cells in the breast.

## Introduction

Fibroadenoma, or fibroadenoma mammae (FAM), is one of the most common types of benign tumours that occur in the breast. Fibroadenoma is round with a firm boundary and has a chewy consistency with a smooth surface [1]. The majority of breast disorders are benign lesions; malignant lesions are only 20% of all abnormalities in the breast. The incidence of this benign disorder begins at the age of the second decade, and the peak is in the fourth and fifth decades of life. A small portion of benign tumours is associated with breast cancer [2].

Cancer is a non-communicable disease characterised by abnormal/continuous and uncontrolled cell growth that can damage the surrounding tissue and can spread to places far from its origin called metastasis. Cancer cells can originate or grow from any cell in the human body. Cancer has become a health problem in the world, including

Indonesia. The type of cancer that many women suffer and fear is breast cancer [3].

Histopathologically most mammary lesions consist of one or more lumps whose shapes and sizes vary greatly. These lumps can be firmly bound or not, single or multiple nodules, soft or hard, can be moved from the bottom or not. This can help distinguish benign lesions or malignant lesions in the breast [4], [1]. However, in molecular biology, there is still a little-known difference in genetic profile between fibroadenoma mammae and Ca mammae (breast cancer). The profile of gene expression in breast cancer has been studied intensively. Gene expression profiling is enabling scientists to understand the heterogeneous nature of breast cancer on a genomic level.

Breast cancer is currently a problem in the health sector because the incidence of breast carcinoma increases from year to year in both developed countries and developing countries like Indonesia. The breast cancer mortality rate also increased sharply [5]. Based on the Globocan estimate, the 2012 International Agency for Research on Cancer (IARC), breast cancer is cancer with the highest percentage of new cases (43.3%) and the highest percentage of deaths (12.9%) in women in the world. Based on data from the Indonesian Ministry of Health (2010), the prevalence of breast cancer in Indonesia reached 0.5 per 1000 women [6].

RhoC is known to be a pro-metastasis gene belonging to the RAS superfamily. RhoC expression increases in gastric cancer, and it will activate the PI3K / Akt pathway to induce the cell invasion. This mechanism is different in some cancers [7]. RhoC is also an effector on the MAPK pathway that increases VEGF, fibroblast growth factors, and regulates the expression of IL-6 and IL-8 [8], [9]. Changes in the expression of RhoC are associated with increased cell proliferation and cause tumours to become malignant [10]. RhoC is a negative mediator from affects the PI3K/Akt and MAPK pathways [11].

PI3Ks are a family of intracellular enzymes that are associated with signal transduction, are involved in cellular functions such as cell growth, proliferation, differentiation, motility, survival and intracellular traffic and are in turn involved in cancer [12]. The PI3K/Akt pathway is activated by changes in the expression of RhoC protein [13]. On the PI3K line, activating Akt is called the PI3K/Akt line. This pathway contains many activators, inhibitors, effectors and second messengers. Several studies have shown that high activity of PI3K/Akt signals will induce resistance of chemotherapy and HER-2 therapy targets. Activation in the PI3K/Akt pathway will promote cell proliferation [14].

Activation of PI3K/Akt causes interference with cell growth control. If there is a change in expression, it will cause metastasis, angiogenesis and therapeutic resistance and reduce PTEN activity [15]. Thus, the molecular mechanism that passes through the complex pathway into the future is considered to be one of the most attractive targets for the development of anticancer agents [16].

Also, PI3K pathways are stimulated as a result of many growth physiological factors and regulators. Whatever the mechanism, PI3K activation will cause a disruption of control of normal cell growth and cell continuity, which contributes to competitive growth, metastatic ability, frequent resistance to therapy. This pathway is an attractive target for the development of new anti-cancer [13]. The expression profile has the potential to differentiate between Fibroadenoma and breast cancer. So, this can be used as a guide for the diagnosis and future prognosis. Therefore, the RhoC inhibition target is possible as an alternative path in activating PI3K. So PI3K has the potential to target the care of breast cancer people [17], [18]. Changes in the level of mRNA, which will be related to expression can be used as biomarkers to detect disease early and can be intervened at the stage of disease progression.

## Material and Methods

### *Sample*

The sample is fresh tissue of breast cancer and benign tumour tissue stored in the BioBank Biomedical Laboratory Tissue. Samples from breast cancer and benign tumour tissue consisted of 30 breast cancer tissues and 30 benign tumour tissues. For breast cancer tissue taken from 30-50 years old. This group was taken because it is the largest population of breast cancer whose tissue is stored in the BioBank Tissue of Faculty of Medicine, Andalas University.

### *Total RNA isolation*

Total RNA from breast cancer tissue and benign tumours (FAM) was isolated using Pure Link Ambion RNA isolation kit (12183018A). The initial stage of sample preparation is homogenised by using the stator-rotor technique to make homogeneous from the tissue. After the isolation process is complete, save the total RNA at -80°C.

### *cDNA synthesis*

The synthesis composition of the total cDNA was 5 µg total RNA, 1 x RT buffer, 20 pmol oligodT, 4 mM dNTP, 10 mM DTT, 40 U SuperScript TMII RTase and $H_2O$-DEPC enzymes with a reaction volume of 20 µl. Total cDNA synthesis was carried out at 52°C for 50 minutes with the work protocol by the manual kit

(Isd cDNA synthesis, Biorad). Check the success of cDNA synthesis using NanoDrop.

**Table 1: Primer design**

| No. | Primer | Nucleotide Sequence |
|---|---|---|
| 1. | RhoC | 5'- GCCCGGCCCGACCCGACCGCACC-3' |
| | | 3'- GGTAACCGATCAGAATGACAACA-5' |
| 2. | PI3K/Akt | 5'- CGACCCAACCCAAGAATCTATC- 3' |
| | | 3'- AGGTGGTCACTTGGTCTTTATTC-5' |

### *Amplification of the real-time PCR of the target gene*

Each gene was amplified with an SYBR Green amplification kit. The PCR program as follows: Predenaturation of 95.0°C for 3 minutes. Denaturation of 95.0°C for 5 seconds, annealing gradient of 50-60°C for 5 seconds, for 39 cycles. Add a melting curve of 65.0°C to 95.0°C with an increase of 0.5°C for 5 seconds. The miRNA is amplified wherein it contains the primary HSA-miR-16-5p LNA PCR set, HSA-MIR-10b-5p LNA ™ PCR primer set and HSA-miR-21-5p primary PCR LNA set (Exiqon, Denmark), ExiLENT SYBR Green master (Exiqon, Denmark). The PCR program as follows Predenaturation 95.0°C for 10 minutes, Denaturation 95.0°C for 10 seconds, gradient annealing 55 – 65°C for 1 minute, for 39 cycles. Add a melting curve of 60.0°C to 95.0°C with an increase of 0.5°C for 5 seconds.

### *Data analysis*

The expression of PI3K and RhoC genes is done by absolute quantification examination. Making standard curves from target genes is based on the Whelan *et al*., 2003 method [19]. Differences in mean expression of PI3K and RhoC between breast cancer tissue and benign tumours were carried out by t-test with 95% significance level at $p \leq 0.05$. Furthermore, the relationship between RhoC and PI3K gene expression was carried out by the Pearson correlation test with a correlation value of 0-1.

## Results

### *qPCR RhoC gene*

Plasmids with the insertion of the RhoC gene for standard curves and target genes together, so that the standard curve is obtained, as shown in Figure 1 below. From the results of run qPCR, the melting curve is obtained, as shown in Figure 1b.

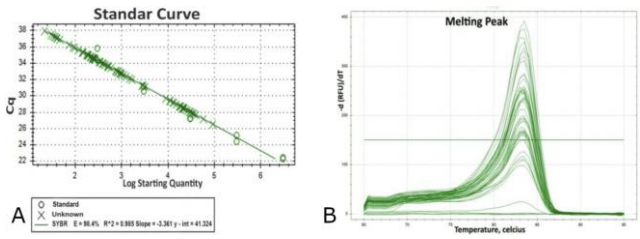



*Figure 1: Standard Curve graph of PI3K gene insertion in vector and graph melting peak RhoC gene from Real-time PCR*

The results of the expression of the RhoC gene from absolute quantification Real-time PCR were obtained in the form of a copy number, and from this study, it was found that the expression of the RhoC gene in breast cancer tissue was lower than the RhoC gene expression in benign tumour tissue (Figure 2).

**Table 2: RhoC gene expression between breast cancer tissue and fibroadenoma tissue**

| Group | Expression | SD | *p* |
|---|---|---|---|
| Breast Cancer Tissue | 3.701 | 0.756 | 0.005 |
| Fibroadenoma Tissue | 4.463 | 0.939 | |

From this study, there was a significant difference in RhoC gene expression between breast cancer tissue and benign tumour tissue with a value of $p < 0.05$ ($p = 0.0001$)

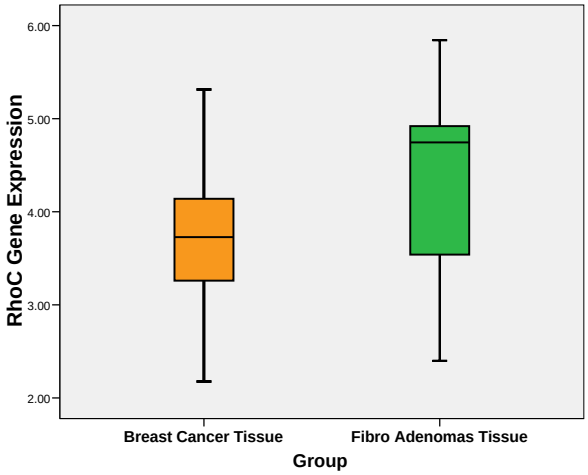



*Figure 2: Boxplot graph of RhoC gene expression in breast cancer tissue and fibroadenoma tissue (benign tumour)*

### *qPCR PI3K gene*

Plasmids with PI3K gene insertion for standard curves and target genes are concurrent so that the standard curve is obtained, as shown in figure 3a below. From the results of running qPCR, the melting curve is obtained, as shown in Figure 3b.

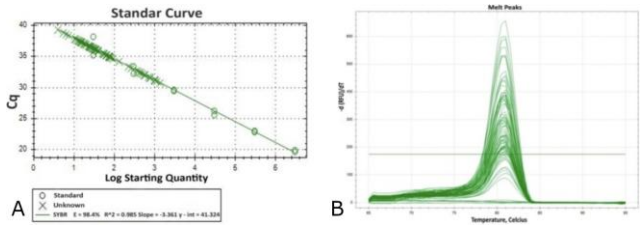



*Figure 3. Standard Curve graph of PI3K gene insertion on vector and graph melting peak PI3K gene from Real-time PCR*

The results of the expression of the PI3K gene from absolute quantification of real-time PCR were obtained in the form of copy number values.

**Table 3: PI3K gene expression between breast cancer tissue and fibroadenoma tissue**

| Group | Expression | SD | *p* |
|---|---|---|---|
| Breast Cancer Tissue | 4.171 | 0.569 | 0.001 |
| Fibroadenoma Tissue | 3.620 | 0.667 | |

From this study, it was found that the expression of the PI3K gene in breast cancer tissue was higher than the PI3K gene expression in benign tumour tissue.

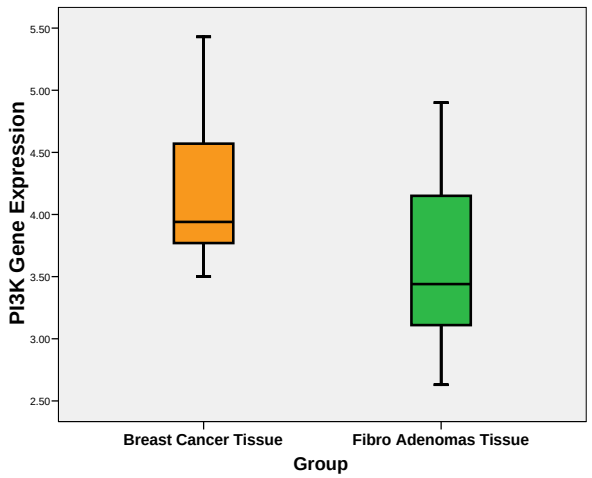



*Figure 4: Boxplot graph of PI3K gene expression in breast cancer tissue and fibroadenoma tissue (benign tumour)*

## Discussion

Molecular changes in the growth of breast cancer are very complex. At first, this change was based on changes in three groups of genes that control cancer growth. The main hereditary gene that influences the growth of breast cancer known today is BRCA1 / 2, which is a suppressor tumour gene. It turns out that this gene only contributes 15% [20].

The expression of the RhoC gene in breast cancer tissue is lower than fibroadenoma tissue (FAMs). The difference in expression of this RhoC gene between breast cancer tissue and fibroadenoma was significant at $p = 0.0001$ ($p < 0.005$). It is suspected that the breast cancer tissue in this sample is still in the early stages of breast cancer and has not experienced metastasis. Lower expression of the RhoC gene in breast cancer tissue than FAMs is likely because breast cancer tissue is a network of primary breast cancer/primary tumour.

Lower expression of the RhoC gene in breast cancer tissue than FAMs is likely because breast cancer tissue is a network of primary breast cancer/primary tumour. But on the other hand, in other studies on breast cancer tissue that had metastasised an increased expression of RhoC when compared to normal tissue. But it is not stated whether the normal network is the source. Because it can be assumed that it is different between normal networks and FAMs tissue, these results can explain the underlying mechanism and provide new therapeutic targets for inhibiting invasion and metastasis from cancer cells [21].

Conversely, if the expression of RhoC is excessive, this causes degradation and reconstruction of the Extracellular Matrix (ECM), which helps cells escape from their tissues. So that it will increase cell motility and increase the ability to be invasive [22]. Uniquely from this protein RhoC, gene damage (mutation) is not found in RhoC. Changes in the expression are not only caused by mutations, but there are other factors involved, such as the effect of epigenetic miRNA and chromosome modification and can also be caused by disturbances at the post-transcription and post-translational stages.

RhoC functions as a button in the signal transduction of the cascades. RhoC promotes the reorganisation of the cytoskeleton, regulates cell shape and cell motility. RhoC can activate formins such as MDIA1 and FMNL2 to change the shape of the cell cytoskeleton [23], [24]. And also, RhoC affects the PI3K pathway, which is a biomarker of proliferation and initiation of invasion [25]. As is known in human's phosphatidylinositol 3-kinase/AKT is a target of the rapamycin (PI3K / Akt / mTOR) pathway which plays an important role in the intracellular signalling system that guides cell growth and cell defence. It is estimated that 70-75% of breast cancers express the ER-associated with this pathway. Many patients with ER + will be more resistant to chemotherapy. Knowing the state of PI3K expression will help in the prognosis for how long to use endocrine hormone therapy and provide an overview of the use or delay of using chemotherapy [26].

In this study, it was found that the expression of PI3K in breast cancer tissue was higher than that of Fibroadenoma. James Ryan and colleagues also found that PI3K expression in breast cancer tissue was higher than in Fibroadenoma [27].

Activation of PI3K activates Akt, so it is known

as PI3K/Akt, which disrupts cell growth control. This is what happens in breast cancer tissue has higher expression of PI3K compared to FAMs tissue. So that if there is a change in expression, it will potentially cause metastasis, angiogenesis and therapeutic resistance. Besides that, the increase in expression of PI3K will reduce PTEN activity [15]. The PI3K/Akt pathway is activated by changes in the expression of RhoC protein. The high intracellular activity on the phosphatidylinositol-3 kinase (PI3K) pathway is common in breast cancer [13]. Thus, the molecular mechanism that passes through the complex pathway into the future is considered one of the most attractive targets for the development of anticancer agents [16]. Activation in the PI3K/Akt pathway will promote cell proliferation [14]. Although the expression of PI3K is higher in breast cancer tissue, when expression of RhoC is still low in breast cancer tissue can be used as a signal that the cancer cell has not yet invaded, because it has not affected the extracellular matrix to change, where changes in the extracellular matrix indicate that the cell cancer will experience invasion and metastasis.

Also, PI3K pathways are stimulated as a result of many growth physiological factors and regulators. Whatever the mechanism, activation of PI3K will cause disruption of control of cell growth and cell continuity, which contributes to competitive growth and metastatic ability. So that resistance often occurs in therapy. This pathway is an attractive target for the development of new cancellers [13]. Therefore, the target of inhibiting PI3K has the potential to target the care of breast cancer people [17], [18]. And it will be even more interesting, the potential for inhibition of genes that are upstream, namely RhoC, will inhibit the process of cancer cells becoming more malignant and undergoing invasion and metastasis. So that RhoC can be used as a good marker to determine breast cancer that is very aggressive and motile and can provide guidance in therapy for intervention before developing into metastasis so that it can identify primary breast cancer cells with breast cancer that have the potential to metastasise [28]. Of research is it can be concluded that an expression of RhoC affect the expression of PI3K so that the thing this is what causes the proliferation and began to provide support aggressive cancer cells in the breast.

## References


1. Utami VL, Muhartono, Fiana DN, Soleha TU. Characteristic of carcinoma mammae at RSUD Dr. H. Abdul Moeloek Bandar Lampung 2010-2012. J Agromed Unila. 2014; 1(1): 1-7.

2. Murshid KR. A review of mastalgia in patients with fibrocystic breast changes and the non-surgical treatment options. Journal of Taibah University Medical Sciences. 2011; 6(1):1-8. https://doi.org/10.1016/S1658-3612(11)70151-2

3. Word Health Organization, 2015. Cancer. (http://www.who.int/mediacentre/factsheets/fs297/en/index.html), diakses 31 Oktober 2015).

4. Underwood JCE, Cross SS. Patologi umum dan sistemik. Edisi ke-2. Jakarta: EGC. 2010: 543-66.

5. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA: a cancer journal for clinicians. 2015; 65(2):87-108. https://doi.org/10.3322/caac.21262 PMid:25651787

6. Kementrian Kesehatan Republik Indonesia. Pedoman Teknis Pengendalian Kanker Payudara dan Kanker Leher Rahim. Keputusan Menteri Kesehatan Republik Indonesia Nomor 796/ Menkes/ SK/ VII/. Jakarta, Hal 4, 2010.

7. Liu Z, Zhu J, Cao H, Ren H, Fang X. miR-10b promotes cell invasion through RhoC-AKT signaling pathway by targeting HOXD10 in gastric cancer. International journal of oncology. 2012; 40(5):1553-60.

8. Van Golen KL, Wu ZF, Qiao XT, Bao LW, Merajver SD. RhoC GTPase, a novel transforming oncogene for human mammary epithelial cells that partially recapitulates the inflammatory breast cancer phenotype. Cancer research. 2000; 60(20):5832-8.

9. Srivastava S, Ramdass B, Nagarajan S, Rehman M, Mukherjee G, Krishna S. Notch1 regulates the functional contribution of RhoC to cervical carcinoma progression. British journal of cancer. 2010; 102(1):196-205. https://doi.org/10.1038/sj.bjc.6605451 PMid:19953094 PMCid:PMC2813755

10. Horiuchi A, Imai T, Wang C, Ohira S, Feng Y, Nikaido T, Konishi I. Up-regulation of small GTPases, RhoA and RhoC, is associated with tumor progression in ovarian carcinoma. Laboratory investigation. 2003; 83(6):861. https://doi.org/10.1097/01.LAB.0000073128.16098.31 PMid:12808121

11. Yang H, ZHOu J, Mi J, Ma K, Fan Y, Ning J, Wang C, Wei X, Zhao H, Li E. HOXD10 acts as a tumor-suppressive factor via inhibition of the RHOC/AKT/MAPK pathway in human cholangiocellular carcinoma. Oncology reports. 2015; 34(4):1681-91. https://doi.org/10.3892/or.2015.4194 PMid:26260613 PMCid:PMC4564083

12. Engelman JA, Luo J, Cantley LC. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. Nature Reviews Genetics. 2006; 7(8):606. https://doi.org/10.1038/nrg1879 PMid:16847462

13. Hennessy BT, Smith DL, Ram PT, Lu Y, Mills GB. Exploiting the PI3K/AKT pathway for cancer drug discovery. Nature reviews Drug discovery. 2005; 4(12):988-1004. https://doi.org/10.1038/nrd1902 PMid:16341064

14. Rafalski VA, Brunet A. Energy metabolism in adult neural stem cell fate. Progress in neurobiology. 2011; 93(2):182-203. https://doi.org/10.1016/j.pneurobio.2010.10.007 PMid:21056618

15. Stahl JM, Sharma A, Cheung M, Zimmerman M, Cheng JQ, Bosenberg MW, Kester M, Sandirasegarane L, Robertson GP. Deregulated Akt3 activity promotes development of malignant melanoma. Cancer research. 2004; 64(19):7002-10. https://doi.org/10.1158/0008-5472.CAN-04-1399 PMid:15466193

16. Chen YL, Law PY, Loh HH. Inhibition of PI3K/Akt signaling: an emerging paradigm for targeted cancer therapy. Current Medicinal Chemistry-Anti-Cancer Agents. 2005; 5(6):575-89. https://doi.org/10.2174/156801105774574649 PMid:16305480

17. Xue B, Huang W, Yuan X, Xu B, Lou Y, Zhou Q, Ran F, Ge Z, Li R, Cui J. YSY01A, a novel proteasome inhibitor, induces cell cycle arrest on G2 phase in MCF-7 cells via eRα and PI3K/Akt pathways. Journal of Cancer. 2015; 6(4):319-326. https://doi.org/10.7150/jca.10733 PMid:25767601 PMCid:PMC4349871

18. Kuger S, Cörek E, Polat B, Kämmerer U, Flentje M, Djuzenova CS. Novel PI3K and mTOR Inhibitor NVP-BEZ235 Radio sensitizes Breast Cancer Cell Lines under Normoxic and Hypoxic Conditions. Breast Cancer. 2014; 8:39-49. https://doi.org/10.4137/BCBCR.S13693 PMid:24678241 PMCid:PMC3964191

19. Whelan JA, Russell NB, Whelan MA. A method for the absolute quantification of cDNA using real-time PCR. Journal of immunological methods. 2003; 278(1-2):261-9. https://doi.org/10.1016/S0022-1759(03)00223-0

20. Couch FJ, Nathanson KL, Offit K. Two decades after BRCA: setting paradigms in personalized cancer care and prevention. Science. 2014; 343(6178):1466-70. https://doi.org/10.1126/science.1251827 PMid:24675953 PMCid:PMC4074902

21. Wang Y, Li Z, Zhao X, Zuo X, Peng Z. miR-10b promotes invasion by targeting HOXD10 in colorectal cancer. Oncology letters. 2016; 12(1):488-94. https://doi.org/10.3892/ol.2016.4628 PMid:27347170 PMCid:PMC4907168

22. Ikoma T, Takahashi T, Nagano S, Li YM, Ohno Y, Ando K, Fujiwara T, Fujiwara H, Kosai KI. A definitive role of RhoC in metastasis of orthotopic lung cancer in mice. Clinical Cancer Research. 2004; 10(3):1192-200. https://doi.org/10.1158/1078-0432.CCR-03-0275 PMid:14871999

23. Kitzing TM, Wang Y, Pertz O, Copeland JW, Grosse R. Formin-like 2 drives amoeboid invasive cell motility downstream of RhoC. Oncogene. 2010; 29(16):2441-8. https://doi.org/10.1038/onc.2009.515 PMid:20101212

24. Vega FM, Fruhwirth G, Ng T, Ridley AJ. RhoA and RhoC have distinct roles in migration and invasion by acting through different targets. The Journal of cell biology. 2011; 193(4):655-65. https://doi.org/10.1083/jcb.201011038 PMid:21576392 PMCid:PMC3166870

25. Sun HW, Tong SL, He J, Wang Q, Zou L, Ma SJ, Tan HY, Luo JF, Wu HX. RhoA and RhoC-siRNA inhibit the proliferation and invasiveness activity of human gastric carcinoma by Rho/PI3K/Akt pathway. World journal of gastroenterology: WJG. 2007; 13(25):3517-22. https://doi.org/10.3748/wjg.v13.i25.3517 PMid:17659701 PMCid:PMC4146790

26. Gil EM. Targeting the PI3K/AKT/mTOR pathway in estrogen receptor-positive breast cancer. Cancer treatment reviews. 2014; 40(7):862-71. https://doi.org/10.1016/j.ctrv.2014.03.004 PMid:24774538

27. Ryan J, Curran CE, Hennessy E, Newell J, Morris JC, Kerin MJ, Dwyer RM. The sodium iodide symporter (NIS) and potential regulators in normal, benign and malignant human breast tissue. PLoS One. 2011; 6(1):e16023. https://doi.org/10.1371/journal.pone.0016023 PMid:21283523 PMCid:PMC3023714

28. Kleer CG, Van Golen KL, Zhang Y, Wu ZF, Rubin MA, Merajver SD. Characterization of RhoC expression in benign and malignant breast disease: a potential new marker for small breast carcinomas with metastatic ability. The American journal of pathology. 2002; 160(2):579-84. https://doi.org/10.1016/S0002-9440(10)64877-8

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Jun 30; 7(12):1917-1925.
https://doi.org/10.3889/oamjms.2019.342
eISSN: 1857-9655
***Basic Science***

# Chromogenic *in Situ* Hybridization Technique versus Immunohistochemistry in Assessment of HER2/neu Status in 448 Iraqi Patients with Invasive Breast Carcinoma

Ali Hussein Mohammed Ali[1], Alaa Qasim Yahya[2*], Haider Latteef Mohammed[3]

*[1]Histopathology Unit, Central Public Health Laboratories, Baghdad, Iraq; [2]Department of Pathology, Al-Kindy College of Medicine, Baghdad, Iraq; [3]Department of Pathology, Al-Wasiti Teaching Hospital, Bagdad, Iraq*

**Citation:** Mohammed Ali AH, Yahya AQ, Mohammed HL. Chromogenic *in Situ* Hybridization Technique versus Immunohistochemistry in Assessment of HER2/neu Status in 448 Iraqi Patients with Invasive Breast Carcinoma. Open Access Maced J Med Sci. 2019 Jun 30; 7(12):1917-1925.
https://doi.org/10.3889/oamjms.2019.342

**Keywords:** ERBB2; HER2/*neu*; Immunohistochemistry; Chromogenic in situ hybridisation; Breast carcinoma

***Correspondence:** Alaa Qasim Yahya. Department of Pathology, Al-Kindy College of Medicine, Baghdad, Iraq. E-mail: alaakasim1983@yahoo.com

**Received:** 07-Apr-2019; **Revised:** 22-May-2019; **Accepted:** 23-May-2019; **Online first:** 30-Jun-2019

**Copyright:** © 2019 Ali Hussein Mohammed Ali, Alaa Qasim Yahya, Haider Latteef Mohammed. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

**Funding:** This research did not receive any financial support

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

**Abbreviation:** IHC: Immunohistochemistry; FISH: Fluorescence in situ hybridisation; HER2: Human epidermal growth factor receptor 2; ASCO: American society of clinical oncology; FDA: Food and drug administration; CEN: centromere

**Abstract**

**BACKGROUND:** The rapidly growing knowledge regarding factors controlling tumour growth, with the new modalities of therapy acting on the biological activity of the tumours draw the attention of most cancer researches nowadays and represent a major focus for clinical oncology practice. For the detection of HER2/neu protein overexpression and gene amplification, immunohistochemistry (IHC) and in-situ hybridisation (ISH) is the recommended techniques, respectively, with high concordance between the two techniques. The current United Kingdom recommendations for HER2/neu testing are either for a two-tier system using IHC with reflex ISH testing in equivocal positive cases, or a one-tier ISH strategy.

**AIM:** To compare the results of HER2/neu gene status in patients with breast carcinoma obtained by chromogenic in situ hybridisation with those obtained by immunohistochemistry, and to compare these results with hormonal receptors expression by immunohistochemistry and with age of patients.

**METHODS:** Immunohistochemistry technique was used for evaluation of status of estrogen receptors (ER) and progesterone receptors (PR) and HER2/*neu* protein expression in 448 Iraqi patients with invasive breast carcinoma with different grades and histological types and then chromogenic in situ hybridization (CISH) technique was applied for all scores of HER2/*neu* to detect the gene status and compare the results in all negative, equivocal and positive cases by immunohistochemistry (IHC). The cases were referred from different centres, and IHC and CISH techniques were done in central public health laboratory in Baghdad over 28 months, from July 2013 to November 2015. A comparison of the results was made to find the relationship between HER2/*neu* and hormone receptors status and other clinical parameters like patients age.

**RESULTS:** The mean age of the study cases was 49.08 years, ranging from 24 to 83 years. Of the 448 cases of breast carcinoma, 44 (9.8%) cases were of score 0 by IHC, none of them (0%) showed HER2/neu gene amplification by CISH. 71(15.8%) cases were of score 1 by IHC, 15 (21.12%) of them showed HER2/neu gene amplification by CISH, all were of low amplification. There were 306 (68.3%) cases of score 2 by IHC, of which 102 (33.33%) cases showed HER2/*neu* gene amplification by CISH, with 79 (25.81%) of them with low amplification and 23 (7.51%) cases with high amplification, while only one case (0.32%) remained in equivocal category. In score 3, all the 27 (6.0%) cases showed gene amplification with 12 (44.44%) cases with low amplification and 15 (55.55) cases with high amplification with overall percentage of gene amplification in score 3 of 100%. There was a significant inverse relationship between hormone receptors (ER and PR) status and HER2/*neu* gene amplification. No significant relationship was found between the patient's age and HER2/*neu* gene amplification.

**CONCLUSION:** Although immunohistochemistry is a widely used, less expensive and reliable test, we strongly advice performance of chromogenic in situ hybridization in assessment of HER2/neu gene status in all cases diagnosed with breast carcinoma as significant number of cases that were reported as negative by immunohistochemistry showed positive amplification by chromogenic in situ hybridization and can get benefit from anti-HER2 targeted treatments.

## Introduction

ERBB2 (HER2/neu), a human epidermal growth factor receptor, is a member of the tyrosine kinases family that is involved in important signalling pathways controlling cell proliferation and tumour growth and survival [1]. Hyperfunction of this receptor, due to gene amplification and protein overexpression, has been reported and evaluated in different types of cancers [2]. In approximately 15% of early invasive breast carcinoma, ERBB2 (HER2/neu) overexpression is present and serve as a poor prognostic marker and an important predictive of response to specific therapy [3]. Tumours with increased levels of the ERBB2 (HER2/neu) are

characterised by more aggressive histological features, more rapid growth rate and a shortened overall survival and relapse-free time, compared with those with no ERBB2 (HER2/neu) overexpression [4].

The great response achieved with anti-ERBB2 (HER2/neu) targeted therapies in ERBB2 (HER2/neu)-positive breast carcinoma cases in addition to serious side effects of these expensive drugs increase the necessity for accurate assessment of ERBB2 (HER2/neu) status [3].

Also, other studies stated that cases with overexpression of ERBB2 (HER2/neu) are associated with impaired responsiveness to tamoxifen (anti-estrogen targeted treatment) in breast carcinoma that is positive for both ERBB2 (HER2/neu) and hormone-receptor [5].

The rapid development of new, clinically advanced information in this subject can induce challenges for oncologists to understand and properly react to these updates [3].

ERBB2 (HER2/neu) status should be determined in all newly diagnosed, recurrent and metastatic breast carcinoma. Bilateral tumours, histologically different ipsilateral tumours or widely separated tumours should each be assessed. Retesting the non-responding stable or progressive ERBB2 (HER2/neu)-negative tumours particularly the high-grade or those with a long interval time between preoperative biopsy and excisional surgery may be considered but are not recommended routinely because of the lack of evidence [3].

By immunohistochemistry, more than 10% complete strong membrane staining defines a positive status of protein overexpression. In situ hybridisation, either bright field chromogenic or fluorescent is used either initially or in immunohistochemistry equivocal positive cases to detect the presence of ERBB2 (HER2/*neu*) gene amplification [3].

Excellent concordance in results between needle core biopsy and surgical specimens has been obtained using immunohistochemistry (IHC) and *in situ* hybridisation (ISH) [6]. In most centers in United Kingdom, ERBB2 (HER2/neu) testing is performed on diagnostic core biopsy specimens, mainly for the results to be available at the time of postoperative multidisciplinary team (MDT) for treatment plan discussion and also to study the use of neoadjuvant treatment which is increasingly given for operable cases with no repeat on excision specimens needed if the test is strongly positive or negative. However; re-performing the assay on incisional or excisional surgical specimens can be considered in the following conditions:

- When the initial ERBB2 (HER2/neu) test result in a core needle biopsy specimen of a primary breast carcinoma is negative, another ERBB2 (HER2/neu) test could be ordered on the excisional specimen if the tumor grade is 3, if small amount of invasive carcinoma is present in the core biopsy specimen, if resection specimen contains carcinoma of higher grade than that in the core, if results of core biopsy is equivocal for ERBB2 (HER2/neu) by both ISH and IHC, or if there is any doubt about handling of the core biopsy specimen (short fixation time, long ischemic time, different fixatives).

- When the initial ERBB2 (HER2/neu) test result in a core needle biopsy specimen of a primary breast carcinoma is positive, another ERBB2 (HER2/neu) test could be ordered on the excisional specimen in histologic grade 1 carcinoma of the following types: ER and PR positive infiltrative ductal or lobular carcinoma, tubular, mucinous, cribriform or adenoid cystic carcinoma. While a new ERBB2 (HER2/neu) test must not be done in previous histologic types if the initial ERBB2 (HER2/neu) test was negative [6], [7].

Cytology specimens from fine needle aspiration of primary breast carcinoma are not ideal for assessment of ERBB2 (HER2/neu) status by IHC as that differentiation between invasive and *in situ* carcinoma cannot be made on these samples. However, if the cytology specimens are the only available material, or in case of metastasis, some studies indicate that ISH is reliable for assessment of ERBB2 (HER2/neu) status in liquid-based and cell block preparations [8].

In the case of bone metastasis, when ERBB2 (HER2/neu) assessment is required, decalcification techniques can detrimentally affect immunohistochemical assessment, and these decalcified specimens should be better tested with ISH techniques [9], [10].

### *Algorithms to be followed for testing ERBB2 (HER2/neu)*

For determination of ERBB2 (HER2/neu) status, IHC for evaluation of protein overexpression and ISH for assessment of gene amplification status are the recommended techniques. High agreement between protein overexpression and gene amplification is reported [4], [11], [12]. The current recommendations in the United Kingdom for ERBB2 (HER2/neu) testing are for “a two-tier system using IHC with reflex ISH testing if required, using the model shown in figure 1, or a one-tier ISH strategy”. In the usual practice for ERBB2 (HER2/neu) testing IHC is used with the analysis of equivocal positive cases by ISH; however, some laboratories use upfront ERBB2 (HER2/neu) ISH testing, especially if the quality of tissue fixation and processing is questionable [13].

ISH has usually been performed using a fluorescence ISH (FISH) technique. Bright-field ISH can be used to assess ERBB2 (HER2/neu) status with a light microscope and is now considered as an acceptable alternative to FISH [14].

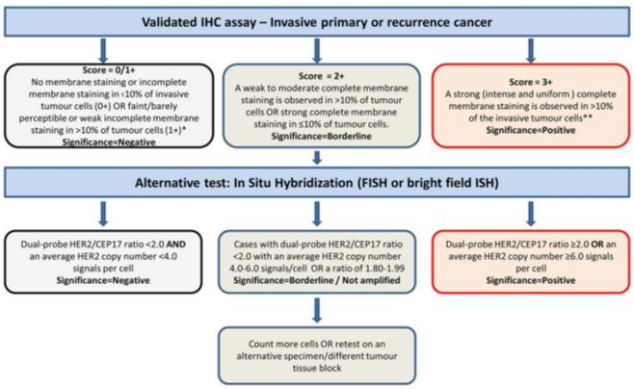



*Figure 1: Recommended ERBB2 (HER2/neu) scoring algorithm for immunohistochemistry (IHC) and in situ hybridisation (ISH); *no sufficient data is available about moderate complete membrane staining in ≤ 10% of tumour cells or strong incomplete membrane staining in > 10% of tumour cells. Repeat on another specimen (tissue block) is advisable; **Membrane staining should be intense and uniform and like chicken-wire. Ignoring incomplete and pale membrane staining in percentage estimation*

The most widely used bright field ISH uses a DNA probe coupled to a silver ISH or chromogenic ISH detection system, or a combination of both [14]. ISH can be performed using either a single probe to enumerate ERBB2 (HER2/neu) copies per nucleus or a dual-probe technique that allows determination of the ERBB2 (HER2/neu): CEP17 ratio and ERBB2 (HER2/neu) gene copy number. Because of this, the use of a chromosome 17 probe is strongly advocated [3].

IHC is a semi-quantitative method for analysis of HER2/neu protein expression, and it is quick, easy and less expensive; however, it is more subjective and susceptible to discrepancies in test results with different laboratory parameters [15]. ISH is a quantitative method for analysis of HER2/neu gene copy number; however, it is more time consuming and costly but more reliable because of its quantitative nature [16], [17]. The choice of most suitable test for HER2/neu status is still an area of discussion with various studies showing and evaluating different opinions regarding the gold standard test algorithm to be followed [18].

### *Chromogenic in situ hybridisation (CISH)*

CISH assays depend on a technique named subtractive hybridisation that uses a DNA probe visualised by a peroxidase reaction. CISH is a new technique that uses a chromogen-labelled probe which offers several advantages. First, simultaneous assessment of tissue morphology and CISH signals at high power using a light microscope. Second, by using the dual probe method, two targets can be detected at the same time. Third, high contrasting between distinct red and green signals. Fourth, it allows quick and easy interpretation of results as compared to IHC, and it is standardised with complete kits and no need for costly fluorescent microscope [19].

### *Fixation and processing of the specimens for HER2/neu assessment*

Proper fixation of specimens used for HER2/neu testing must be ensured and the cold ischemic time (the time between removal of the tissue from the patient and its placing in fixative) should be as short as possible, typically less than one hour [20]. Formalin-fixed, paraffin-embedded tumour tissue samples are suitable for the assay. Tumour samples must be fixed in buffered formalin and embedded in paraffin wax; fixatives that contain alcohol will result in staining of normal tissue and the use of Bouin's fixative will prevent testing by fluorescence in situ-based methods. Other methods of fixation can also badly affect antigen reactivity [3].

At least six hours of fixation are recommended for the core biopsies. Surgical specimens have to be incised as near as possible through the carcinoma to allow good penetration of the fixative and then dissected into 5 – 10 mm slices to ensure rapid penetration and fixation. Tissue must be placed in an adequate volume (typically 1:10; tissue: fixative) of fixative for at least twenty-four hours and not more than three days. Centres that use rapid fixation and processing methods should validate their methodology for HER2/neu assessment [3].

Sections must be stained within one to two days of cutting and drying. Excessive section drying time will lead to loss of HER2/neu expression, and therefore, it is recommended that freshly cut sections are either dried at 60°C for one hour or 37°C overnight [21].

## Material and Methods

Immunohistochemistry technique was used for evaluation of ER, PR status and HER2/neu protein expression in 448 Iraqi patients with invasive breast carcinoma with different grades and histological types and then CISH technique was applied for all scores of HER2/neu to detect the gene status and compare the results with all 115 (25.6%) negative, 306 (68.3%) equivocal and 27 (6.0%) positive cases by immunohistochemistry. The cases were referred from different centres, and IHC and CISH techniques were done in central public health laboratory in Baghdad over three years, from July 2013 to November 2015. A comparison of the results was made to find the relationship between HER2/neu and hormone receptors status and other clinical parameters like patients age. IHC test kit was provided by (Dako, Glostrup, Denmark) and the CISH test kit was provided by Zytovision medical company, ZytoDot ® 2C SPEC ERBB2/CEN 17 Probe.

### *IHC for ER, PR and HER 2*

Multiple sections of (3-4 µm) thickness were cut from breast carcinoma tissue blocks and placed on positively charged slides to overnight incubation at 56ºC. Sections were de-paraffinized via xylene and further rehydrated with graded alcohols to distilled water. After blocking endogenous peroxidase activity with 3% hydrogen peroxide in methanol, antigen retrieval was achieved through heating the slides in 10 mmol/l citrate buffer (pH 6) by using a water bath. Rabbit monoclonal anti-HER-2/*neu* primary antibody (Dako, Glostrup, Denmark) were applied for 60 minutes at dilution of 1:800. The Envision Kit (Dako) was used for the application of the secondary antibody. Signals were developed with Diaminobenzidine (DAB) followed by light nuclear counterstaining by haematoxylin. Each test was run with a known positive and negative control.

For evaluating the immunohistochemistry for the ERBB2 antibody, ASCO/CAP guidelines [6]. Were followed, as shown in Table 1.

**Table 1: Interpretation of results of immunohistochemistry for the ERBB2 antibody**

| Staining pattern | Score | HER2/neu protein overexpression assessment |
|---|---|---|
| No membrane staining or incomplete membrane staining in < 10% of the invasive tumour cells | 0 | Negative |
| A weak to moderate complete membrane staining is observed in > 10% of tumour cells OR strong complete membrane staining in ≤10% of tumour cells | 1+ | Negative |
| A weak to moderate complete membrane staining is observed in > 10% of tumour cells OR strong complete membrane staining in ≤10% of tumour cells | 2+ | Weakly positive/equivocal |
| A strong (intense and uniform) and complete membrane staining in > 10% of invasive tumour cells | 3+ | Strong positive |

For estrogen and progesterone receptor detection, Heat Induced Epitope Retrieval (HIER) was achieved through heating the slides in EDTA buffer (pH 9) for 25 minutes at 95ºC. Primary antibody clones used for ER and PR were DAKO 1D5 (1:200) and DAKO PR 88 (1:200) respectively.

**Table 2: Allred system of scoring for estrogen and progesterone receptors**

| Score | Intensity of staining |
|---|---|
| 0 | Negative |
| 1 | Weak |
| 2 | Intermediate |
| 3 | Strong |
| Score | Percentage of stained cells |
| 0 | No cells are ER/PR positive |
| 1 | < 1% cells are ER/PR positive |
| 2 | 1-10% of cells are ER/PR positive |
| 3 | 11-33% of cells are ER/PR positive |
| 4 | 34-66% of cells are ER/PR positive |
| 5 | 67-100% of cells are ER/PR positive |
| Allred score (intensity + percentage) | Effect of hormone therapy |
| 0-1 | No effect |
| 2-3 | Small (20%) chance of benefit |
| 4-6 | Moderate (50%) chance of benefit |
| 7-8 | Good (75%) chance of benefit |

Interpretation of results was made as per ER/PR reporting guidelines of the Allred scoring system depending on the proportion of stained cell (PS), given score of 0-5 , and intensity of staining (IS), given score of 0-3, in a combined score (AS), from 0/8 to 8/8, results from their summation as shown in table 2 [21]. Proper controls were used as indicated.

### *CISH HER2/neu*

The ZytoDot ® 2C SPEC ERBB2/CEN 17 probe was designed for the simultaneous detection of ERBB2 and centromere 17 in formalin-fixed, paraffin-embedded tissue sections or cell samples.

### *Probe Description*

The Zyto*Dot*® 2C SPEC ERBB2/CEN 17 probe is a mixture of a Digoxigenin-labeled probe specific for the ERBB2 gene at 17q12 and a Dinitrophenyl-labeled CEN 17 probe specific for the alpha satellite centromeric region of chromosome 17 (D17Z1). Using the ZytoDot® 2C SPEC ERBB2/CEN 17 Probe Kit, two green (ERBB2) and two red (CEN 17) signals are expected in a normal interphase nucleus. In a cell with amplification of the ERBB2 gene locus, multiple copies of the green signal or green signal clusters will be observed. The results interpretation followed the manufacturer instructions of ZytoDot 2C SPEC HER2/CEN 17 Probe Kit.

The UK recommendation when using dual probe CISH is to report the HER2/CEP17 signal ratio and HER2/neu copy number [3].

Tumours with a HER2/CEP17 signal ratio ≥ 2.0 and/or a mean HER2/neu gene, copy number ≥ 6 are said to be positive. Cases with a HER2/CEP17 signal ratio < 2.0 with an average HER2/neu copy number < 4.0 signals/cells are said to be negative. The controversy is still present about cases with HER-2/*neu* gene copy number ≥ 6, but the HER2/CEP17 ratio is < 2 [6]. Selection and assessment of normal breast tissue cells to confirm proper hybridisation, successful detection and visualisation, before testing of the invasive carcinoma were done. The numbers of HER2/neu and chromosome 17 signals were scored and recorded, and the mean HER2/neu to chromosome 17 copy ratio is assessed in 20 – 60 cells, where possible, using at least three distinct tumour fields. In cases where either obvious amplification is observed, or the ratio is below 1.5, scoring of just 20 tumour cells is sufficient.

Cells to be assessed and scored are only those with identifiable nuclear borders. Over-damaged, digested, or truncated nuclei were excluded. Only cells with minimum one copy of HER2/neu and CEP17 were scored. In cases with tumour heterogeneity (coexistence of different population of tumour cells having distinct HER2/neu amplification patterns within the same tumour), or the ratio is close to 2.0, or the average copy number is between ≥ 4.0 and < 6.0 signals/cell, additional cells were scored (at least 40).

**Table 3: Evaluation of HER2/neu gene status using chromogenic in situ hybridisation**

| Amplification | Her-2/*neu* gene status |
|---|---|
| High-level | > 10 copies or large cluster of amplicon per nucleus in > 50% of cancer cells |
| Low-level | 6–10 copies or small cluster of amplicon per nucleus in > 50% of cancer cells |
| None | 1–5 copies per nucleus of cancer cells |

## Results

### *Patients' age*

The mean age of the 448 cases of invasive breast carcinoma studied in this study was 49.08 years, ranging from 24 to 83 years old.

### *Estrogen and Progesterone receptors status by IHC*

Expression of estrogen receptors was seen in 279 (62.27%) cases. 66 (14.7%) were with weak expression (AS score 2-3), 143 (31.9%) were with moderate expression (AS score 4-6) and 70 (15.6%) with strong expression (AS score 7-8). Expression of progesterone receptors was seen in 304 (67.85%) cases. Forty-nine (10.9%) were with weak expression (AS score 2-3), 151 (33.7) were with moderate expression (AS score 4-6) and 104 (23.2%) with strong expression (AS score 7-8). As in Table 4.

**Table 4: Estrogen and Progesterone receptors status by IHC**

| Hormone receptors expression | NO expression (AS score 0-1) | Weak expression (AS score 2-3) | Moderate expression (AS score 4-6) | Strong expression (AS score 7-8) |
|---|---|---|---|---|
| ER status | 169 (37.7%) | 66 (14.7%) | 143 (31.9%) | 70 (15.6%) |
| PR status | 144 (32.1%) | 49 (10.9%) | 151 (33.7) | 104 (23.2%) |

### *HER2/neu protein overexpression by IHC*

HER2/neu protein overexpression was positive (score + 3) in 27 (6.0%) cases, equivocally positive (score + 2) in 306 (68.3%) cases and negative (score + 1/0) in 115 (25.6%) cases of the studied specimens. As in Table 5.

**Table 5: HER2/neu protein overexpression by IHC**

| HER2/neu score | No. of the cases | A percentage of the total number |
|---|---|---|
| Score 0 | 44 | 9.8% |
| Score + 1 | 71 | 15.8% |
| Score + 2 | 306 | 68.3% |
| Score + 3 | 27 | 6.0% |

### *CISH results for HER2/neu gene amplification*

HER2/neu gene amplification was seen in 144 (32.14%) specimens of the 448 studied cases. HER2/neu gene was not amplified in all cases (0%) of score 0 by IHC, while all the 27 cases (100%) with score +3 by IHC showed gene amplification by CISH, 12(44.4%) cases showed low amplification and 15 (55.5%) cases showed high amplification. Within cases of score + 1 by IHC, 15 (21.1%) cases show low gene amplification and no case show high amplification.

**Table 6: CISH results for HER2/neu gene amplification**

| Ihc Results for HER2/neu Protein Over-Expression | Cish Results for HER2/neu Gene Amplification | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Total No. of cases | Not amplified | Percentage (%) | Low amplification | Percentage (%) | High amplification | Percentage (%) | Equivocal | Percentage (%) |
| Score 0 | 44 | 44 | 100% | 0 | 0% | 0 | 0% | 0 | 0% |
| Score +1 | 71 | 56 | 78.8% | 15 | 21.1% | 0 | 0% | 0 | 0% |
| Score +2 | 306 | 203 | 66.3% | 79 | 25.8% | 23 | 7.5% | 1 | 0.3% |
| Score +3 | 27 | 0 | 0% | 12 | 44.4% | 15 | 55.5% | 0 | 0% |

Among equivocal positive cases of score + 2 by IHC, 102 (33.3%) cases show HER2 gene amplification, 79 (25.8%) cases with low amplification and 23 (7.5%) cases with high amplification while one (0.3%) case was equivocal. As in Table 6 and Table 7.

**Table 7: Percentage of positive cases for HER2/neu gene amplification by CISH within each score**

| HER2/neu protein expression by IHC | Percentage of positive cases for HER2/neu gene amplification by CISH within each score |
|---|---|
| Negative score 0 | 0 % |
| Negative score 1 | 21.1 % |
| Equivocal positive score 2 | 33.3 % |
| Positive score 3 | 100 % |

### *Relationship between HER2/neu and Estrogen receptor*

HER2/neu gene amplification was present in 70 (41.4%) cases of those with no estrogen receptor expression, while 75 (26.9%) cases of those expressing estrogen receptors showed no HER2/neu gene amplification. There is a significant negative relationship between HER2/neu status and ER expression with a p-value of 0.015. As shown in Table 8.

**Table 8: Relationship between HER2/neu and Estrogen receptors**

| Relationship between HER2/neu and Estrogen receptor | | CISH results | | | | P value |
|---|---|---|---|---|---|---|
| | | Not amplified | percentage | Amplified | percentage | |
| ER status | No expression | 99 | 58.6% | 70 | 41.4% | 0.015 |
| | expression | 204 | 73.1% | 75 | 26.9% | |

### *Relationship between HER2 and Progesterone receptor*

HER2/neu gene amplification was present in 64 (44.4%) cases of those with no progesterone receptor expression, while 81 (26.6%) cases of those expressing progesterone receptors, showed no HER2/neu gene amplification.

There is a significant negative relationship between HER2/neu status and PR expression with a p-value of 0.001. As shown in Table 9.

**Table 9: Relationship between HER2 and Progesterone receptor**

| Relationship between HER2/neu and Progesterone receptor | | CISH results | | | | P value |
|---|---|---|---|---|---|---|
| | | Not amplified | percentage | Amplified | percentage | |
| PR status | No expression | 80 | 55.5% | 64 | 44.4% | 0.001 |
| | expressed | 223 | 73.3% | 81 | 26.6% | |

### *Relationship between HER2/neu and patients age*

HER2/neu gene was amplified in 67 (36.4%) specimens from young patients (45 years and younger), and it was amplified in 78 (29.5%) specimens from old patients (more than 45 years) with no significant relationship between patient's age and HER2/neu gene amplification. As shown in Table 10.

**Table 10: Relationship between HER2 and patients age**

| Relationship between HER2/neu and patients age | | CISH results | | | | P value |
|---|---|---|---|---|---|---|
| | | Not amplified | percentage | Amplified | percentage | |
| Patients age | ≤ 45 years | 117 | 63.6% | 67 | 36.4% | 0.126 |
| | > 45 years | 186 | 70.5% | 78 | 29.5% | |

## Discussion

In Iraq, breast cancer is the most frequent cancer in females with 4824 cases were reported during 2015, with an annual incidence of 13.3 per 100000 population [23].

Overexpression of human epidermal growth factor receptor type 2 (HER2/neu or ErbB-2), a 185-kD receptor first described three decades ago, occurs in many tumours like 48.38% of endometrioid endometrial carcinoma and 20% to 30% of invasive breast carcinomas [24], [25]. Generally, patients with carcinomas that overexpress HER2/neu or that have a high copy number of its gene have worsened overall survival and might have different responses to a variety of hormonal and chemotherapeutic agents [26], [27], [28]. Thus, therapies aimed to target HER2/neu appear to be crucial in treating breast cancer. One such treatment is trastuzumab (Herceptin, Genentech), which is a humanised monoclonal antibody. Trastuzumab binds to an extracellular juxtamembrane domain of HER2/neu and inhibits proliferation and survival of HER2/neu-dependent tumours. It has been approved by the Food and Drug Administration (FDA) for patients with invasive breast carcinoma with HER2/neu overexpression. The choice of a standard gold method for testing HER2/neu status had been an area of argument for a long time with controversy regarding the use of immunohistochemistry with the application of in situ hybridisation in equivocal cases or the use of in situ hybridisation from the start in all cases diagnosed with invasive breast carcinoma [13].

In this study, IHC was done to ascertain the score of HER2/neu protein expression in tumour samples. About two thirds of the cases (306; 68.3%) were equivocally positive (2+ reactivity) whereas 115 (25.6%) case were negative (0, 1+ reactivity) and 27 (6.0%) cases were positive (3+ reactivity).

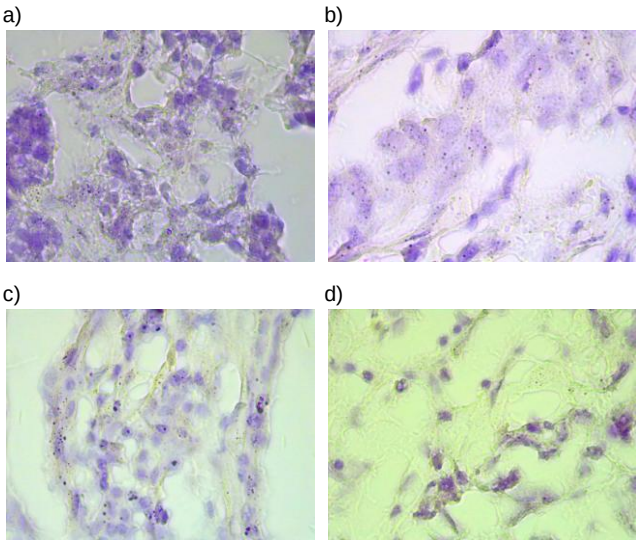



*Figure 2: a) HER2/neu high amplification by CISH in the equivocal case by IHC; b) HER2/neu low amplification by CISH in the equivocal case by IHC; c) HER2/neu equivocal by CISH in the equivocal case by IHC; d) HER2/neu non-amplification by CISH in the equivocal case by IHC*

These figures differ from that reported in other studies like Eswarachary V et al., that showed approximately a third of the cases (143/432, 33.10%) being positive (3+ reactivity) while 47 cases (10.88%) were negative (0, 1+ reactivity) with a substantial number of cases (242/432; 56.01%) showed equivocal positivity (2+ reactivity) [29]. In Varga Z et al., study, 12% of the cases were 3+ on IHC (184 of 1522 cases), 26.5% of the cases were 2+ (404 of 1522), and 61.4% of the cases were negative 0/1+ on IHC (934 of 1522) [30]. This may be attributed to sampling bias as most of the cases in this study were of score 2+ that are routinely submitted for in situ hybridisation to test the HER2/neu gene amplification.

Expression of estrogen receptors was seen in 279 (62.27%) cases in this study, and the expression of progesterone receptors was seen in 304 (67.85%) cases. This is in agreement with Eswarachary V et al., where approximately two thirds (284/432, 65.74%) of their cases were positive for ER receptor expression whereas 59.72% (258/432) of the cases were positive for PR receptor expression [29]. The same results were obtained by Alwan N et al., showing the registered rates of positive ER and PR tumour contents of 67.8% and 65.3% respectively [31].

Analysing the correlation between CISH HER2/neu status and ER/PR expression demonstrated that ER and PR expressions were

higher in HER2/neu negative tumours compared to HER2/neu positive tumours. These inverse associations between the expression of ER/PR and HER2/neu amplification were significant with p-value 0.015(with ER) and p-value 0.001(with PR). This is similar to what was obtained by Eswarachary V et al., but with a still significant number of HER2/neu positive tumours, which also expressed ER/ PR [29]. The inverse relationship between ER/PR status and HER2/neu is logic in that HER2/neu hyper-functioning usually associates with high-grade aggressive tumours in contrary to ER and may explain the cause of poor responsiveness to tamoxifen therapy in a patient who express both ER and HER2/neu [29].

Regarding the correlation between age of the patient and HER2/neu status by CISH, there was no significant correlation between them with p-value 0.126. This is in agreement with Barros et al., [32], while Jabbar N et al., found in their study significant correlation between female age and her HER2/neu amplification by CISH; where increased age was associated more with gene amplification [33].

HER2/neu gene amplification rates in different scores of HER2/neu protein expression by IHC in this study were as follow: 0% in score 0, 21.1% in score 1+ (all were with low amplification), 33.3% in score 2+ (25.8% with low amplification and 7.5% with high amplification with one (0.3%) case equivocal) and 100% in score3+. In a study done by Vocaturo et al., there was no case of 0/1+ by IHC score showed gene amplification by CISH, 53% of score 2+ showed gene amplification and 64% of cases with score 3+ were amplified by CISH [34]. In another study from Iraq done by Khashman B et al., for equivocally positive cases only, 24.5% (12 out of 49) showed low amplification results while 8.1% (4 out of 49) showed high amplification results with 32.6% overall percentage of CISH positive cases within score 2+ [35]. Manuelito A Madrid et al., in their study of 160 cases of invasive breast carcinoma for HER2/neu status, 80 were IHC positive (score 2+ and 3+), and 80 were IHC negative (score 0 and score 1+). With the CISH assay, 58 (36.25%) of their 160 cases showed HER2/neu gene amplification. No case (0%) of the negative ones show gene amplification by CISH, while all cases (100%) of score 3+ showed positive results with CISH, 9 (22.5%) cases with low amplification and 39 (97.5%) cases with high amplification, 18 cases (45%) of score 2+ were positive by CISH, 8 (20%) cases with low amplification and 10 (25%) cases with high amplification [36]. The subjectivity of the immunohistochemical method for assessment of HER2/neu expression lies behind this discrepancy in results between different studies. This may result from up scoring or down the scoring of the expression, so that cases with score 1+ may be reported by others as 2+ or underestimate score 3+ as 2+. For that reason, the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP), human epidermal growth factor receptor 2 (HER2) testing Expert Panel aimed to improve the analytic validity of HER2/neu testing and the clinical utility of HER2/neu as a predictive biomarker for the potential responsiveness to therapies targeting the HER2/neu protein [37]. Eswarachary V et al., in their similar research, but with use of fluorescent in situ hybridisation (FISH) technique for HER2/neu gene status testing, HER2/neu was non-amplified in more than half of their cases (223/432, 51.6%) while it was amplified in 46.3% cases (200/432). IHC was done to evaluate the expression of HER2/neu protein in tumour samples. Approximately one-third of the samples (143/432, 33.1%) were positive (3+ reactivity), whereas 47 samples (10.8%) were negative (0/1+ reactivity). Also, a substantial number of patients (242/432, 56%) showed equivocal 2+ reactivity. Of the equivocal IHC cases, 68 (28.10%) cases were amplified for HER2/neu by FISH, 168 (69.42%) cases were not amplified, and the rest (2.5%) were FISH equivocal. Within score 3+, 91.6% of the cases showed amplification by FISH and, within score 0/1+, only 2.1% of the cases were positive [29].

*Study Limitations:* The pre-analytical variables, including fixation time, environment and tissue processing, could be controlled because the significant number of samples was referred from other centres.

In conclusion, although IHC is a widely used, less expensive and reliable test, we strongly advice performance of CISH in assessment of HER2/nu gene status in all cases diagnosed with breast carcinoma as significant number of cases that were reported as negative by IHC showed positive amplification by CISH and can get benefit from anti-HER2 targeted treatments. In addition to the subjectivity and the several limitations in the interpretation of the IHC test results.

## References

1. Arteaga CL, Sliwkowski MX, Osborne CK, Perez EA, Puglisi F, Gianni L. Treatment of HER2-positive breast cancer: current status and future perspectives. Nature Reviews Clinical Oncology. 2011; 9: 16-32. https://doi.org/10.1038/nrclinonc.2011.177 PMid:22124364

2. Gravalos C, Jimeno A. HER2 in gastric cancer: a new prognostic factor and a novel therapeutic target. Annals of Oncology. 2008; 19:1523-29. https://doi.org/10.1093/annonc/mdn169 PMid:18441328

3. Rakha EA, Pinder SE, Bartlett JM, Ibrahim M, Starczynski J, Carder PJ, Provenzano E, Hanby A, Hales S, Lee AH, Ellis IO. Updated UK Recommendations for HER2 assessment in breast cancer. Journal of clinical pathology. 2015; 68(2):93-9. https://doi.org/10.1136/jclinpath-2014-202571 PMid:25488926 PMCid:PMC4316916

4. Pauletti G, Dandekar S, Rong H. Assessment of methods for tissue-based detection of the HER-2/neu alteration in human breast cancer: a direct comparison of fluorescence in situ

hybridization and immunohistochemistry. J Clin Oncol. 2000; 18:3651-64. https://doi.org/10.1200/JCO.2000.18.21.3651 PMid:11054438

5. Elledge RM, Green S, Ciocca D. HER-2 expression and response to tamoxifen in estrogen receptor-positive breast cancer: a Southwest Oncology Group Study. Clin Cancer Res. 1998; 4:7-12.

6. Wolff AC, Hammond ME, Hicks DG. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. J Clin Oncol. 2013; 31:3997-4013. https://doi.org/10.1200/JCO.2013.50.9984 PMid:24101045

7. Greer LT, Rosman M, Mylander WC. Does breast tumor heterogeneity necessitate further immunohistochemical staining on surgical specimens? J Am Coll Surg. 2013; 216:239-51. https://doi.org/10.1016/j.jamcollsurg.2012.09.007 PMid:23141136

8. Durgapal P, Mathur SR, Kalamuddin M. Assessment of her-2/neu status using immunocytochemistry and fluorescence in situ hybridization on fine-needle aspiration cytology smears: Experience from a tertiary care centre in india. Diagn Cytopathol. 2014; 42:726-31. https://doi.org/10.1002/dc.23088 PMid:24376261

9. Zustin J, Boddin K, Tsourlakis MC. HER-2/neu analysis in breast cancer bone metastases. J Clin Pathol. 2009; 62:542-6. https://doi.org/10.1136/jcp.2008.059717 PMid:19474354

10. Penault-Llorca F, Coudry RA, Hanna WM. Experts' opinion: recommendations for retesting breast cancer metastases for HER2 and hormone receptor status. Breast. 2013; 22:200-2. https://doi.org/10.1016/j.breast.2012.12.004 PMid:23352656

11. Walker RA, Bartlett JM, Dowsett M. HER2 testing in the UK: further update to recommendations. J Clin Pathol. 2008; 61:818-24. https://doi.org/10.1136/jcp.2007.054866 PMid:18381380

12. Press MF, Sauter G, Bernstein L. Diagnostic evaluation of HER-2 as a molecular target: an assessment of accuracy and reproducibility of laboratory testing in large, prospective, randomized clinical trials. Clin Cancer Res. 2005; 11:6598-607. https://doi.org/10.1158/1078-0432.CCR-05-0636 PMid:16166438

13. Sauter G, Lee J, Bartlett JM. Guidelines for human epidermal growth factor receptor 2 testing: biologic and methodologic considerations. J Clin Oncol. 2009; 27:1323-33. https://doi.org/10.1200/JCO.2007.14.8197 PMid:19204209

14. Arnould L, Roger P, Macgrogan Gl. Accuracy of HER2 status determination on breast core-needle biopsies (immunohistochemistry, FISH, CISH and SISH vs FISH). Mod Pathol. 2012; 25:675-82. https://doi.org/10.1038/modpathol.2011.201 PMid:22222637

15. Olsson H, Jansson A, Holmlund B, Gunnarsson C. Methods for evaluating HER2 status in breast cancer: comparison of IHC, FISH, and real-time PCR analysis of formalin-fixed paraffin-embedded tissue. Pathology and Laboratory Medicine International. 2013; 5:31-37. https://doi.org/10.2147/PLMI.S44976

16. Rosa FE, Santos RM, Rogatto SR, Domingues M. Chromogenic in situ hybridization compared with other approaches to evaluate HER2/neu status in breast carcinomas. Brazilian Journal of Medical and Biological Research. 2013; 46:207-16. https://doi.org/10.1590/1414-431X20132483 PMid:23558859 PMCid:PMC3854374

17. Kaufman PA, Bloom KJ, Burris H, Gralow JR, Mayer M, Pegram M, et al. Assessing the discordance rate between local and central HER2 testing in women with locally determined HER2-negative breast cancer. Cancer. 2014; 120:2657-64. https://doi.org/10.1002/cncr.28710 PMid:24930388 PMCid:PMC4232097

18. Allred DC. Issues and updates: evaluating oestrogen receptor, progesterone receptor, and HER2 in breast cancer. Modern Pathology. 2010; 23:S59. https://doi.org/10.1038/modpathol.2010.55 PMid:20436503

19. Reliable and simple detection of genomic alterations using light microscopy. ZytoDotR 2CTM - 2- Color CISH for the detection of genomic alterations. A user manual provided by ZytoVision GmbH - Fischkai 1, 27572 Bremerhaven- Germany. ZytoVision molecular diagnostics simplified, 2019:182-3. www. Zytovision.com

20. Lee AH, Key HP, Bell JA. The effect of delay in fixation on HER2 expression in invasive carcinoma of the breast assessed with immunohistochemistry and in situ hybridisation. J Clin Pathol. 2014; 67:573-5. https://doi.org/10.1136/jclinpath-2013-201978 PMid:24737400

21. Lundgaard Hansen B, Winther H, Moller K. Excessive section drying of breast cancer tissue prior to deparaffinisation and antigen retrieval causes a loss in HER2 immunoreactivity. Immunocytochemistry. 2008; 6:117-22.

22. Allred DC, Bustamante MA, Daniel CO, Gaskill HV, Cruz AB Jr. Immunocytochemical analysis of estrogen receptors in human breast carcinomas. Evaluation of 130 cases and review of the literature regarding concordance with biochemical assay and clinical relevance. Arch Surg. 1990; 125:107-13. https://doi.org/10.1001/archsurg.1990.01410130113018 PMid:1688490

23. Iraqi cancer registry 2015 (page 29). Ministry of Health and Environment/Iraq- Iraqi Cancer Board, 2015. Accessed at: https://moh.gov.iq/upload/upfile/ar/833.pdf

24. Abd Ali Z, Hassan F, Yahya A. Immunohistochemical expression of HER2/neu receptors in Iraqi patients with endometrioid carcinoma. Journal of Clinical and Diagnostic Research. 2018, 12(11):5-8.

25. Schechter AL, Stern DF, Vaidyanathan L. The neu oncogene: an erb-B-related gene encoding a 185,000-Mr tumour antigen. Nature. 1984; 312:513-516. https://doi.org/10.1038/312513a0 PMid:6095109

26. Albanell J, Bellmunt J, Molina R. Node-negative breast cancers with p53 (-)/HER2-neu (-) status may identify women with very good prognosis. Anticancer Res. 1996; 16:1027-32.

27. Ellis MJ, Coop A, Singh B. Letrozole is more effective neoadjuvant endocrine therapy than tamoxifen for ErbB-1- and/or ErbB-2-positive, estrogen receptor-positive primary breast cancer: evidence from a phase III randomized trial. J Clin Oncol. 2001; 19:3808-16. https://doi.org/10.1200/JCO.2001.19.18.3808 PMid:11559718

28. Berry DA, Muss HB, Thor AD. HER-2/neu and p53 expression versus tamoxifen resistance in estrogen receptor-positive, node-positive breast cancer. J Clin Oncol. 2000; 18:3471-79. https://doi.org/10.1200/JCO.2000.18.20.3471 PMid:11032587

29. Eswarachary V, Mohammed I, Jayanna P, PatilOkaly G, Nargund A, Dhondalay G. HER2/neu testing in 432 consecutive breast cancer cases using FISH and IHC - A comparative study. Journal of Clinical and Diagnostic Research. 2017, 11(4):1-5. https://doi.org/10.7860/JCDR/2017/25625.9521 PMid:28571140 PMCid:PMC5449786

30. Varga Z, Noske A, Ramach C, Padberg B and Moch H. Assessment of HER2 status in breast cancer: overall positivity rate and accuracy by fluorescence in situ hybridization and immunohistochemistry in a single institution over 12 years: a quality control study. BMC Cancer. 2013; 13:615. https://doi.org/10.1186/1471-2407-13-615 PMid:24377754 PMCid:PMC3879657

31. Alwan N, Tawfeeq F, Muallah F. Breast cancer subtypes among Iraqi patients: identified by their Er, Pr and Her2 Status. J Fac Med Baghdad. 2017; 59(4):303-07. https://doi.org/10.32007/med.1936/jfacmedbagdad.v59i4.6

32. Barros FF. Biological characterisation of HER2 amplified breast cancer (Doctoral dissertation, University of Nottingham).

33. Jabbar N, Altaee M, Naji R. Correlation of Her-2/neu gene amplification by FISH and CISH with clinicopathological parameters of Iraqi breast carcinoma patients at central public health laboratories. Bio--Genetics Journal. 2016; 4(4):73-77.

34. Vocaturo A, Novelli F, Benevolo M, Piperno G, Marandino F, Cianciulli A, et al. Chromogenic in situ hybridization to detect HER-2/neu gene amplification in histological and thin prep®-processed

breast cancer fine-needle aspirates: A sensitive and practical method in the trastuzumab era. The Oncologist. 2006; 11:878-86. https://doi.org/10.1634/theoncologist.11-8-878 PMid:16951391

35. Khashman B, Abdul-Sattar S. Determination of HER2 gene amplification using chromogenic in situ hybridization (CISH) in Iraqi patients with breast carcinoma. International Journal of Science and Research (IJSR). 2017; 6(8).

36. Manuelito A Madrid and Raymundo W Lo. Chromogenic in situ hybridization (CISH): a novel alternative in screening archival breast cancer tissue samples for HER-2/neu status. Breast Cancer Research. 2004; 6( 5):1. https://doi.org/10.1186/bcr915 PMid:15318940 PMCid:PMC549176

37. Wolff AC, Hammond ME, Hicks DG. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. Arch Pathol Lab Med. 2014; 138:241-56. https://doi.org/10.5858/arpa.2013-0953-SA PMid:24099077 PMCid:PMC4086638

38. Goud KI, Dayakar S, Vijayalaxmi K, Babu SJ, Reddy PV. Evaluation of HER-2/neu status in breast cancer specimens using immunohistochemistry (IHC) & fluorescence in-situ hybridization (FISH) assay. Indian J Med Res. 2012; 135(3):312-7.

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Jun 30; 7(12):1926-1930.
https://doi.org/10.3889/oamjms.2019.551
eISSN: 1857-9655
***Basic Science***

# Sputum Quality Assessment Regarding Sputum Culture for Diagnosing Lower Respiratory Tract Infections in Children

Gorica Popova[1*], Katerina Boskovska[2], Ivana Arnaudova-Danevska[2], Olga Smilevska-Spasova[2], Tatjana Jakovska[2]

*[1]Department of Clinical Microbiology, Institute for Respiratory Diseases in Children, Skopje, Republic of Macedonia; [2]Pediatric Department, Institute for Respiratory Diseases in Children, Skopje, Republic of Macedonia*

**Citation:** Popova G, Boskovska K, Arnaudova-Danevska I, Smilevska-Spasova O, Jakovska T. Sputum Quality Assessment Regarding Sputum Culture for Diagnosing Lower Respiratory Tract Infections in Children. Open Access Maced J Med Sci. 2019 Jun 30; 7(12):1926-1930. https://doi.org/10.3889/oamjms.2019.551

**Keywords:** Sputum culture; Quality assessment; *Streptococcus pneumoniae; Escherichia coli*

***Correspondence:** Gorica Popova. Department of Clinical Microbiology, Institute for Respiratory Diseases in Children, Skopje, Republic of Macedonia. E-mail: gorica.popova@yahoo.com

**Received:** 03-May-2019; **Revised:** 17-Jun-2019; **Accepted:** 18-Jun-2019; **Online first:** 30-Jun-2019

**Copyright:** © 2019 Gorica Popova, Katerina Boskovska, Ivana Arnaudova-Danevska, Olga Smilevska-Spasova, Tatjana Jakovska. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

**Funding:** This research did not receive any financial support

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

**Abstract**

**BACKGROUND:** The clinical relevance of specimens from the lower airways is often debatable. However, they are most commonly examined for diagnosing lower respiratory tract infections (LRTIs).

**AIM:** This study aimed to determine the diagnostic value of sputum quality assessment about sputum culture for diagnosing LRTIs in children.

**METHODS:** In six months, a total of 1485 sputum samples were quality assessed by using Bartlett's grading system. All samples, regardless of their quality, were cultured, identified, and antimicrobial susceptibility testing was performed by Kirby-Bauer disc-diffusion method.

**RESULTS:** Among the acceptable category, defined by Bartlett's grading system, 132 (63.2%) samples showed culture positivity of which *Streptococcus pneumoniae* 48 (36.4%) was most commonly isolated, followed by *Moraxella catarrhalis* 22 (16.7%) and *Haemophilus influenza* 21 (15.9%). Among the non-acceptable category, 185 (14.5%) samples were culture positive of which most commonly isolated were *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* with 64 (34.6%), 54 (29.2%) and 28 (15.1%), respectively.

**CONCLUSION:** Sputum quality assessment is a useful tool for distinguishing the true respiratory pathogens from possible colonising flora for which antibiotic treatment should not be highly considered.

## Introduction

Bacteriological examination of the specimens obtained from the lower respiratory tract is quite challenging for medical microbiologists. All expectorated sputa are contaminated by the oropharyngeal flora, which may include potential pathogens. The decision of whether the isolated pathogenic bacteria represent true causes of lower respiratory tract infection or are only colonisers of the upper respiratory tract is often impossible. This dilemma is particularly present when young children are in question because obtaining sputum with good quality from this age group is an especially difficult task. This is due to several reasons. First of all, instead of expectorating the sputum, children tend to swallow it and usually have difficulty producing an adequate amount of specimen [1]. Moreover, compared to adults, children have higher colonisation rate of the respiratory tract with bacteria such as *Streptococcus pneumoniae, Haemophilus influenza* and especially in infants, high colonisation rate with *Escherichia coli* [2], [3], [4]. The latter increases the likelihood of contamination of the sputum specimen during its collection.

Involving the method for assessment of the sputum quality allows us to estimate the amount of oropharyngeal contamination. This method is performed by microscopic examination of the cellular components in a stained smear of the specimen, seen under the low power field magnification (LPF). A presence of two cell-types: *s*quamous *e*pithelial *c*ells (SEC) and inflammatory cells, primarily polymorphonuclear leukocytes, is taken into consideration. SEC is found only in the upper respiratory tract, so this finding suggests oropharyngeal-contamination, whereas the presence of polymorphonuclear leukocytes, suggests material derived from the site of active infection [5].

There are several published criteria for assessing the quality of sputum. According to Murray and Washington [6] and Geckler et al., [7], sputum quality assessment should be dependent only on the presence of the SEC, seen microscopically at low-power field (LPF) magnification, regardless of the number of the white blood cells (WBC). On the other hand, Van Scoy [8] states that specimens with more than 25 leukocytes per LPF should be accepted regardless of the number of the SEC.

The variations in the thickness of the material in different areas of the same slid causes inconsistencies when only one type of cells is taken into account. This can be overcome by assessment of the sputum quality according to the WBC-SEC ratio, as Bartlett [9] recommends.

Heineman and Radano [10] describe a similar scheme for screening sputum specimens, and the criterion for acceptability is the presence of more than ten WBC per SEC, seen microscopically at LPF.

In the present study, 1485 sputum specimens were quality assessed according to criteria of Bartlett et al., [9] and these findings were compared with the results of the bacteriological examination of the same specimens.

## Methods

The studied population was children suspected having lower respiratory tract infections, aged from 0 to 14 years, treated in ambulatory and hospital settings at the Institute for Respiratory Diseases in Children, Skopje.

In six months, from 01.07.2017 to 30.12.2017, a total of 1485 sputa were quality assessed after slides of the specimens had been gram-stained and microscopically examined at 100x magnification. The sputa quality was assessed according to the criteria of Bartlett et al., [9], based on the relative number of SEC and inflammatory cells, seen microscopically per LPF. According to these criteria, for every specimen, a Q-score (sum of "+" and "–"assigned values) was calculated, following the scheme: + 2 if > 25 WBC were seen per LPF, + 1 if 10-25 WBC were seen per LPF; on the other hand, - 2 was assigned if > 25 SEC were seen per LPF and - 1 if 10-25 SEC were seen per LPF. The "+" Q-score indicated material derived from the site of an active infection and these samples were categorized as acceptable. A "0" or "-" Q-score suggested low sputum quality, excessive oropharyngeal contamination and these samples were categorized as non-acceptable.

All sputum specimens, regardless of their quality, were further processed, using standard microbiological procedures for bacterial isolation and identification. Sputa were inoculated on the same day on 5% sheep blood agar, chocolate agar and *Candida albicans*-chromogenic agar. Plates were incubated at 37°C overnight in 5% $CO_2$ and were observed up to 48 hours. If no growth of relevant bacteria was seen, the cultures were declared negative. Preliminary identification of the bacteria was based on colony morphology and cultural characteristics on selective and differential media. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc-diffusion method according to the methodology proposed by the European Committee on Antimicrobial Susceptibility Testing [11]. Interpretation of zones of inhibition around the antibiotic discs was made according to EUCAST- breakpoint tables for interpretation of MICs and zone diameters [12].

The results of the bacteriological examination were evaluated against the assessment of the sputa quality - assigned as a value of the Q-score.

## Results

The results of sputa quality assessment are given in Figure 1.

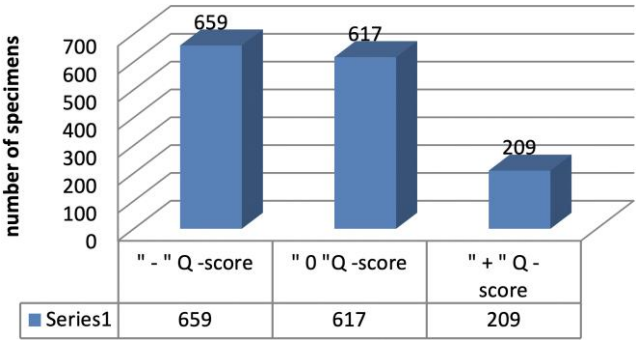



*Figure 1: Sputa quality assessment*

Based on Bartlett's screening criteria, out of 1485 processes sputum samples, 209 (14.1%) were good quality (acceptable category), and 1276 (85.9%) were low-quality (non-acceptable category). Of a total

of 209 sputa with good quality (acceptable category), 132 were culture positive (63.2%) and of 1276 sputa with low quality (non-acceptable category), 185 were culture positive (14.5 %), p = 0,000.

Distribution of isolated potential pathogenic bacteria from sputa with good quality (acceptable category) is given in Figure 2.

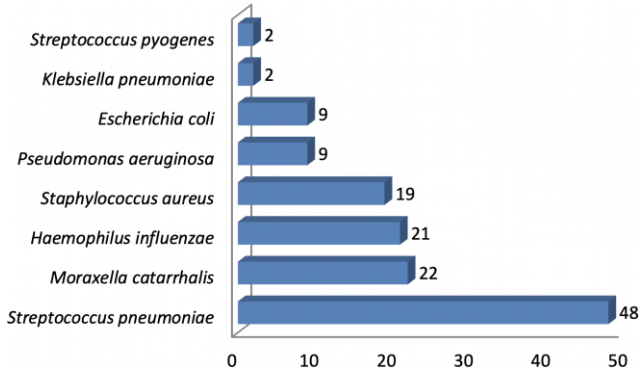



*Figure 2: Distribution of isolated bacterial species from sputa with good quality (acceptable category)*

The most frequently isolated bacteria from sputa with good quality (acceptable category) were *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Haemophilus influenzae* with 36.4%, 16.7% and 15.9%, respectively.

Distribution of isolated potential pathogenic bacteria from sputa with low quality (non-acceptable category) is given in Figure 3.

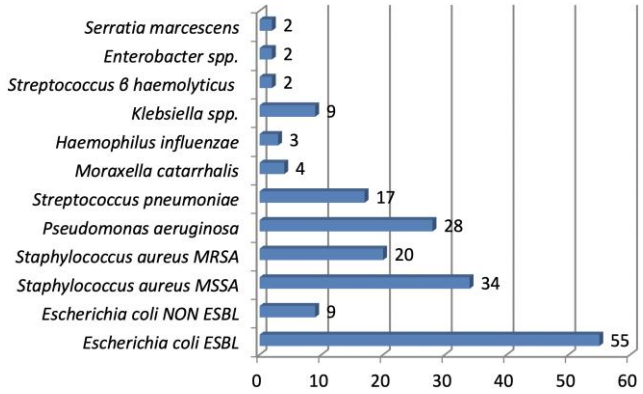



*Figure 3: Distribution of isolated bacterial species from sputa with low quality (non-acceptable category); MSSA – Methicillin-Sensitive Staphylococcus aureus; MRSA – Methicillin-Resistant Staphylococcus aureus; ESBL – Extended Spectrum β-Lactamases*

The most commonly isolated bacterium from low-quality sputum was *Escherichia coli* with 34.6% (64/185), of which ESBL (Extended Spectrum β-Lactamases)-producers were 85.9% (55/64). The second most often isolated was *Staphylococcus aureus* with 29.2% (54/185), of which MRSA (Methicillin-Resistant *Staphylococcus aureus*) was 37% (20/54).

Of all reported antimicrobial susceptibility test results, 23.6% (75/317) were reported for multidrug-resistant bacteria such as MRSA and ESBL-*Escherichia coli*, while microscopic examination of the specimens reviled low sputum quality.

Table 1 contains the number of isolated *Escherichia coli* and bacteria other than *Escherichia coli* from sputa with “0/-” Q-score (non-acceptable category) and sputa with “+” Q-score (acceptable category).

**Table 1: The probability of *Escherichia coli* isolation in the function of sputum quality**

| | E. coli | Isolated Organisms other than E. coli |
|---|---|---|
| Non-acceptable category | 64 | 121 |
| Acceptable category | 9 | 123 |

$$RR(relative\ risk) = \frac{64 : (64 + 121)}{9 : (9 + 123)} = 5.07$$

The low-quality sputum (as a risk factor) increases the relative risk for *Escherichia coli* isolation by 5 times.

Table 2 displays the number of isolated *Staphylococcus aureus* and bacteria other than *Staphylococcus aureus* from sputa with “0/-” Q-score (non-acceptable category) and sputa with “+” Q- score (acceptable category).

**Table 2: The probability of *Staphylococcus aureus* isolation in the function of sputum quality**

| | S. aureus | Organisms other than S. aureus |
|---|---|---|
| Non-acceptable category | 54 | 131 |
| Acceptable category | 19 | 113 |

$$RR(relative\ risk) = \frac{54 : (54 + 131)}{19 : (19 + 113)} = 2.07$$

Low sputum quality, as a risk factor, increases the relative risk for *Staphylococcus aureus* isolation by 2 times.

## Discussion

The role of medical microbiologists is to isolate a causative agent of a certain infection which is highly dependent on the quality of the specimen. Among adults with pneumonia, about 60% can produce an adequate sputum specimen for microbiologic evaluation [13]. When infants and young children are considered having LRTIs, collecting good sputum specimen is hardly feasible. In this study, according to Bartlett’s grading system, the majority (nearly 86%) of the sputum specimens obtained from young children was low-quality. Therefore rejection of such a huge number of specimens tends to be complex. Most of them were declared negative (with

the remark that the specimens were with low quality, so the possibility for false negative results is not excluded). However, the biggest concern was 14.5% culture positive specimens (among non-acceptable category) where the majority of isolated bacteria were multidrug resistant (MDR) bacteria, such as ESBL-producing *Escherichia coli* and Methicillin-Resistant *Staphylococcus aureus* (MRSA). According to the results of this study, the probability for *Escherichia coli* isolation is 5 times increased if the sputum is low-quality, whereas the probability for isolating *Staphylococcus aureus* is doubled.

The isolation of MDR-organisms from low-quality sputum promotes unnecessary prescription of antibiotics, which should be overcome by combined efforts of clinicians and microbiologists.

It is noteworthy that 9% (17/185) of positive cultures in the non-acceptable category were identified as *Streptococcus pneumoniae*. The latter is due to the significant *Streptococcus pneumoniae* colonisation rate of the respiratory tract in childhood. According to Abdullahi et al., [3] nasopharyngeal pneumococcal carriage prevalence among groups aged from 0-4 years was 57% in contrast to 6.4% in groups aged from 10-80 years.

However, the greatest benefit of the sputum assessment was its assistance in the further interpretation of the sputum culture. Antimicrobial Susceptibility Test (AST) results were released for all potential pathogens isolated from low-quality sputa, but it was annotated that, according to the microscopic examination of the specimen, the isolated bacteria are probably part of the colonising flora of the upper respiratory tract. Hence, antibiotic treatment is very uncertain. From a clinical perspective, knowing whether the isolated bacteria are the cause of LRTI or they are merely colonising flora, could be salient in deciding whether the antibiotic therapy should be prescribed. Under these conditions, modified reporting, with the additional explanation that the isolated bacteria are probably part of colonising flora, could be a good practice. Moreover, automatic reporting of AST results could be excluded, similarly, as it is explained for asymptomatic bacteriuria in a work of Leis et al., [14]. This type of reporting will certainly lead to a more rational prescription of antimicrobial therapy.

The problem with the low quality of sputum is evident, and, perhaps, it may be overcome by introducing the procedures for the collection of induced sputum specimen [15]. According to results from Pneumonia Etiology Research for Child Health (PERCH) study, good quality sputum specimen can be collected from children with pneumonia, aged from 1 to 59 months, through saline nebulization induction [1], [15]. It has already been said that < 10 SEC per LPM is the best measure of induced sputum quality in children with pneumonia [15].

In contrast to sputa with low quality, where the culture positivity was 14.5%, sputa with good quality were culture positive in 63.2%, p = 0.00. Other studies are reporting similar culture positivity. In the studies of Anevlavis et al., [13], Rana et al., [16], Mariraj et al., [17], acceptable sputa were culture positive in 72%, 77% and 63%, respectively. According to the results of this study, the most frequently isolated was *Streptococcus pneumoniae* – 36.1%. This finding is in correlation with the results from other studies [15], [18], [19], [20], which implies that *Streptococcus pneumoniae* represents the most important respiratory pathogen in community-acquired lower respiratory tract infections in children.

## References


1. Grant LR, Hammitt LL, Murdoch DR, O, Brien KL, Scott A. Procedures for Collection of Induced Sputum Specimens From Children. Clin Infect Dis. 2012; 54(S2):S140-5. https://doi.org/10.1093/cid/cir1069 PMid:22403228 PMCid:PMC3297553

2. Millar EV, Watt JP, Bronsdon MA, Dallas J, Reid R, Santosham M, O'Brien KL. Indirect effect of 7-valent pneumococcal conjugate vaccine on pneumococcal colonization among unvaccinated household members. Clin Infect Dis. 2008; 47(8):989-96. https://doi.org/10.1086/591966 PMid:18781875

3. Abdullahi O, Nyiro J, Lewa P, Slack M, Scott JA. The descriptive epidemiology of Streptococcus pneumoniae and Haemophilus influenzae nasopharyngeal carriage in children and adults in Kilifi district, Kenya. Pediatr Infect Dis J. 2008; 27(1):59-64. https://doi.org/10.1097/INF.0b013e31814da70c PMid:18162940 PMCid:PMC2382474

4. Popova G, Jankuloski D, Félix B, Boskovska K, Stojanovska-Dimzovska B, Tasic V, Blagoevska K. Pulsed-Field-Gel-Elecrophoresis used for typing of extended-spectrum-β-lactamases-producing Escherichia coli isolated from infant's respiratory and digestive system. Mac. Vet. Rev. 2018; 41:133-141. https://doi.org/10.2478/macvetrev-2018-0016

5. Wong LK, Barry AL, Horgan SM. Comparison of six different criteria for judging the acceptability of sputum specimens. J Clin Microbiol. 1982; 16:627-631.

6. Murray PR, Washington JA. Microscopic and bacteriologic analysis of expectorated sputum. Mayo Clin Proc. 1975; 50:339-344. https://doi.org/10.1080/00357529.1975.11762852

7. Geckler RW, Gremillion DH, McAllister CK, Ellenbogen E. Microscopic and bacteriological comparison of paired sputa and transtracheal aspirates. J Clin Microbiol. 1977; 6:518-527.

8. Van Scoy RE. Bacterial sputum cultures, a clinician's viewpoint. Mayo Clin Proc 1977; 52:39-41.

9. Bartlett JG, Breiman RF, Mandell LA, File TM. Community-acquired pneumonia in adults: guidelines for management. Clin Infect Dis. 1998; 26:811-838. https://doi.org/10.1086/513953 PMid:9564457

10. Heineman HS, Radano RR. Acceptability and cost savings of selective sputum microbiology in a community teaching hospital. J Clin Microbiol. 1979; 10:567-73.

11. Matuschek, E, Brown DFJ, Kahlmeter G. Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories. Clin Microbiol Infect. 2014; 20:O255-O266. https://doi.org/10.1111/1469-0691.12373 PMid:24131428

12. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone

diameters. Version 6.1, 2016. http://www.eucast.org

13. Anevlavis S, Petroglou N, Tzavaras A, et al. A prospective study of the diagnostic utility of sputum Gram stain in pneumonia. J Infect. 2009; 59:83-9. https://doi.org/10.1016/j.jinf.2009.05.011 PMid:19564045

14. Leis JA, Rebic GW, Daneman SM, Lo P, Larocquen M, Shojania KG, McGeer A. Reducing antimicrobial therapy for asymptomatic bacteriuria among noncatheterized inpatients: a proof-of-concept study. Clin Infect Dis. 2014; 58(7):980-3. https://doi.org/10.1093/cid/ciu010 PMid:24577290

15. Murdoch DR, Morpeth SC, Hammitt LL, et al. Microscopic Analysis and Quality Assessment of Induced Sputum from Children with Pneumonia in the PERCH Study. Clin Infect Dis. 2017; 64(S3):S271-S279.

16. Rana A, Sharma A, Pandey G. Diagnostic value of sputum Gram's stain and sputum culture in lower respiratory tract infections in a tertiary care hospital. Int J Curr Microbiol App Sci. 2017; 6:4310. https://doi.org/10.20546/ijcmas.2017.607.448

17. Mariraj J, Surekha Asangi Y, Krishna S, Suresh Sonth B, Ramesh, Shanmugam. Sputum Gram,s stain assessment in relation to sputum culture for respiratory tract infections in a tertiary care hospital. J Clin Diag Res. 2011; 5(8):1699-1700.

18. Khan S, Priti S, Ankit S. Bacteria Etiological Agents Causing Lower Respiratory Tract Infections and Their Resistance Patterns. Iran Biomed J 2015; 19(4):240-6.

19. Rudan I, Boschi-Pinto C, Biloglav Z, Mulholland K, Campbell H. Epidemiology and etiology of childhood pneumonia. Bull World Health Organ. 2008; 86(5):408-16. https://doi.org/10.2471/BLT.07.048769 PMid:18545744 PMCid:PMC2647437

20. Rudan I, O'Brien KL, Nair H, et al. Epidemiology and etiology of childhood pneumonia in 2010: estimates of incidence, severe morbidity, mortality, underlying risk factors and causative pathogens for 192 countries. J Glob Health. 2013; 3(1):010401. https://doi.org/10.7189/jogh.03.010101

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Jun 30; 7(12):1931-1934.
https://doi.org/10.3889/oamjms.2019.554
eISSN: 1857-9655
***Basic Science***

# Impact of the *Neuregulin* rs35753505 C/T Polymorphisms on Neuregulin 1 Levels in Preterm Infants

Bugis Mardina Lubis[1*], Sjarif Hidajat Effendi[2], Ratna Akbari Ganie[3], Oke Rina Ramayani[1]

*[1]Department of Child Health, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia; [2]Department of Child Health, Faculty of Medicine, Universitas Padjajaran, Bandung, Indonesia; [3]Department of Clinical Pathology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia*

**Citation:** Lubis BM, Effendi SH, Ganie RA, Ramayani OR. Impact of The Neuregulin rs35753505 C/T Polymorphisms on Neuregulin 1 Levels in Preterm Infants. Open Access Maced J Med Sci. 2019 Jun 30; 7(12):1931-1934. https://doi.org/10.3889/oamjms.2019.554

**Keywords:** Neuregulin; Polymorphism; Preterm infant

***Correspondence:** Bugis Mardina Lubis. Department of ChildHealth, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia; 2Department of ChildHealth, Faculty of Medicine, Universitas Padjajaran, Bandung, Indonesia. E-mail: bugismardina@yahoo.com

**Received:** 13-Apr-2019; **Revised:** 19-Jun-2019; **Accepted:** 20-Jun-2019; **Online first:** 30-Jun-2019

**Copyright:** © 2019 Bugis Mardina Lubis, Sjarif Hidajat Effendi, Ratna Akbari Ganie, Oke Rina Ramayani. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

**Funding:** This research did not receive any financial support

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

**Abstract**

**BACKGROUND:** Neuregulin (NRG) 1 plays an important role in the development of various organ systems in human. Single nucleotide polymorphisms rs35753505 C/Tof the gene encoding NRG1 evident as allele C and T with genotypes of CT, CC, and TT are believed to have an impact on NRG1 levels.

**AIM:** To determine the impact of the NRGrs35753505 C/T polymorphisms on NRG1 levels in preterm infants.

**METHODS:** A cross-sectional study was conducted from February to December 2018, whereas 48 eligible preterm infants with a gestational age of 32- < 37 weeks were enrolled. An umbilical cord blood specimen was collected for determination of NRG1 levels with enzyme-linked immunosorbent assay (ELISA) and NRG1 polymorphisms with polymerase chain reaction (PCR). Statistical analysis was performed with 95%CI and P value of < 0.05 was considered statistically significant.

**RESULTS:** Median value of NRG1 levels (174.4 pg/ml) served as a cut off value. NRG 1 polymorphisms composed distribution of CC (31%), CT (42%), TT (27%) genotypes and distribution of C and T alleles were 52% and 48%. The median NRG1 levels in CC and CT genotypes were significantly lower compared to TT genotype (151.1 pg/ml vs 407.2 pg/ml, P = 0.005 and 159.1 pg/ml vs 407.2 pg/ml, P = 0.009). Subjects with C allele had significantly lower median NRG1 levels than T allele (151.1 pg/ml vs 407.2 pg/ml, P = 0.002). Subjects with CC and CT genotypes had higher risk to develop lower NRG1 levels compared to TT genotype (OR = 8.25, P = 0.016 and OR = 10.74, P = 0.005, respectively).

**CONCLUSION:** Allele C is associated with lower NRG1 levels. Preterm infants with CC and CT genotypes pose a higher risk to have lower NRG1 levels.

## Introduction

The neuregulin (NRG) protein, encoded by the neuregulin gene, is a membrane glycoprotein that mediates inter-cell signals and plays an important role in the growth and development of multiple organ systems. It has ligand to thyroxine kinase receptors ERBB3 and ERBB4. It concomitantly recruits co-receptors ERBB1 and ERBB3, which stimulate thyroxine phosphorylation and activation of ERBB receptors. These isoforms affect the growth and differentiation of epithelial cells, glial cells, neurons, and muscle cells; induces expression of acetylcholine receptors in synaptic vesicles during neuromuscular junction generation; stimulates lobuloalveolar budding and milk production; stimulates Schwann cell proliferation; myocardial development such as trabeculation during heart development; neuronal migration and regulation of neurotransmitter receptors on neurons; play a role in the development of motor and sensory neurons [1], [2].

Neuregulin 1 is a trophic factor which is a subclass of transmembrane GF polypeptide owned by family epidermal growth factor (EGF) signalled by stimulating ERBB receptor tyrosine kinases. It is a family of GF encoded from four different genes of NRG-1, NRG-2, NRG-3, and NRG-4, where NRG1 is the best characteristic. EGF-like domains are located within the membrane-proximal region of the

extracellular region, which allows the activation of the ErbB receptor tyrosine kinase. NRG1 plays a role in stimulating a family of single-transmembrane receptor tyrosine kinases called ErbB proteins [3].

The last important finding is sequencing and the identification of all NRG 1 genes in human that have been successfully identified. It has a length of ≈ 1.4 megabases (≈ 1/2000$^{th}$ of the genome); less than 0.3% of its length encodes the protein. Due to the many alternative splicing and multiple promoters, there are at least 15 different NRG isoforms produced by a single NRG1 gene. The three important structural characteristics that distinguish isoforms in in-vivo functions and biological cells are types of EGF-like domains (αorβ), N-terminal sequences (type I, II, or III) or whether the isoform is directly synthesised as a transmembrane or non-membrane protein [4]. Single nucleotide polymorphisms rs35753505 C/Tof the gene encoding NRG1evident as allele C and T with genotypes of CT, CC, and TT are believed to have an impact on the NRG1 levels.

## Methods

A cross-sectional study was conducted from February to December 2018, whereas 48 preterm infants from 5 hospitals in Medan with a gestational age of 32- < 37 weeks were enrolled. Infants with severe congenital malformation were excluded. Umbilical cord blood specimens were collected immediately after birth in an EDTA tube for determination of NRG1 levels with enzyme-linked immunosorbent assay (ELISA). A blood sample was centrifuged and frozen at a temperature of -700°C until laboratory testing commenced. ELISA was performed using the ®DuoSetHuman NRG1-β1/HRG-β. As much as 100 µl sample was added in each well-containing reagent diluents and incubated for 2 hours. Detection antibody (100 µl) was added, and the solution was incubated for 2 more hours. Working dilution (100 µl) was then added into the solution and incubated for 20 minutes. The solution was incubated for 20 more minutes after the addition of 100 µl substrate solution. At the last step, 50 µl of stop solution was added to each well. After being centrifuged, the solution's optical density was determined using a microplate reader set to 450 nm.

Polymerase Chain Reaction was performed on 100 of genomic DNA using primer 5'-ACC TAA GAT GTC CAA GAG ACA G-3' forward and 5'-GAC TGG AAG CCA TGT ATC TTT ATT GT-3' reverse (®Integrated DNA Technologies) and Master mix Go Taq® Green Master Mix (®Promega). Thirty-six cycles were performed with 15 minutes denaturation at 95°C, 1-minute annealing at 68°C and 1-minute extension at 72°C. The difference in NRG1 levels was analysed using the Mann-Whitney test. The association between polymorphisms and NRG1 levels was analysed using the chi-square test. Statistical analysis was performed using statistical software at 95% CI, and a P value of < 0.05 was considered significant. This study was approved by the Health Research Ethical Committee, Medical School, University of Sumatera Utara.

## Results

The median value of NRG1 levels was 174.4 pg/ml and served as a cut off value. Neuregulin 1 polymorphisms rs35753505 C/Tcomposed distribution of CC, CT, and TT as many as 31%, 42%, and 27%, respectively. Distribution of C and T alleles were 52% and 48%. Baseline characteristics of subjects are shown in Table 1.

**Table 1: Baseline characteristics of subjects**

| Characteristics | n (%) |
|---|---|
| Mode of delivery, n (%) | |
| Normal | 2 (4.2%) |
| Caesarean section | 48 (95.8%) |
| Indication for preterm labor, n (%) | |
| Medical indication | 28 (58.3%) |
| Spontaneous | 20 (41.7%) |
| Gestational age, week, n (%) | |
| 32 – < 35 weeks | 16 (33.3%) |
| 35 – < 37 weeks | 32 (66.7%) |
| Gender, n (%) | |
| Male | 25 (52.1%) |
| Female | 23 (47.9%) |
| Birth weight, gram, n (%) | |
| < 2.500 | 27 (56.3%) |
| ≥ 2.500 | 21 (43.8%) |

Mann-Whitney test was used to determine the differences in serum NRG1 levels between genotypes and alleles of neuregulin rs35753505 C/T polymorphisms. The median NRG1 levels in CC and CT genotypes were significantly lower compared to TT genotype (151.1 pg/ml vs 407.2 pg/ml, P = 0.005 and 159.1 pg/ml vs 407.2 pg/ml, P = 0.009). Subjects with C allele had significantly lower median NRG1 levels than their counterparts (151.1 pg/ml vs 407.2 pg/ml, P = 0.002) (Figure 1).

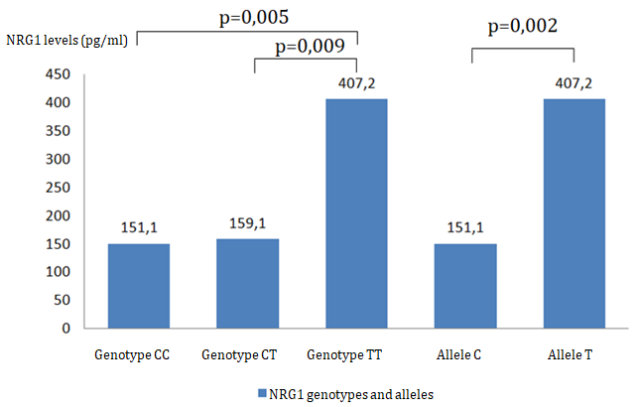



*Figure 1: The differences in serum NRG1 levels between genotypes and alleles of NRG rs35753505 C/T polymorphisms*

Chi-square test was conducted to determine the association between NRG1 rs35753505 C/T polymorphisms and NRG1 levels in this study. We found a significant association between NRG rs35753505 C/T polymorphism with NRG1 levels. Subjects with CC and CT genotypes had higher risk to have lower NRG1 levels compared to TT genotype (OR = 8.25, 95%CI = 1.32-51.26, P = 0.016 and OR = 10.74, 95% CI = 1.74-59.65, P = 0.005, respectively). Subjects with C allele had higher risk to have lower NRG1 levels compared to T allele (OR = 2.78, 95% CI = 1.21-6.36, P = 0.014) (Table 2).

**Table 2: The association between NRG rs35753505 C/T polymorphism with NRG1 levels**

| NRG rs35753505 C/T polymorphism | NRG1 levels | | *P* | OR (95%CI) |
|---|---|---|---|---|
| | Low n (%) | High n (%) | | |
| CC genotype | 9 (37.5) | 6 (23.0) | 0.016# | 8.25 (1.32 – 51.26) |
| CT genotype | 13 (54.1) | 7 (26.9) | 0.005# | 10.74 (1.74 – 59.65) |
| TT genotype | 2 (8.3) | 13 (50.0) | | |
| C allele | 31 (64.6) | 19 (39.6) | 0.014* | 2.78 (1.21 – 6.36) |
| T allele | 17 (35,4) | 29 (60,4) | | |

*$P < 0.05$; #significant compared to TT genotype.

## Discussion

The presence of NRG1 has been widely studied and has been shown to play a role in fetal development. Low NRG1 levels will inhibit surfactant formation and affect heart development, nerves, and the immune system in premature infants [3], [5], [6], [7], [8], [9], [10]. There is no literature that publishes the normal cut-off levels of NRG1 in children. Studies conducted on the adult population with coronary heart disease shows the median NRG1β level in plasma and serum in individuals with ischemia of 4100 pg/mL and 2400 pg/mL, respectively, whereas for individuals without ischemia at 3300 pg/mL and 1600 pg/mL. The study also states that NRG1β levels in plasma are more accurate than those in serum [11]. Studies conducted in clinically healthy populations obtained a mean serum NRG1 levels of 217 ng/mL with a range of 32 ng/mL to 473 ng/mL [12]. Neuregulin 1 is also found in the human cornea; prior studies using the PCR showed no difference in the replication of the NRG1 coding gene in the cornea and epithelium. This demonstrates that NRG1 is produced by relatively equal numbers by epithelial and stromal cells [13]. The median NRG1 level in this study was 174.4 pg/mL. Using the median value as a cut off to determine high and lower NGR1 levels, an equal proportion of subjects with high and lower NRG1 levels was obtained. This is different from the previous study. The previous study has suggested that NRG1 levels will be lower as more premature the baby is [14]. The NRG1 gene, together with the NRG2, NRG3, and NRG4 genes, functions to encode the NRG1 protein [7]. In these genes, DNA sequences variations can occur in the form of SNPs that can affect NRG1 production [10]. A study conducted by Hoffmann, et al., showed the presence of NRG 221533 coding gene polymorphism, which resulted in 77% of infants having at least one C allele [14]. By Hardy-Weinberg law, the polymorphism will be passed to the next generation in the same proportion if no mutation occurred. The impact of these polymorphisms will also persist in the next generation until other mutations or genetic changes occurred [15]. In this study, a genetic examination was conducted to determine the NRG rs35753505 C/T polymorphism. Based on the results of the examination, it was found that 42% of subjects had CT genotypes, while CC and TT genotypes were detected in 31% and 27% of subjects, respectively.

There was an association between genotypes and alleles of the NRG rs35753505 C/T polymorphism with NRG1 levels in this study. Subjects with CC and CT genotypes had lower NRG1 levels than subjects with TT genotype (P = 0.005 and P = 0.009). The presence of C allele was associated with a decrease in NRG1 levels in the study subjects (P = 0.02). The NRG1 rs35753505 polymorphism was associated with a decrease in NRG1 levels in this study. Subjects with CC and CT genotypes showed a decrease in NRG1 levels of 8.25 times (P = 0.016) and 10.74 times (P = 0.005) compared to subjects with TT genotypes. The presence of C allele will increase the risk of decreasing NRG1 levels by 2.78 times (P = 0.014). The previous study reported different results. This is different from the results of previous studies where neonates, who had C alleles were less often born prematurely and had higher NRG levels than neonates who had T alleles [14].

In conclusion, the median value of NRG1 levels in the study subjects was 174.4 pg/ml. The most frequent NRG rs35753505 C/T polymorphism in this study was heterozygous CT genotype (42%). The c allele was present in 52% of subjects. The serum neuregulin level was lower in subjects with CC and CT genotype than subjects with TT genotypes. Subjects with C allele showed lower serum NRG1 levels compared to subjects with T allele. There was a significant relationship between NRG rs35753505 C/T polymorphism and NRG1 levels. Subjects with CC genotype had a risk of 8.25 times higher having lower NRG1 levels than subjects with TT genotype while subjects with CT genotype had 10.74 times higher risk. The presence of C allele had a risk of 2.78 times higher for low NRG1 levels.

## References

1. US National Library of Medicine. NRG1 gene. Available from: https://ghr.nlm.nih.gov/gene/NRG1#synonyms. [cited June 1, 2018]

2. Nawar R, Asif H, Khan A, Ishtiaq H, Shad FK, Siddiqui S. Genetic polymorphism in NRG1 SNP rs35753505 genes

associated with schizophrenia in Pakistani patients. Int J Genetics. 2013; 5:142-8.

3. Nicodemus KK, Law AJ, Luna A, Vakkalanka R, Straub RE, Kleinman JE, Weinberger DR. A 5′ promoter region SNP in NRG1 is associated with schizophrenia risk and type III isoform expression. Molecular psychiatry. 2009; 14(8):741-3. https://doi.org/10.1038/mp.2008.150 PMid:19626024 PMCid:PMC3271936

4. Falls DL. Neuregulins: functions, forms, and signaling strategies. Experimental Cell Research. 2003; 284:14-30. https://doi.org/10.1016/S0014-4827(02)00102-7

5. Li C, Liang Z, Blom MS, Wang Q, Shen X, Zhang H, et al. Temporal trends of preterm birth in Shenzen, China: a retrospective study. Reprod Health. 2018; 15:1-10. https://doi.org/10.1186/s12978-018-0477-8 PMid:29534760 PMCid:PMC5851155

6. McIntosh AM, Job D, Lymer GKS, Maniega SM, Mckirdy J, Sussmann JED. The effect of a neuregulin 1 variant on white matter density and integrity. Molecular Psychiatry. 2008; 13:1054-9. https://doi.org/10.1038/sj.mp.4002103 PMid:17925794

7. Damman O, Leviton A, Gressens P, Dammann E. Neuregulin 1: a potential endogenous protector in perinatatal brain white matter damage. Neonatology. 2008; 93:182-7. https://doi.org/10.1159/000111119 PMid:17993737 PMCid:PMC2910616

8. Lemmens L, Doggen K, De Keulenaer GW. Role of neuregulin 1/ErbB signaling in cardiovascular physiology and disease: implications for therapy of heart failure. Circulation. 2007; 116: 954-60. https://doi.org/10.1161/CIRCULATIONAHA.107.690487 PMid:17709650

9. Patel NV, Acarregui MJ, Snyder JM, Klein JM, Sliwkowski MX. Neuregulin 1 and human epidermal growth factor receptors 2 and 3 play a role in human lung development in vitro. Am J Respir Cell Mol Biol. 2000; 22:432-40. https://doi.org/10.1165/ajrcmb.22.4.3854 PMid:10745024

10. Pleickhardt EP, Celandine A, Davis J, Chen M, Schurmann P, Dork T. Neuregulin 1 high-producer genotype is associated with a decreased risk of admission to the neonatal intensive care unit. Early Human Development. 2010; 86:299-304. https://doi.org/10.1016/j.earlhumdev.2010.03.010 PMid:20472376

11. Geisberg CA, Wang G, Safa RN, Smith HM, Anderson B, Peng X. Circulating neuregulin-1β levels vary according to the angiographic severity of coronary artery disease and ischemia. Coron Artery Dis. 2011; 22:577-82. https://doi.org/10.1097/MCA.0b013e32834d3346 PMid:22027878 PMCid:PMC3217257

12. Moondra V, Sarma S, Buxton T, Safa R, Cote G, et al. Serum neuregulin-1β as a biomarker of cardiovascular fitness. Open Biomark J. 2009; 2:1-5. https://doi.org/10.2174/1875318300902010001 PMid:20634924 PMCid:PMC2903891

13. Brown DJ, Lin B, Holguin B. Expression of neuregulin 1, a member of the epidermal growth factor family, is expressed as multiple splice variants in the adult human cornea. Invest Ophthalmol Vis Sci. 2004; 45:3021-9. https://doi.org/10.1167/iovs.04-0229 PMid:15326116

14. Hoffmann I, Bueter W, Zcheppang K, Brinkhaus M, Liese A, Riemke S. Neuregulin 1, the fetal endothelium, and brain damage in preterm newborns. Brain, Behavior, and Immunity Journal Elsevier. 2010; 24:784-91. https://doi.org/10.1016/j.bbi.2009.08.012 PMid:19733651 PMCid:PMC2885532

15. Stern C. The Hardy-Weinberg law. Science. 1943; 97:137-8. https://doi.org/10.1126/science.97.2510.137 PMid:17788516

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Jun 30; 7(12):1935-1939.
https://doi.org/10.3889/oamjms.2019.480
eISSN: 1857-9655
***Basic Science***

# Ethyl acetate Fraction of *Garcinia Mangostana* L Rind Study as Antimalaria and Antioxidant in *Plasmodium berghei* Inoculated Mice

Susy Tjahjani*, Yonathan Biantoro, Rita Tjokropranoto

*Faculty of Medicine, Maranatha Christian University, Bandung, Indonesia*

**Citation:** Tjahjani S, Biantoro Y, Tjokropranoto R. Ethyl acetate Fraction of *Garcinia Mangostana* L Rind Study as Antimalaria And Antioxidant in *Plasmodium berghei* Inoculated Mice. Open Access Maced J Med Sci. 2019 Jun 30; 7(12):1935-1939. https://doi.org/10.3889/oamjms.2019.480

**Keywords:** Antimalarial; Antioxidant; G. mangostana L rind; Ethylacetate fraction; In vivo

***Correspondence:** Susy Tjahjani. Faculty of Medicine, Maranatha Christian University, Bandung, Indonesia. E-mail: susy_tjahjani@yahoo.com

**Received:** 03-Apr-2019; **Revised:** 24-Jun-2019; **Accepted:** 25-Jun-2019; **Online first:** 30-Jun-2019

**Copyright:** © 2019 Susy Tjahjani, Yonathan Biantoro, Rita Tjokropranoto. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

**Funding:** This research was financially supported by the Directorate General of Higher Education of the Indonesian Ministry of Education for "Hibah Unggulan Perguruan Tinggi" grant

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

**Abstract**

**BACKGROUND:** Drug resistance to malaria is still a problem in various regions, and there have even been developments in resistance to the ACTs (artemisinin-based combination therapies) as standard antimalarial drugs included to artemisinin's partner drugs. Ethyl acetate fraction of *G. mangostana* L rind, containing xanthones as an antioxidant, has antimalarial activity in vitro which has a synergistic effect with artemisinin. That's why the activities of this fraction are needed to be studied in vivo.

**AIM:** To explore the antimalarial and antioxidant activity of ethyl acetate fraction of *Garcinia mangostana* L rind in mice.

**METHODS:** This was a complete randomised design true experimental study. Six groups of mice: a healthy mice group and 5 groups of *Plasmodium berghei* inoculated mice treated with various doses of the sample for 3 days compared to artemisinin. Parasitemia and total antioxidant status were examined and analysed using ANOVA, and probit analysis were done.

**RESULTS:** The parasitemia level in all of the treatment groups were lower than the positive control group without treatment ($p < 0.01$) and the parasitemia level was the lowest in artemisinin group which was not significantly different from the 100 mg/kg body weight dose group ($p > 0.05$). The parasitemia level in 20 and 4 mg/kg body weight dose groups were higher than the artemisinin group ($p < 0.01$). Parasite growth inhibition rate from the highest to the lowest consecutively was: artemisinin, 100 mg/kg body weight, 20 mg/kg body weight, 4 mg/kg body weight, and positive control group ($p < 0.05$) and ED50 was 3.396 mg/kg body weight. Total antioxidant status was the highest in 20 mg/ kg body weight dose and higher than the negative control group ($p < 0.05$) while the lowest total antioxidant status was in the positive control group.

**CONCLUSION:** Ethyl acetate fraction of *G. mangostana* L rind potentially showed antimalarial and antioxidant activity in vivo. Further study is needed to explore the detail of its mechanism of action and its quantitative phytochemical analysis to find the leading compound in it.

## Introduction

The main issue of malaria is the number of cases that are resistant to conventional antimalarial drugs. To overcome this issue, artemisinin-based combination regimen has been used. But resistance to the available artemisinin-based combination drugs has been reported in some countries [1]. Resistance also happens against artemisinin partner drugs such as piperaquine in dihydroartemisinin-piperaquine regimen in Cambodia [2], also against lumefantrine in artemether-lumefantrine regimen [3]. Therefore, it is necessary to find other alternative reserved drugs that could potentially be paired with artemisinin. Part of the mangosteen (*G. mangostana* L) fruit, i.e. the rind, contains xanthones [4]. A synthetic xanthone, i.e. 2,3, 4,5,6-pentahydroxy xanthone prevent hemozoin formation by inhibiting heme polymerization [5]. Xanthones are also antioxidant and are possibly suitable for use in malaria because malaria decreases levels of antioxidant [6]. *In vitro*, it has been proven that some xanthone compounds worked synergistically with artemisinin as antimalaria against a 3D7 clone of *Plasmodium falciparum* [7]. The previous study showed that ethanolic extract and ethylacetate fraction of *G. mangostana* L rind had antimalarial activity and worked synergistically with artemisinin against a 3D7 clone of *P. falciparum in vitro* [8]. In the previous study, *in vivo* antimalarial effect of ethanolic extract of the *G. mangostana* L rind also has been studied against *P. berghei* inoculated mice [9] but there is no report about the antimalarial activity of the ethyl acetate fraction *in vivo*. By the

way, reported by other researchers that alphamangostin, which is an antioxidant, containing in this preparation caused this fraction to have SPF [10]. This fact supports the background of this study to explore the antioxidant activity besides the antimalarial activity of the *G. mangostana* L rind ethylacetate fraction *in vivo* in *P. berghei* inoculated mice.

## Material and Methods

### *Ethical Issue*

This study was approved by the ethics committee of Maranatha Christian University-Hospital Immanuel according to SK NO: 040/KEP/IV/2014 on the principle of 3 R (reduction, replacement, refinement).

### *Plant Sample*

Mangosteen fruit was collected from Subang, West Java, Indonesia in March 2012, and has been identified by Djuandi, a curator at the Herbarium Bandungense, Sekolah Tinggi Imu Hayati, Bandung Institute of Technology (ITB), Bandung, Indonesia. The fruit was ripe with dark purple colour. The voucher specimen has been deposited by Dr J S Rahajoe in a publicly available herbarium, the Herbarium Bogoriense, Research Center of Biology, Indonesian Institute of Sciences in 2012 with deposition number of 1143/IPH.1.02/lf.8/VII/2012. After fruit washing, the rind has been proximately analysed previously [8].

### *G. mangostana L Rind extraction, fractionation, and treatment preparation*

The rind was cut into small pieces, air dried, pulverised and macerated with 96% alcohol, followed by evaporation to obtain paste like extract according to the standard procedure [11]. The extract was macerated again with hexane and ethyl acetate consecutively using the same method to obtain paste like ethyl acetate fraction from the hexane fraction. This fraction was stored in -20°C freezer until used. For this study, the artemisinin, as well as this fraction, were dissolved in dimethyl sulfoxide (DMSO, Sigma Aldrich, IL, USA) to make a stock solution. Ethyl acetate fraction stock solution was diluted to adjust each of the treatment doses in 0.1 mL solution. The doses were 100 mg/kg body weight, 20 mg/kg body weight, and 4 mg/kg body weight daily. Artemisinin dose as a positive control dose was 50 mg/kg body weight/day in 0.1 mL.

### *Animals and treatment*

A group of 24 DDY strain male mice, 8 weeks of age with 20-25 grams weight, were obtained from the Biopharma Institute, Bandung. They were divided into 6 treatment groups randomly in complete randomised design consisting of 5 groups of *P. berghei* inoculated, and 1 group of healthy non inoculated mice as a negative control group (NC). Each group contained 4 replications according to a certain formula from Hanafiah [12]. The mice were fed with pellets and water *ad libitum*. The *P. berghei* was obtained from the Malaria Laboratory, The Eijkman Insitute for Molecular Biology, Jakarta and was inoculated intraperitoneally into a donor mouse until the minimal parasitemia of 5-10%, and then this mouse was terminated by cervical dislocation, and cardiac puncture was done to obtain the parasites. All of the 24 experimental mice were adapted to the condition of biology laboratory for 1 week in condition: 12 hours light/ dark cycle, 23-24°C, 60-70% relative humidity, then each of the 20 mice of the 5 inoculated groups were inoculated intraperitoneally with $10^7$ parasitized red blood cells (pRBC) from the donor in 200 µL PBS solution. After reaching about 5% parasitaemia, around the 4-5$^{th}$ day, these mice were divided into five groups.

Each of these groups was orally treated every day, once daily, for 3 days consecutively as follows: aquadest (the positive control group = PC), 50 mg/kg body weight/day of artemisinin (artemisinin control group = AC), 100 mg/kg body weight/day of ethyl acetate fraction (the first dose group = A1), 20 mg/kg body weight/ day of ethyl acetate fraction (second dose group = A2), and 4 mg/kg body weight/day of ethyl acetate fraction (third dose group = A3). Parasitaemia was calculated before and after the treatment on the fourth day by determining the parasites amount microscopically per 5,000 red blood cells in Giemsa stain thin blood smears. The rest 4 healthy non inoculated mice were used as a negative control (NC) for the antioxidant test. After completion of the experiment, all of these mice in six groups were terminated by neck dislocation, and their blood was taken from cardiac puncture for total antioxidant levels examination (total antioxidant status = TAS).

### *Total antioxidant status analysis*

Each of the mice serum samples was analysed to determine total antioxidant status using Cayman Antioxidant Assay Kit accordingly. The absorbances were read at 750 nM by ELISA plate reader.

### *Statistical analysis*

The data of parasitaemia and total antioxidant status were analysed using ANOVA followed by Tukey HSD ($\alpha = 0.05$) and $ED_{50}$ (effective dose 50) of

the fraction as antimalaria was analyzed using probit analysis.

## Results

### *The effect of the ethyl acetate fraction of G. mangostana L Rind against parasitaemia in mice suffering berghei malaria*

The parasitemia level in the 5 treatment groups on the day before treatment, day 4 (after 3 days treatment), and parasite growth inhibition is shown in following Table 1.

**Table 1**: **Parasitemia level in percentage in the 5 treatment groups on the day before treatment, day 4 (after 3 days of treatment), and parasite growth inhibition**

| Treatment Groups | Parasitemia level average ± stdev (%) before treatment | Parasitemia level average ± stdev (%) on day 4 (after 3 days of treatment) | Parasite growth inhibition average ±stdev (%) |
|---|---|---|---|
| PC | (5.22 ± 0.5)$^{a}$ | (15.9 ± 3.34)$^{a}$ | |
| AC | (5.32 ± 1.8)$^{a}$ | (0.24 ± 0.11)$^{b}$ | 98 ± 0.01$^{a}$ |
| A1 | (5.42 ± 1.2)$^{a}$ | (3.02 ± 0.33)$^{b}$ | 81 ± 0.02$^{b}$ |
| A2 | (5.3 ± 0.93)$^{a}$ | (6.1 ± 0.75)$^{c}$ | 62 ± 0.05$^{c}$ |
| A3 | (5.2 ± 0.98)$^{a}$ | (7.3 ± 0.85)$^{c}$ | 54 ± 0.05$^{d}$ |

Notes: the same alphabet superscript in each column shows no significant difference (p > 0.05); PC = mice suffering berghei malaria without treatment; AC = mice suffering berghei malaria treated with artemisinin 50 mg/kg body weight; A1 = mice suffering berghei malaria treated with 100 mg/kg body weight of ethylacetate fraction; A2 = mice suffering berghei malaria treated with 20 mg/kg body weight of ethyl acetate fraction; A3 = mice suffering berghei malaria treated with 4 mg/kg body weight of ethylacetate fraction.

Parasitemia levels of the five groups after 3 days of treatment (day 4) could be compared because each of the parasitemia levels on day 0 was no significant difference (p > 0.05). This was shown in the data of Figure 1.

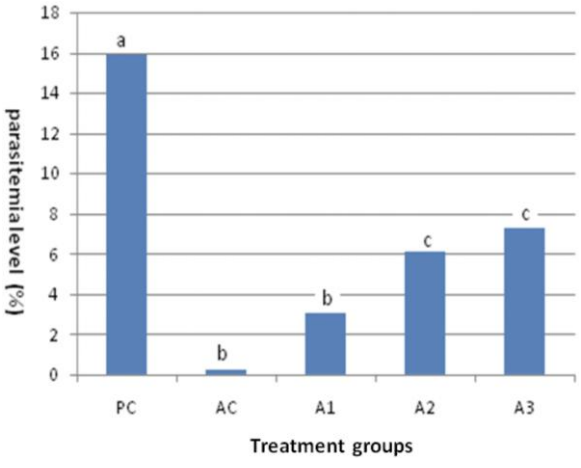



*Figure 1: Parasitaemia level in percentage in the 5 treatment groups after 3 days of treatment (day 4). The same letter above each of the columns indicates that there is no significant difference (P > 0.05); PC = mice suffering berghei malaria without treatment; AC = mice suffering berghei malaria treated with artemisinin 50 mg/kg body weight; A1 = mice suffering berghei malaria treated with 100 mg/kg body weight of ethyl acetate fraction; A2 = mice suffering berghei malaria treated with 20 mg/kg body weight of ethyl acetate fraction; A3 = mice suffering berghei malaria treated with 4 mg/kg body weight of ethyl acetate fraction*

Parasite growth inhibition rate after 3 days of treatment (day 4) indicates that the highest to the lowest group was: AC, A1, A2, and A3 consecutively (p < 0.05). This was shown in the following Figure 2.

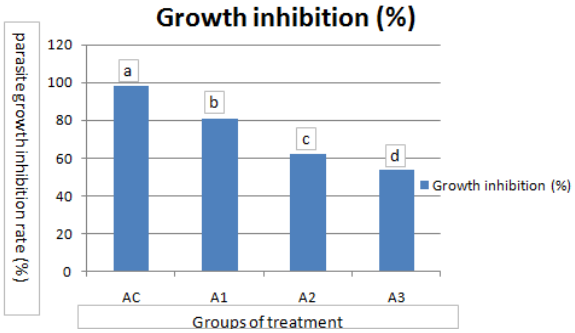



*Figure 2: The parasite growth inhibition rate (%) in various treatment. The same letter above each of the columns indicates that there is no significant difference (P > 0.05); PC = mice suffering berghei malaria without treatment; AC = mice suffering berghei malaria treated with artemisinin 50 mg/kg body weight, A1 = mice suffering berghei malaria treated with 100 mg/kg body weight of ethyl acetate fraction; A2 = mice suffering berghei malaria treated with 20 mg/ kg body weight of ethylacetate fraction; A3 = mice suffering berghei malaria treated with 4 mg/kg body weight of ethyl acetate fraction*

As shown in Figure 1, dose 1, dose 2, and dose 3 of ethyl acetate fraction of *G. mangostana* L rind showed antimalarial activity (p < 0.05) and dose 1 was the strongest one which had an equivalent antimalarial activity as artemisinin (p > 0.05).

$ED_{50}$ (effective dose 50), which indicates the level of antimalarial activity could be calculated from the parasite growth inhibition data shown in Figure 2 using probit analysis. The correlation between the log of doses and probit of the percentage of parasite growth inhibition was shown in Figure 3.

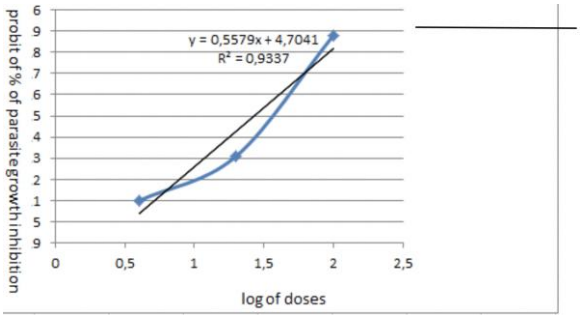



*Figure 3: Correlation between the probit of the percentage of parasite growth inhibition and the log of doses. According to the formula shown in the figure, $ED_{50}$ was 3.396 mg/kg body weight*

### *Effect of the ethyl acetate fraction of G. mangostana* L rind *against total antioxidant status in mice suffering berghei malaria*

Total antioxidant status in the sera of mice suffering berghei malaria after various treatments are shown in the following Table 2.

**Table 2: Total antioxidant status in the sera of mice suffering berghei malaria after various treatments**

| | Total antioxidant level (mMTrolox) | | | | Average±SD |
|---|---|---|---|---|---|
| PC | 0.070 | 0.067 | 0.081 | 0.099 | $0.079^{a} \pm 0.015$ |
| NC | 0.221 | 0.222 | 0.276 | 0.222 | $0.235^{b} \pm 0.027$ |
| AC | 0.193 | 0.231 | 0.203 | 0.191 | $0.204^{b} \pm 0.018$ |
| A1 | 0.121 | 0.146 | 0.112 | 0.108 | $0.122^{c} \pm 0.017$ |
| A2 | 0.2316 | 0.2732 | 0.2393 | 0.3578 | $0.276^{a} \pm 0.058$ |
| A3 | 0.2046 | 0.2240 | 0.2039 | 0.2288 | $0.215^{b} \pm 0.013$ |

Notes: the different superscript alphabet means that there is a significant difference ($p < 0.05$). NC = healthy mice without treatment; PC = mice suffering berghei malaria without treatment; AC = mice suffering berghei malaria treated with artemisinin 50 mg/kg body weight, A1 = mice suffering berghei malaria treated with 100 mg/kg body weight of ethyl acetate fraction; A2 = mice suffering berghei malaria treated with 20 mg/kg body weight of ethyl acetate fraction; A3 = mice suffering berghei malaria treated with 4 mg/kg body weight of ethyl acetate fraction; SD = standard deviation. The TAS level from the highest to the lowest consecutively was: A2, NC/AC/A3, A1, PC.

As shown in Table 2, the TAS level of ethyl acetate fraction groups was higher than PC group ($p < 0.05$) which meant that the fraction had antioxidant activity and could rise the TAS level and possibly could encounter the lack of antioxidant status in mice suffering malaria. The highest serum antioxidant level was in A2 treatment, not in A1. That's mean that there was an optimum dose of this fraction, which leads to the maximum level of the serum antioxidant status. This was consistent with our previous research on co-cultivation of 3D7 clone of *Plasmodium falciparum*-HUVEC (human umbilical vein endothelial cell) which were incubated with $IC_{50}$ of artemisinin and various concentrations of L ascorbic acid as mentioned in the discussion chapter.

## Discussion

Qualitative phytochemical analysis of ethyl acetate fraction of *G. mangostana* L rind was done according to the reported method. It contains several phytochemicals: tannins, terpenoids, triterpenoids, and flavonoids [13], [14].

The antimalarial activity of this ethyl acetate fraction of *G. mangostana* L rind *in vivo* supports the previous study indicating this antimalarial activity *in vitro* [8]. Similar *in vivo* study in Swiss Webster mice suffering berghei malaria showed that ethyl acetate fraction of *G. mangostana* L rind also had a very good antimalarial activity even more active than hexane or methanol fraction [15]. This antimalarial action of the ethyl acetate fraction of *G. mangostana* L rind may be caused by alphamangostin containing in it [10], and alphamangostin itself is a kind of xanthones which had good antimalarial action *in vitro* against 3D7 clone of *P. falciparum* working synergistically with artemisinin [7]. Xanthones could inhibit aggregation of heme, block hemozoin formation, and they bind to the soluble heme cause intra food vacuole osmotic pressure enhancement, and the lysis [5]. Transmission electron microscopical image also showed the inhibition of hemozoin formation of alphamangostin [16]. The result of this study is closely correlated with the qualitative phytochemical analysis, which is needed to be followed by quantitative analysis in further study. This antimalarial action of ethyl acetate fraction of *G. mangostana* L rind was supported the fact that besides gammamangostin, alphamangostin was the most abundant kind of xanthones in mangosteen rind [17].

According to *P. falciparum* foot printing study, mangosteen rind extract targeted several metabolic pathways, particularly glucose and TCA metabolisms [18].

According to Figure 3 and the $ED_{50}$ of this ethyl acetate fraction against *P. berghei in vivo*, the fraction has potentially a very good antimalarial activity [19]. Further study is needed to observe this activity for a longer period for detecting the possibility of recrudescence. Treatment with higher dose is also needed to find out the dose that can inhibit 100% parasite growth.

The previous research indicated that L ascorbic acid as an antioxidant could improve the viability of HUVEC and produce the highest level of GSH at optimum supplementation and not at the maximum [20]. Another study also showed the similar result about the antioxidant as well as prooxidant effect of curcumin which was concentration dependent that's mean in low concentration it had antioxidant effect in contrast against high concentration which had pro-oxidant effect [21]. This phenomenon is very interesting to be studied further.

In conclusion, ethylacetate fraction of *G. mangostana* L rind potentially showed antimalarial and antioxidant activity *in vivo*. Further study is needed to explore the detail of its mechanism of action and its quantitative phytochemical analysis to find the leading compound in it. Longer period of parasitaemia observation and higher doses are also needed to detect recrudescence possibility and to explore the dose which can cause 100% inhibition of parasite growth.

## Acknowledgements

We acknowledge the Directorate General of Higher Education of Indonesian Ministry of Education for "Hibah Unggulan Perguruan Tinggi" grant.

## References

1. Wongsrichanalai C, Meshnick SR. Declining artesunate-mefloquine efficacy against falciparum malaria on the Cambodia-Thailand border. Emerging Infectious Diseases. 2008; 14(5):716-719. https://doi.org/10.3201/eid1405.071601 PMid:18439351

PMCid:PMC2600243

2. Amaratunga C, Lim P, Suon S, Sreng S, Mao S, Sopha C, et al. Dihydroartemisinin-piperaquine resistance in Plasmodium falciparum malaria in Cambodia: a multisite prospective cohort study. LancetInfect Dis. 2016; 16:357-365. https://doi.org/10.1016/S1473-3099(15)00487-9

3. Farnert A, Ursing J, Tolfvenstam T, Rono J, Karlsson L, Sparrelid E, et al. Artemether-lumefantrine treatment failure despite adequate lumefantrine day 7 concentration in a traveller with Plasmodium falciparum malaria after returning from Tanzania. Malar J. 2012; 11:176. https://doi.org/10.1186/1475-2875-11-176 PMid:22632033 PMCid:PMC3416680

4. Zarena AS and Udaya Sankar K.Screening of xanthone from mangosteen (Garcinia mangostana L.) peels and their effect on cytochrome c reductase and phosphomolybdenum activity. Journal of Natural Products. 2009; 2:23-30.

5. Ignatushchenko MV, Winter RW and Riscoe M. Xanthones as antimalarial agents: Stage specificity. Am J Trop Med Hyg. 2000; 62:77-81. https://doi.org/10.4269/ajtmh.2000.62.77 PMid:10761728

6. Percário S, Moreira DR, Gomes BAQ, Ferreira MES, Gonçalves ACM, Laurindo PSOC, Vilhena, TC, et al. Oxidative stress in malaria. Int J Mol Sci. 2012; 13:16346-16372. https://doi.org/10.3390/ijms131216346 PMid:23208374 PMCid:PMC3546694

7. Tjahjani S and Widowati W. Potensi beberapa senyawa xanthone sebagai antioksidan dan anti-malaria serta sinergisme dengan artemisinin in vitro. J Indon Med Assoc. 2013; 63.

8. Tjahjani S. Antimalarial activity of Garciniamangostana L rind and its synergistic effect with artemisinin in vitro. BMC complementary and Alternative Medicine. 2017; 17:1-5. https://doi.org/10.1186/s12906-017-1649-8 PMid:28241761 PMCid:PMC5329916

9. Marito DR, Tjahjani S and Khiong K. The effect of ethanol extract from mangosteen pericarps towards parasitemia in Plasmodium berghei-inoculated mice. Journal of Medicine and Health. 2016; 1:319-325. https://doi.org/10.28932/jmh.v1i4.528

10. Liandhajani, Iwo MI, Sukrasno S, Soemardji AA and Hanafi M. Sunscreen activity of α -mangostin from the pericarps of Garcinia mangostana Linn. Journal of Applied Pharmaceutical Science. 2013; 3:70-73.

11. Mahdi S, Altikriti Y. Extraction of Natural Products. Sweden: Biologiskt Aktiva Natuprodukter, Uppsala University, 2010.

12. Hanafiah KA. Rancangan percobaan: teori & aplikasi. Jakarta: PT Raja Grafindo Persada, 1997.

13. Morsy N. Phytochemical analysis of biologically active constituents of medicinal plants. Main Group Chemistry. 2014; 13(1):7-21.

14. Mishra P, Yadav KS, Gautam G. Comparative qualitative and quantitative phytochemical analysis of Calotropis gigantea and Calotropis proceraroots. Journal of Drug Delivery & Therapeutics. 2018; 8(4):179-184. https://doi.org/10.22270/jddt.v8i4.1757

15. Iqbal M, Effendi Z, Aamruna Y and Suryawati S. Uji aktivitas antimalaria in vivo dari beberapa fraksi ekstrak kulit buah manggis (Garcinia manggostana Linn) pada mencit (Mus musculus) yang diinfeksi dengan Plasmodium berghei. [e-Proceeding PIMNAS], 2013.

16. Tjahjani S, Syafruddin, Tjokropranoto R. Interaction of alphamangostin and curcumin. IOP Conf Series: Earth and Environmental Science. 2018; 125:1-6. https://doi.org/10.1088/1755-1315/125/1/012017

17. Gutierrez-Orozco F and Failla ML. Biological activities and bioavailability of mangosteen xanthones: a critical review of the current evidence. Nutrients. 2013; 5:3163-3183. https://doi.org/10.3390/nu5083163 PMid:23945675 PMCid:PMC3775248

18. Chaijaroenkul W, Mubaraki MA, Ward SA and Na-Bangchang K. Metabolite footprinting of Plasmodium falciparum following exposure to Garcinia mangostana Linn. crude extract. Exp Parasitol. 2014; 145:80-86. https://doi.org/10.1016/j.exppara.2014.07.013 PMid:25102435

19. Munoz V, Sauvain M, Bourdy G, Callapa J, Bergeron S, Rojas I, et al. A search for natural bioactive compounds in Bolivia through a multidisciplinary approach: Part I. Evaluation of the antimalarial activity of plants used by the Chacobo Indians. J Ethnopharmacol. 2000; 69:127-137. https://doi.org/10.1016/S0378-8741(99)00096-3

20. Tjahjani S, Achmad TH and Syafruddin D. Asam L askorbat meningkatkan viabilitas HUVEC dalam kultur P. falciparum yang diinkubasi dengan artemisinin bergantung konsentrasi. Majalah Kedokteran Bandung. 2011; 43(2):66-71. https://doi.org/10.15395/mkb.v43n2.53

21. Banerjee A, Kunwar A, Mishra B and Priyadarsini KI. Concentration dependent antioxidant/pro-oxidant activity of curcumin studies from AAPH induced hemolysis of RBCs. Chem Bio Interact. 2008; 174:134-139. https://doi.org/10.1016/j.cbi.2008.05.009 PMid:18571152

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Jun 30; 7(12):1940-1945.
https://doi.org/10.3889/oamjms.2019.548
eISSN: 1857-9655
***Basic Science***

# The Correlation of *KRAS* Gene Expression and *P53* Immunoexpression in Colorectal Adenocarcinoma

Meike Rachmawati[1,2*], Herry Yulianti[1], Bethy S. Hernowo[1], Sri Suryanti[1], Indra Wijaya[3], Nur Rahadiani[4], Didik S. Heriyanto[5], Irianiwati Irianiwati[5]

*[1]Department of Pathology Anatomy, Oncology and Stem Cells Research Centre, Padjadjaran University, Dr Hasan Sadikin Hospital, Bandung, Indonesia; [2]Department of Pathology Anatomy, Universitas Islam Bandung, Bandung, Indonesia; [3]Department of Interne Medicine, Padjadjaran University, Dr Hasan Sadikin, Hospital Bandung, Bandung, Indonesia; [4]Department of Pathology Anatomy, Indonesia University, Cipto Mangun Kusumo Hospital, Jakarta, Indonesia; [5]Department of Pathology Anatomy, Gajah Mada University, Sardjito Hospital, Jogjakarta, Indonesia*

**Citation:** Rachmawati M, Yulianti H, Hernowo BS, Suryanti S, Wijaya I, Rahadiani N, Heriyanto DS, Irianiwati I. The Correlation of *KRAS* Gene Expression and *P53* Immunoexpression in Colorectal Adenocarcinoma. Open Access Maced J Med Sci. 2019 Jun 30; 7(12):1940-1945. https://doi.org/10.3889/oamjms.2019.548

**Keywords:** KRAS; p53; Adenocarcinoma Colorectal (ADCCR)

***Correspondence:** Meike Rachmawati. Department of Pathology Anatomy/Oncology and Stem Cells Research Centre, Padjadjaran University/Dr. Hasan Sadikin Hospital, Bandung, Indonesia; Department of Pathology Anatomy, Universitas Islam Bandung, Bandung, Indonesia. E-mail: meikerachmawati@gmail.com

**Received:** 01-Apr-2019; **Revised:** 11-Jun-2019; **Accepted:** 12-Jun-2019; **Online first:** 30-Jun-2019

**Copyright:** © 2019 Meike Rachmawati, Herry Yulianti, Bethy S. Hernowo, Sri Suryanti, Indra Wijaya, Nur Rahadiani, Didik S. Heriyanto, Irianiwati Irianiwati. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

**Funding:** This research was funded by an internal grant for Padjadjaran University, Indonesia batch 2 no: 4851/UN6.C/LT/2018

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

**Abstract**

**BACKGROUND:** Colorectal Adenocarcinoma (ADCCR) is the third most cancer not only in the world but also in Indonesia. There were 623 cases of ADCCR at Dr Hasan Sadikin hospital within 2015-2017. Both KRAS and TP53 mutation are known as genes which involve in carcinogenesis through the same pathway, namely the chromosomal instability pathway. In West Java, researches focusing on mutation KRAS and p53 also a correlation between both biomarkers among ADCCR patients are still limited.

**AIM:** Therefore, this research aimed to perceive a correlation between KRAS gene expression with p53 immunoexpression in ADCCR.

**METHODS:** Cross section research design was performed to 62 cases of ADCCR as paraffin block taken from 4 hospitals in West Java, including Dr Hasan Sadikin hospital Bandung, Santosa hospital Bandung, Borromeus hospital Bandung and Syamsudin hospital Sukabumi from January 1st 2014 to 31s November 2018. KRAS mutation gene data taken from secondary data at molecular laboratory in Ciptomangunkusumo Hospital Jakarta and Dr Sardjito Hospital Jogjakarta, while the detection of p53 immunoexpression data using immunohistochemical staining was carried out in the Laboratorium of Anatomical Pathology of Padjadjaran University (Dr Hasan Sadikin Hospital). All data were analysed using Chi-Square test with p-value < 0,05 of significant level then proceeded with Stata ver.11 for windows.

**RESULTS:** The results of this study showed that KRAS gene expressions from 62 sample consist of 39 wild type KRAS (62.39%) and 23 mutant KRAS (37.1%). The p53 immunoexpression consists of 27 negative cases (non-mutant p53) and 35 mutant p53, which includes 10 cases as focal expression (16.33%) and 25 cases as diffuse expressions (40.33%). There is a significant association between KRAS gene expression and p53 immunoexpressions in ADCCR (p = 0.04), with mild positive correlation (Rho 0.28).

**CONCLUSION:** This study concluded that KRAS and p53 mutations are involved in carcinogenesis, and the p53 mutation is a more dominant risk factor than KRAS mutation among West Java people. P53 mutations with diffuse pattern tend to express mutant KRAS while p53 negative and having a focal pattern tend to express wt KRAS.

## Introduction

Colorectal carcinoma (CRC) is a malignant epithelial tumour originating in the large bowel. Colorectal carcinoma ranks as the third most frequent cancer not only in the world but also in Indonesia [1], [2]. The worldwide mortality rate is about 608.000 deaths [2]. In Indonesia, the mortality rate is about 9.5% of all cancer deaths [3], [4]. According to data from the Department of Anatomy Pathology Dr Hasan Sadikin Hospital Bandung, the frequency of CRC is about 224 cases in 2015, 187 cases in 2016 and 212 cases in 2017.

Colorectal Adenocarcinoma (ADCCR) is the most frequent type 0f CRC in the world [1]. There are many mutation genes occur in ADCCR, such as Adenomatous Polyposis Coli (APC), TP53, Kirsten rat sarcoma virus (KRAS), PIK3CA, etc. [5]. These genes are involved in carcinogenesis through three major pathways such as chromosomal instability, mismatch repair and CpG island methylator phenotype (CIMP).

The most frequent pathway is chromosomal instability, about 65% [6], [7]. Three genes are involved in this pathway; they are: 1) APC gene mutation as an initiator in the early phase of carcinogenesis. 2) Kirsten rat sarcoma virus gene mutation, which occurs in 50% of ADCCR from intermediate phase to advance phase. 3) TP53 gene mutation in the late phase of carcinogenesis is about 40-60% (Figure 1) [6].

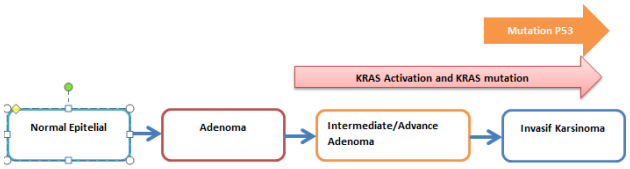



*Figure 1: KRAS mutation can occur together with p53 mutation in the late phase of carcinogenesis. Both of them involved in the same pathway of carcinogenesis (instability chromosome pathway)*

KRAS gene expression consists of wild type (wt) KRAS and mutant KRAS. Wild type KRAS gene is located in short arm of chromosome number 12, in 12 p12.1. This product is wt KRAS protein, which can flip back and forth between an excited signal transmitting state and quiescent state. RAS is inactive when bound to GDP, but stimulation of cells by growth factors such as EGF and PDGF leads to an exchange of GDP for GTP and subsequent conformational changes that generate active RAS and stimulate downstream regulators of cells proliferation [6], [8].

This excited signal emitting state, in short, lived' however, because the intrinsic guanosine triphosphatase (GTPase) activity of wt RAS hydrolyses GTP to GDP return the active wt KRAS to its inactive state [8]. In ADCCR wt KRAS will always be active because EGF stimulates continuously. ADCCR patients with wt KRAS have a good response to anti-epidermal Growth Factor Receptor (EGFR) drugs such as cetuximab and panitumumab [1], [9], [10], [11]. In contrast, mutant KRAS was not responsive to anti-EGFR inhibition [1], [3], [12]. Mutant KRAS can be active continuously although having limited EGFR stimulation. This gene has GTP-ase activity lower than wt KRAS, resulting in a decrease of hydrolysing activity 3-9 times lower than wt KRAS. This situation makes the irreversible signal to downstream regulation to provide uncontrolled proliferation and differentiation cells [8].

Mutant KRAS gene occurs in exon 2, 3 and 4. Exon 2 is the frequent site of mutation [12] — mutant KRAS gene as a predictive negative response from anti-EGFR therapy. Patient with mutant KRAS has a worse prognosis than those with wt KRAS. Product of mutant KRAS gene is mutant KRAS protein [1], [9], [10], [12].

In ADCCR another abnormality is Tumor Protein (TP) 53 pathway consists of loss of function wt TP53 and the increase of TP53 mutant concentration. Wild type TP53 is normally in the latent phase and will be stabilised and activated by genotoxic agent and another stress signal. Regulatory of TP53 is Murine Double minute 2 (Mdm2), a protein which can bind TP53 in unstable or inactivated conditions. TP53 gene can be activated by the detachment of TP53 from Mdm2. This condition makes this gen stop the cell cycle and repairing DNA breakdown and involving in apoptosis and ageing [6].

In the state of activated p53 due to release from Mdm2, p53 will have the ability to trigger cell cycle discontinuation, repair DNA damage and be involved in the process of apoptosis and cell ageing [6]. One of the p53 gene mutations is caused by the loss of alleles on the chromosome on the short arm of chromosome 17, resulting in genomic disorders that occur in the later phase of tumour progression [8]. Most TP53 mutations occur in exons 5 to 8, which encode residues 130-286, which are important and responsible for folding and stabilising tertiary structures of proteins [8]. Mutations in the P53 gene can result in loss of wild type TP53 ability for DNA repair, apoptosis, checkpoints, and cell ageing processes [8], [9], [10]. Also, the TP53 mutation produces a mutant P53 gene which acts as an oncogene, thereby increasing the aggressiveness, survival and metastasis ability of the tumour cell [13], [14]. The product of the P53 gene mutation is a mutant p53 protein that can be examined by several methods, including the Immunohistochemical (CPI) method [8], [9], [10].

The expression of the KRAS and TP53 genes are mostly associated with pathological features, lymph node metastasis, distant metastasis and ADCCR therapy response [10, [15], [16], [17]. The role of these two molecular markers is widely used as a predictor of prognosis of ADCCR therapy. The Assesment of the anti p53 antibody with immunohistochemistry methods is lower in cost but more effective and efficient. Thus it can be considered as one alternative to predict KRAS gen expressions in ADCCR patients. However, studies involving the relationship of KRAS mutations as one of the stress signals in tumour cells with mutant Immunoexpression p53 are still not widely performed and documented [6], [10], [13], [14], [15], [16], [17].

The objective of this study is to understand the relationship and correlation between KRAS gene expression and p53 immunoexpression in ADCCR.

## Methods

Samples were obtained from tissue biopsy and or colectomy of surgery patients registered at Dr Hasan Sadikin Hospital Bandung, Santosa hospital Bandung, Borromeus hospital Bandung and Syamsudin hospital Sukabumi. Histopathologically

diagnosed with ADCCR from January 1st, 2014 until November 31st, 2018. Samples were attained after approved by the Ethical Committee with assessment number 105/UN6.KEP/EC/2019, and then drawn according to inclusion criteria.

Sixty-two samples of KRAS gene expression had been checked in the molecular laboratory, Ciptomangunkusumo Hospital Jakarta and Sardjito Hospital Jogjakarta. To see if mutation in exon 2 occur, the KRAS mutation using PCR-HRM (High-Resolution Melting-Polymerase Chain Reaction) methods was combined with RFLP (Restriction fragment length polymorphism) and to see if mutation in exon 3 and 4 occur, the KRAS mutation using PCR-HRM (High-Resolution Melting-Polymerase Chain Reaction) methods was combined with direct DNA sequencing. KRAS mutation is positive if there is 'split peaks' in the melting curve that shows two populations (wild type and mutant allele) and occurs in at least 1 out of 3 exons. KRAS mutation is negative (wild type) if there are no 'split peaks. These gene expressions data were recorded and collected as secondary data in this research

P53 immunoexpression had been checked by immunohistochemistry staining procedure at the Laboratorium of Anatomical Pathology of Padjadjaran University (Dr. Hasan Sadikin Hospital. Immunohistochemistry staining using mouse monoclonal antibody p53 (Santa Cruz, Pab-1801, SC-98) with dilution 1:150 was used in standard immunohistochemistry (IHC) staining procedure. Immunoexpression of p53 was categorised by calculating the distribution of the cells that showed immunoreactivity, which was the nucleus-stained cells. Distribution score was explained as 0 = negative; Focal = 1-50%; Diffuse = ≥ 50%. Intensity compares with breast cancer cells as an internal positive control. IHC staining result was examined by two experts in the IHC technique using light microscope Olympus CX31.

### *Statistical analysis*

The data obtained from this research was analysed using Chi-Square test and Spearman Rho correlation. A significant association was interpreted from p-value where $p \leq 0.05$ showed a statistically significant association, while $p \geq 0.05$ showed otherwise. If the Spearman rho was not equal to 0, the significant correlation showed. If Spearman rho = 0, it showed otherwise. The data attained from laboratory procedure was recorded in a distinct form, and Stata ver 11 *for Windows* was used to be analysed statistically.

## Results

The characteristics of the research subjects are based on age, gender and the degree of ADCCR differentiation as mention in table 1. Age < 40 years as many as 8 cases (12.90%), age 40-60 years as many as 35 cases (56.45%) and age > 60 years as many as 19 cases (30.65%). The youngest is 26 years old, and the oldest is 81 years old, and the middle value is 54 years. The males are 33 people (53.23%), and females are 29 people (46.77%). Histological degree in ADCCR consists of 42 well-differentiated cases (67.74%), 10 cases for each moderately and poorly differentiated (16.13%).

**Table 1. Research patient's characteristic**

| Characteristics | ADCCR (n = 62) |
|---|---|
| Age (years) | |
| Mean ± Std | 54 ± 12 |
| Median (min, maks) | 54 (26.81) |
| ≤ 40 years | 8 (12.9%) |
| 40-60 years | 35 (56.45%) |
| ≥ 60 years | 19 (30.65%) |
| Sex | |
| Male | 33 (53.23%) |
| Female | 29 (46.77%) |
| Histological degree | |
| Well | 42(67.74%) |
| Moderately | 10 (16.13%) |
| Poorly | 10 (16.13%) |

Based on secondary data from PCR examination, KRAS gene expression includes wt KRAS (negative mutation) and KRAS mutants. P53 immunoexpression in this study was assessed from the brown stained of the tumour cell nucleus. The result of p53 immunohistochemical staining was assessed based on tumour cell distribution consisting of negative, focal and diffuse. The results of the data and calculations are shown in Table 2.

Table 2 shows the descriptive data distribution regarding the frequency of the KRAS gene expression and p53 immunoexpression. It is shown that the most visible KRAS gene expression is wt KRAS as many as 39 cases (62.39%), KRAS mutations occur in only 23 cases (37.1%). The highest p53 Immunoexpression is in the negative distribution of 27 cases (43.55%), diffuse distribution of 25 cases (40.32%), and the focal distribution of 10 cases (16.13%).

**Table 2: KRAS Gene Expression and p53 Immunoexpression**

| Characteristics | ADCCR (n = 62) |
|---|---|
| KRAS gene expression | |
| Wt KRAS (negative mutation) | 39 (62.39%) |
| Mutant KRAS | 23 (37.1%) |
| P53 immunoexpression | |
| Negative | 27 (43.55%) |
| Focal | 10 (16.13%) |
| Diffuse | 25 (40.33%) |

In this study, the statistical tests were using Chi-square to analyse the relationship between expression of the KRAS gene and immunoexpression of TP53 in ADCCR and using Spearman Rho to analyse the correlation between expression of the KRAS gene and immunoexpression of TP53 in ADCCR. The result is a significant relationship with a value of p = 0.04 (p < 0.05)), with positive mild correlation (Rho 0.28) as shown in Table 3.

**Table 3: Correlation of KRAS Gene Expression with p53 Immunoexpression**

| KRAS | P53 Immunoexpression | | | P value (Chi Square) | Spearman Rho (Correlation) | P(Spearman) |
|---|---|---|---|---|---|---|
| | Negative | Focal | Diffuse | | | |
| Wt | 20 (74.07%) | 8 (80%) | 11 (44%) | | | |
| Mutant | 7 (25.93%) | 2 (20%) | 14 (56%) | 0.04 | 0.28 | 0.03 |

Immunoexpression of p53 was categorised by calculating the distribution of the cells that showed immunoreactivity, which was the nucleus-stained cells. Distribution score was explained as 0 = negative; Focal = 1-50%; Diffuse = ≥ 50%. The figure below showed the distribution of p53 in tumour cells more than 50% categorised as Diffuse distribution.

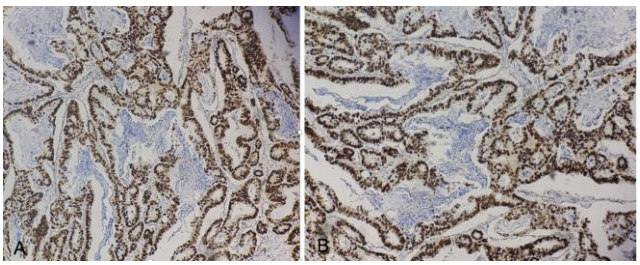



*Figure 2: A) Immunoexpression p53 diffuse distribution (magnification 40 x ); B) Immunoexpression p53 diffuse distribution (magnification 100 x)*

## Discussion

The collection of KRAS gene expression examination data in all ADCCR cases was carried out. The result is that the KRAS mutation only occurs in 23 cases (37.1%). This figure is lower than the data obtained from the literature (50%), but it is higher than the research conducted by Sameer et al. in Kashmir ethnicity which was 22.6% [4], [6]. The results of the KRAS secondary data collection showed that 62.9% of ADCCR in this study has a wild type gene expression profile. In ADCCR through the wt KRAS pathway, continuous KRAS activation will occur because it is stimulated continuously by growth factors, one of which is EGF which will bind to EGFR to subsequently induce various proteins that help KRAS phosphorylation process. Phosphorylated KRAS is KRAS, which is bound to GTP, which will give hyperproliferative signals to cancer cells [1], [6], [18].

ADCCR patients with wt KRAS status have a good response to anti-EGFR therapy [8], [18]. This study shows that 62.9% of ADCCR cases in this study had a good response to anti-EGFR, such as cetuximab and panitumumab [1], [3], [11], [18].

Patients with wt KRAS will have a better prognosis than patients with mutant KRAS [1], [8], [18]. The ability of tumour cells to proliferate, differentiate, invade, and metastasis is not as bad as in ADCCR patients with mutant KRAS status. This is by the most differentiation degrees in this study, namely low degrees with better prognosis (67.74%). KRAS mutations only occurred in 23 cases (37.1%). These results indicate that it is necessary to consider other alternative carcinogenesis pathways in ADCCR in West Java in addition to the CIN pathway.

In ADCCR, the TP53 pathway can experience abnormalities, including loss of function wt TP53 and increased mutant TP53 levels [10]. The products of the two genes are wt p53 and p53 mutant proteins. In this study, the measured protein is the p53 mutant using the IHK method, and the results of the assessment are thus referred to as p53 immunoexpression. The results of this study showed that the highest p53 immunoexpression was 27 cases of negative distribution (43.55%), 25 cases of diffuse distribution (40.32%) and 10 cases of focal distribution (16.13%). These results showed mutant p53 occurred in 35 cases (56, 45%). These results are in line with the literature, which states that p53 mutations can occur in as many as 40-60% of ADCCR cases [10]. It was higher than the incidence of ADCCR which was identified as having a TP53 gene mutation in Saudi Arabia of 33.7% but data from WHO showed a higher mutation of 80% [1], [16].

In this study, the statistical tests were using Chi-square to analyse the relationship between expression of the KRAS gene and immunoexpression of TP53 in ADCCR and using Spearman Rho to analyse the correlation between expression of the KRAS gene and immunoexpression of TP53 in ADCCR. The result is a significant relationship with a value of p = 0.04 (p < 0.05)), with positive mild correlation (Rho 0.28). This may be due to the activation of the wt KRAS by excessive growth factors signal that will cause cell stress due to hyperproliferative signals caused by stimulation of proteins involved in the KRAS pathway which activates the cell nucleus for the process of transcription and proliferation [13], [14]. This causes various activation of E2F protein, a protein involved in triggering the role of tumour suppressor genes such as TP53, RB, and P21 [13], [14].

The signalling pathway that connects the KRAS mutation with wildtype TP53 has been widely documented, one of which is research on mice that have become experimental animal models for lung cancer. The results of the study stated that the KRAS mutations that occur could lead to excessive cell

proliferation. This Hyperproliferative signal is considered a stress signal which triggers the release of wt TP53 from Murine Double Minute 2 (Mdm2). This results in wt TP53 being able to stop the cell cycle and trigger the apoptosis program and protect cells from abnormal growth factors [13].

One study reported that the correlation of KRAS mutations in ADCCR patients had a low prevalence of TP53 mutations. This result contrary to this study, which wt KRAS tend to show high frequent of negative (20/27; 74.07%) and focal (8/10; 80%) TP53 mutant. This is possible because the mutations occurred in ADCCR in the study were indeed low so that the wt KRAS predominant as hyperproliferative stress compare than mutant KRAS and will trigger the wt TP53 pathway, especially for well-differentiated and less aggressive tumours, characterised by slow growth and low metastatic ability. This was caused by the fact that TP53 still functions as a tumour suppressor and have a little oncogenic ability [13], [14], [20]. In this study, the proportion of KRAS mutations was low in the wt KRAS, whereas positive p53 immunoexpression (mutant p53) was higher than the negative (non-mutant p53). This is possible because the KRAS mutation in ADCCR in this study had little role in carcinogenesis in the CIN pathway compared to TP53 mutations. This result shows that ADCCR among west java people mostly occurred sporadically.

In conclusion, a mild correlation occurs in the relationship between the expression of the KRAS gene and p53 immunoexpression in ADCCR In the case of ADCCR with diffuse p53 immunoexpression, it expresses or predicts the expression of mutant KRAS genes. On the contrary, in the case of ADCCR with negative and focal p53 immunoexpression, it tends to express or predict the expression of the wt KRAS gene.

## Acknowledgement

This research was funded by an internal grant for Padjadjaran University batch 2 no: 4851/UN6.C/LT/2018. We thanked Mr Wahyudin, Mrs Nur, Mr Herman, for excellent technical assistance. We also thanked Mrs Arief Budi Yulianti and Mr Fajar Awaliya and for excellent manuscript writing and statistician assistance.

## References

1. Bosman FT, Carneiro F, Hruban RH, Theise ND. WHO classification of tumours of the digestive system. World Health Organization; 2010:131-181.

2. Mahasneh A, Al-Shaheri F, Jamal E. Molecular biomarkers for early diagnosis, effective treatment and prognosis of colorectal cancer: Current updates. Experimental and molecular pathology. 2017; 102(3): 475-83. https://doi.org/10.1016/j.yexmp.2017.05.005 PMid:28506769

3. Kementrian Kesehatan RI. Panduan Penatalaksanaan Kanker kolorektal. 2015; 1-67.

4. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018; 68(6):394-424. https://doi.org/10.3322/caac.21492 PMid:30207593

5. Testa U, Pelosi E, Castelli G. Colorectal cancer: genetic abnormalities, tumour progression, tumor heterogeneity, clonal evolution and tumor-initiating cells. Medical Sciences. 2018; 6(2):31. https://doi.org/10.3390/medsci6020031 PMid:29652830 PMCid:PMC6024750

6. Kumar V, Abbas AK, Aster JC. Robbins basic pathology. ed-10. Elsevier Health Sciences; 2017:261-71.

7. Abdullah M, Sudoyo AW, Utomo AR, Fauzi A, Rani AA. Molecular profile of colorectal cancer in Indonesia: is there another pathway?. Gastroenterology and Hepatology from bed to bench. 2012; 5(2):71-5.

8. Tan C, Du X. KRAS mutation testing in metastatic colorectal cancer. World journal of gastroenterology: WJG. 2012; 18(37):5171

9. Sui X, Zhu J, Tang H, Wang C, Zhou J, Han W, Wang X, Fang Y, Xu Y, Li D, Chen R. p53 controls colorectal cancer cell invasion by inhibiting the NF-κB-mediated activation of Fascin. Oncotarget. 2015; 6(26):22869. https://doi.org/10.18632/oncotarget.5137 PMid:26362504 PMCid:PMC4673205

10. Chaar I, Amara S, Elamine OE, Khiari M, Ounissi D, Khalfallah T, Mzabi S, Bouraoui S. Biological significance of promoter hypermethylation of p14/ARF gene: relationships to p53 mutational status in Tunisian population with colorectal carcinoma. Tumor Biology. 2014; 35(2):1439-49. https://doi.org/10.1007/s13277-013-1198-9 PMid:24065196 PMCid:PMC3932170

11. Levi M, Prayogi G, Sastranagara F, Sudianto E, Widjajahakim G, Gani W, Mahanadi A, Agnes J, Khairunisa BH, Utomo AR. Clinicopathological associations of K-RAS and N-RAS mutations in Indonesian colorectal cancer cohort. Journal of gastrointestinal cancer. 2018; 49(2):124-31. https://doi.org/10.1007/s12029-016-9901-x PMid:28044264

12. Van Cutsem E, Kohne CH, Láng I, Folprecht G, Nowacki MP, Cascinu S, Shchepotin I, Maurel J, Cunningham D, Tejpar S, Schlichting M. Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: updated analysis of overall survival according to tumor KRAS and BRAF mutation status. J Clin Oncol. 2011; 29(15):2011-9. https://doi.org/10.1200/JCO.2010.33.5091 PMid:21502544

13. Suh YA, Post SM, Elizondo-Fraire AC, Maccio DR, Jackson JG, El-Naggar AK, Van Pelt C, Terzian T, Lozano G. Multiple stress signals activate mutant p53 in vivo. Cancer research. 2011; 71(23):7168-75. https://doi.org/10.1158/0008-5472.CAN-11-0459 PMid:21983037 PMCid:PMC3320147

14. Doyle B, Morton JP, Delaney DW, Ridgway RA, Wilkins JA, Sansom OJ. p53 mutation and loss have different effects on tumourigenesis in a novel mouse model of pleomorphic rhabdomyosarcoma. The Journal of pathology. 2010; 222(2):129-37. https://doi.org/10.1002/path.2748 PMid:20662002

15. Ross JS. Clinical implementation of KRAS testing in metastatic colorectal carcinoma: the pathologist's perspective. Archives of pathology & laboratory medicine. 2012; 136(10):1298-307. https://doi.org/10.5858/arpa.2011-0478-RA PMid:22272560

16. Al-Kuraya KS. KRAS and TP53 mutations in colorectal carcinoma. Saudi journal of gastroenterology. 2009; 15(4):21-7. https://doi.org/10.4103/1319-3767.56087 PMid:19794264 PMCid:PMC2981835

17. Mármol I, Sánchez-de-Diego C, Pradilla Dieste A, Cerrada E,

Rodriguez Yoldi M. Colorectal carcinoma: a general overview and future perspectives in colorectal cancer. International journal of molecular sciences. 2017; 18(1):197. https://doi.org/10.3390/ijms18010197 PMid:28106826 PMCid:PMC5297828

18. Arrington AK, Heinrich EL, Lee W, Duldulao M, Patel S, Sanchez J, Garcia-Aguilar J, Kim J. Prognostic and predictive roles of KRAS mutation in colorectal cancer. International journal of molecular sciences. 2012; 13(10):12153-68. https://doi.org/10.3390/ijms131012153 PMid:23202889 PMCid:PMC3497263

19. Nyiraneza C, Jouret-Mourin A, Kartheuser A, Camby P, Plomteux O, Detry R, Dahan K, Sempoux C. Distinctive patterns of p53 protein expression and microsatellite instability in human colorectal cancer. Human pathology. 2011; 42(12):1897-910. https://doi.org/10.1016/j.humpath.2010.06.021 PMid:21665242

20. Knickelbein K, Zhang L. Mutant KRAS as a critical determinant of the therapeutic response of colorectal cancer. Genes & diseases. 2015; 2(1):4-12. https://doi.org/10.1016/j.gendis.2014.10.002 PMid:25815366 PMCid:PMC4372129

21. Duldulao MP, Lee W, Nelson RA, Li W, Chen Z, Kim J, et al. Mutations in specific codons of the KRAS oncogene are associated with variable resistance to neoadjuvant chemoradiation therapy in patients with rectal adenocarcinoma. Annals of surgical oncology. 2013; 20(7):2166-71. https://doi.org/10.1245/s10434-013-2910-0 PMid:23456389 PMCid:PMC5584556

22. Muller PAJ, Vousden KH. Mutant p53 in cancer: new functions and therapeutic opportunities. Cancer cell. 2014; 25(3):304-17. https://doi.org/10.1016/j.ccr.2014.01.021 PMid:24651012 PMCid:PMC3970583

23. Lukashchuk N, Vousden KH. Ubiquitination and degradation of mutant p53. Molecular and cellular biology. 2007; 27(23):8284-95. https://doi.org/10.1128/MCB.00050-07 PMid:17908790 PMCid:PMC2169174

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Jun 30; 7(12):1946-1950.
https://doi.org/10.3889/oamjms.2019.545
eISSN: 1857-9655
***Clinical Science***

# The Effect of Adding Lidocaine to Patient Controlled Analgesia with Morphine on Pain Intensity after Caesarean Section with Spinal Anesthesia: A Double-Blind, Randomized, Clinical Trial

Ali Habibi[1], Abbas Alipour[2], Afshin Gholipour Baradari[3], Abdolmajid Gholinataj[3], Mohammad Reza Habibi[3], Saloumeh Peivandi[4*]

*[1]Student Research Committee, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran; [2]Department of Epidemiology, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran; [3]Department of Anesthesiology, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran; [4]Department of Gynecology and Obstetrics, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*

**Citation:** Habibi A, Alipour A, Gholipour Baradari A, Gholinataj A, Habibi MR, Peivandi S. The Effect of Adding Lidocaine to Patient Controlled Analgesia with Morphine on Pain Intensity after Caesarean Section with Spinal Anesthesia: A Double-Blind, Randomized, Clinical Trial. 2019 Jun 30; 7(12):1946-1950. Open Access Maced J Med Sci. https://doi.org/10.3889/oamjms.2019.545

**Keywords:** Lidocaine; Morphine; Caesarean Section; Gynecologic Surgical Procedures; Analgesia; Patient-Controlled

***Correspondence:** Saloumeh Peivandi. Department of Gynecology and Obstetrics, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran. E-mail: s.peivandi@mazums.ac.ir

**Received:** 16-May-2019; **Revised:** 16-Jun-2019; **Accepted:** 17-Jun-2019; **Online first:** 26-Jun-2019

**Copyright:** © 2019 Ali Habibi, Abbas Alipour, Afshin Gholipour Baradari, Abdolmajid Gholinataj, Mohammad Reza Habibi, Saloumeh Peivandi. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

**Funding:** This research has been financially supported by the deputy of research and technology, Mazandaran University of Medical Sciences, Sari, Iran

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

**Abstract**

**BACKGROUND:** Caesarean section is one of the commonest gynaecological surgeries.

**AIM:** Given the importance of pain relief after caesarean section surgery as well as contradictions in the studies conducted on intravenous lidocaine analgesic effects, this study aimed to evaluate the effect of adding lidocaine to patient-controlled analgesia (PCA) with morphine on pain intensity after caesarean section surgery.

**MATERIAL AND METHODS:** In a double-blinded, randomised clinical trial, 80 women who were scheduled for caesarean section surgery with spinal anaesthesia at Sari Imam Khomeini Hospital in 2017 were randomly assigned into two intervention and control groups. After surgery, all patients were connected to a morphine PCA pump. The PCA solution (total volume = 100 ml) in intervention group contained 50 ml of 2% lidocaine and 30 mg (3 ml) of morphine in 47 ml normal saline. In the control group, the PCA pump contained 30 mg (3 ml) of morphine, and the rest (97 cc) was normal saline. Patients' pain intensity was assessed at 2, 4, 6, 12, 18 and 24 hours after surgery using a visual analogue scale (VAS). Additionally, their postoperative nausea and vomiting, duration of hospitalisation, duration of ileus relapse after surgery, and patients' satisfaction after surgery were evaluated. Data were analysed using SPSS version 22 software.

**RESULTS:** The mean and standard deviation of pain intensity in all patients at the intervals of 2, 4, 6, 12, 18 and 24 hours after surgery were 5.91 ± 1.57, 4.97 ± 1.55, 3.84 ± 1.60, 3.54 ± 1.45, 2.56 ± 1.70 and 0.94 ± 1.70, respectively. Data analysis revealed that, regardless of the groups, postoperative pain intensity significantly decreased ($P < 0.0001$). However, there were no significant differences between the two groups in terms of mean postoperative pain intensity at any time interval ($p > 0.05$). Also, there was no significant difference between the two groups in terms of frequency of receiving the diclofenac suppositories after the surgery ($p > 0.05$). Additionally, there was no statistically significant difference between the two groups in terms of postoperative nausea and vomiting, duration of hospitalisation, duration of postoperative ileus relapse and patients' satisfaction ($p > 0.05$).

**CONCLUSION:** Based on the results of this study, it seems that adding lidocaine to PCA with morphine, compared with morphine PCA alone, do not have a significant effect on reducing the pain intensity after cesarean section using spinal anaesthesia. Although, further studies with larger sample size are warranted.

## Introduction

Caesarean section is one of the commonest gynaecological surgeries. Its prevalence of sectarian section is increasing due to various causes, such as increased marriage age and socioeconomic status of the community; so that 65% of Iranian pregnant women underwent cesarean section and about 22.5% of all deliveries in the world are performed by cesarean section. Thus, cesarean section surgery is one of the health priorities of the community [1]. After surgery, patients experience pain inevitably at varying levels. Following caesarian section surgery, patients suffer from acute pain due to a complex physiological response to tissue damage, visceral dilatation, and uterus contractions [2], [3]. Postoperative pain results in undesirable physiological effects, such as the lack of discharge of respiratory secretions, ileus, and

prolonged bed rest, leading to increased risk of deep vein thrombosis and delay in onset of breastfeeding. Therefore, finding a way to minimise complications and bring the highest pain relief for the patient is one of the most important issues after the cesarean section surgery [4], [5]. Opioids, especially in their injectable forms, are highly used to relieve acute postoperative pain, including cesarean section [4], [6]. Moreover, pain is a multifactorial phenomenon which is not completely controlled merely by opioids. Additionally, using opioids is associated with dose-related complications such as respiratory depression, nausea, vomiting, urinary retention, itching, drowsiness or ileus [7].

Thus, it seems reasonable to use compounds that can mitigate the pain intensity in the patients without causing side effects caused by using opioids. Lidocaine is one of the drugs used to relieve pain after surgery. When the use of opioids causes insufficient pain relief or excessive side effects, the use of lidocaine is an appropriate option to control visceral and central pains [8]. Various studies have used lidocaine for postoperative pain control, which has led to the controversial results [9], [10], [11], [12], [13], [14]. Also, doing further studies to investigate the effects of intravenous lidocaine in controlling acute postoperative pain in various surgical procedures have been recommended [15]. It has been previously confirmed that the excretion of lidocaine in breast milk is low and the potential disadvantages caused by child breastfeeding of a mother who has received lidocaine is negligible [16], [17]. Therefore, considered to the abovementioned issues and the importance of pain control after cesarean section surgery, as well as the contradictions in the literature regarding the effect of intravenous lidocaine, as an adjuvant for postoperative pain management, this study was conducted aiming to evaluate the effect of adding lidocaine to patient-controlled analgesia (PCA) with morphine on pain intensity after caesarean section surgery.

## Material and Methods

After obtaining approval from the institutional Ethics Committee and informed written consent from patients, 80 women who were scheduled for caesarean section surgery with spinal anaesthesia at Sari Imam Khomeini Hospital were included in this double-blind, randomised, clinical trial. The study was carried out between October and December 2018 and registered in the Iranian Registry of Clinical Trials Database (IRCT20100713004365N23).

The inclusion criteria were candidate for non-emergency cesarean section under the spinal anaesthesia, age between 20 and 45 years, American Society of Anesthesiologists (ASA) class I, first or second pregnancy, lack of sensitivity to lidocaine or bupivacaine, no previous intra-abdominal surgery, lack of multiple pregnancy, lack of preoperative pain, lack of substance abuse and psychotropic drugs uses, and body mass index (BMI) less than 40. Exclusion criteria were the patient's unwillingness to continue studying at any time, midline surgical incision for any cause, the occurrence of any unusual complications during surgery and lack of willingness to perform spinal anaesthesia. Of all patients who evaluated, 80 patients met the inclusion criteria and randomly allocated into two groups using computer-generated random numbers. This study was double-blinded study, in which the statistician, the project executor and the patients were unaware of the assignment of patients in the treatment groups, and the data collection form was completed by a project executor collaborator who was unaware of the study groups. Before the surgery, patients' demographic characteristics such as age, educational level, weight (kg), height (cm), duration of surgery, getting out of bed after surgery were also evaluated and recorded. Detailed explanations were provided on the way of measuring the severity of postoperative pain and nausea and vomiting using the Visual Analog Scale (VAS) as well as the use of the PCA pump, and the necessary training was provided in this regard. Before the anaesthetic procedure, a good peripheral vein was taken from a non-dominant hand. Thirty minutes before the procedure, 7 ml/kg of crystalloid serum was given to these patients, and then spinal anaesthesia was performed. Patients were placed in the sitting position, and spinal punctures were performed in the L2-L4 and L4-L5 space using Quinke G25 spinal needle and midline approach.

The PCA pump in the intervention group contained 50 ml of lidocaine 2% plus 30 mg of morphine (3 ml) and the rest was 47 cc normal saline (total volume of the pump was 100 ml). In the control group, the PCA pump contained 30 mg of morphine (3 ml), and the rest (97 cc) was normal saline. The pumps were numbered 1 and 2 and were similar in terms of volume and appearance, and only the nurse who was the project contributor was aware of it, and other staffs including surgeon and anesthesiologist were not aware of the two groups. After completion of anaesthesia and recovery of patients, all patients were connected to a morphine PCA pump. The settings for the PCA pump were as follows: bolus dose of 0.5 cc and 15 minutes of lockout interval and background infusion rate of 2 cc/h.

The primary outcome of this study was the postoperative pain intensity that was assessed by a nurse using VAS at times of 2, 4, 6, 12, 18, 24 hours after surgery when the patient was at the resting position. The secondary outcomes were patient satisfaction from pain control, postoperative nausea and vomiting, duration of patient hospitalisation, and the duration of ileus relapse after surgery.

### *Statistical Analysis*

Data were analysed by using SPSS software version 22. Frequency and percentage were used to show qualitative variables and mean, and the standard deviation was used to show the quantitative variables. To examine the correlation between quantitative variables, the Pearson or Spearman coefficients were calculated, and Chi-Square test was used to examine the relationship between qualitative variables. The P value < 0.05 was considered as significant level.

## Results

A total of 91 consecutive patients were screened during the study period. Of these, 8 patients did not meet the inclusion criteria, and 3 patients declined to participate in the study. The remaining 80 patients were randomly allocated to two equal-sized groups (Figure 1).

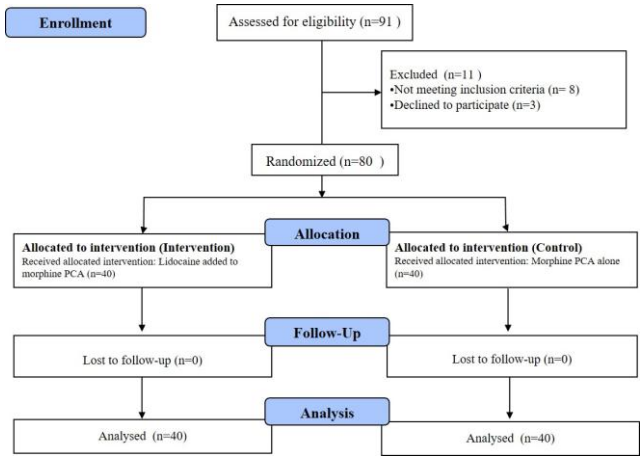



*Figure 1: Flow chart of the study*

The mean age of patients was 29.83 ± 3.65 years. 92.5% and 100% of the women in the intervention and control groups had a diploma degree, respectively. A total of 20% of the subjects were a candidate for the first cesarean section with spinal anaesthesia, 43.8% of whom had 1 child, 33.8% had two children, and 2.5% had 3 children (p > 0.05). The mean BMI of the subjects was 31.18 ± 3.91 and 30.93 ± 3.24 in the intervention and control groups, respectively (p = 0.487). The mean duration of surgery in the intervention group was 63.6 ± 10.4 and 65.75 ± 7.8 minutes in the control group. There was no statistically significant difference between the two groups in this regard (P = 0.301). The duration of ileus relapse was 18.73 ± 3.73 hours in the intervention group and 18.87 ± 4.29 hours in the control group, which was not statistically significant between the two groups (p = 0.868). The mean duration of hospitalisation in the control group was slightly higher than that in the intervention group, although there was no significant difference between the two groups (1.90 ± 0.30 days in the control group and 1.82 ± 0.38 days in the intervention group, p = 0.336). The mean and standard deviation of the pain intensity of the patients in the two groups at the specified intervals of 2, 4, 6, 12, 18, and 24 hours after the surgery was 5.91 ± 1.57, 4.97 ± 1.55, 3.84 ± 1.60, 3.54 ± 1.45, 2.56 ± 1.70 and 0.94 ± 1.70, respectively (Figure 2). The trend of the changes was statistically significant (P < 0.0001).

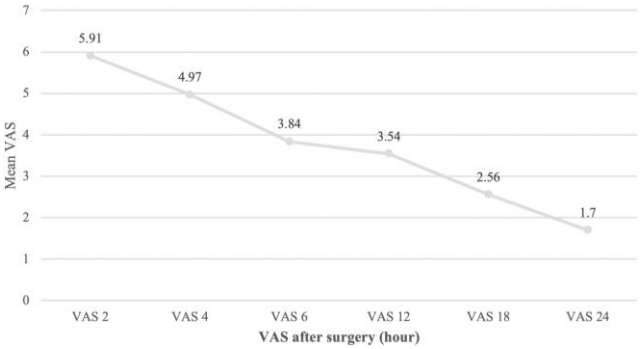



*Figure 2: The mean VAS pain intensity score at specified intervals after the surgery in all patients*

The mean pain intensity of patients after the surgery at specified intervals in both intervention and control groups is shown in Table 1 and Figure 1.

**Table 1: The mean VAS pain intensity score at specified intervals of 2, 4, 6, 12, 18, and 24 hours after surgery in two groups**

| Variable | Intervention group | Control group | P value |
|---|---|---|---|
| VAS 2 (Pain severity 2 hours after the surgery) | 5.75 ± 1.67 | 6.08 ± 1.47 | P = 0.360 |
| VAS 4 (Pain severity 4 hours after the surgery) | 5.07 ± 1.55 | 4.87 ± 1.55 | P = 0.567 |
| VAS 6 (Pain severity 6 hours after the surgery) | 3.93 ± 1.67 | 3.75 ± 1.54 | P = 0.628 |
| VAS 12 (Pain severity 12 hours after the surgery) | 3.60 ± 1.44 | 3.48 ± 1.48 | P = 0.704 |
| VAS 18 (Pain severity 18 hours after the surgery) | 2.65 ± 1.35 | 2.48 ± 1.21 | P = 0.545 |
| VAS 24 (Pain severity 24 hours after the surgery) | 1.85 ± 0.94 | 1.55 ± 0.94 | P = 0.158 |

As seen, there was no significant difference between the two groups in terms of pain intensity at the first 24 hours after surgery in any of the studied periods.

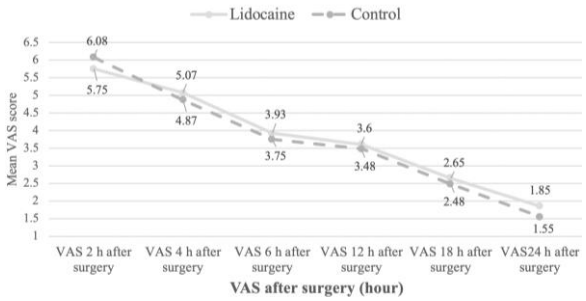



*Figure 3: The mean pain severity based on the VAS criterion at specified intervals of 2, 4, 6, 12, 18, and 24 hours after surgery in intervention and control groups*

The results of the study showed that there

was no significant difference between the two groups in terms of the frequency of receiving diclofenac suppositories after the surgery (Table 2).

**Table 2: The number of frequencies of receiving diclofenac suppositories in all of the patients in the intervention and control groups**

| Frequency of receiving diclofenac suppositories | 0 | 1 | 2 | 3 | 4 | P-value |
|---|---|---|---|---|---|---|
| Intervention group | 7 (%17.5) | 10 (%25) | 11 (%27.5) | 11 (%27.5) | 1 (%2.5) | P = 0.756 |
| Control group | 5 (%22.5) | 9 (%22.5) | 16 (%40) | 9 (%22.5) | 1 (%2.5) | |

In the control group, none of the patients reported postoperative nausea and vomiting. In the intervention group, only one patient reported nausea and vomiting (2.5%), which was resolved after 6 hours. All patients in both groups had 100% satisfaction with cesarean section.

## Discussion

The results of this study revealed that the pain intensity after the cesarean section was significantly decreased in both groups before and after 24 hours after the surgery, and the severity of postoperative pain in all hours in both intervention and control groups decreased significantly compared to the first hour of pain. In general, the pain severity in the first 6 hours after surgery in the intervention group was lower than that in the control group. However, there was no significant difference between the two groups in terms of pain intensity at first 24 hours after surgery in any of the studied intervals. Choi et al. examined the effects of lidocaine infusion on postoperative pain intensity after breast surgery. For patients in the intervention group, about thirty minutes before the incision, lidocaine was injected at a dose of 1.5 mg/kg. Then, lidocaine infusion with a dose of 1.5 mg/kg was performed up to incision closure. The results showed that intravenous lidocaine had no significant effect on postoperative pain severity in patients [13]. Also, another study in patients undergoing hip arthroplasty showed that continuous infusion of low dose lidocaine (1.5 mg/kg) compared to placebo, did not affect the pain intensity and reducing the opioid consumption after surgery [9]. The results of Moslemi et al. on patients undergoing gynecologic laparoscopic surgery indicate that infusion of lidocaine (1.5 mg/kg) could significantly affect postoperative pain reduction [18]. Also, it has been shown that adding lidocaine to morphine PCA, compared to morphine PCA alone, has no significant efficacy in postoperative pain reduction after abdominal surgery [19]. The results of these studies are consistent with our study. In a study conducted by Alebouyeh et al, the effects of adding lidocaine to morphine in three groups of lidocaine 1% with 10 mg of morphine, 1% lidocaine with 20 mg of morphine and the control group with just 20 mg of morphine showed that the mean pain intensity decreased significantly in the groups in which lidocaine was added to morphine [12]. Choi et al., evaluate the effects of intravenous lidocaine infusion on the duration of the ileus and duration of hospitalisation and the severity of postoperative pain. The results revealed that the use of lidocaine had a significant effect on the narcotic drug consumed, intestinal function and duration of hospitalisation [13]. Another study showed that the administration of lidocaine (bolus dose and then infusion dose) 30 minutes before surgery for up to 1 hour after surgery led a reduction in duration of hospitalisation [20]. Also, it has been revealed that infusion of adding lidocaine, at a dose of 1.5 mg/kg, to morphine which began 30 minutes before the surgical incision and continued until 1 hour after the completion of the surgery, had significant effect on reduction of pain intensity and postoperative morphine consumption after major abdominal surgery [10]. Yardeni et al. showed a significant effect of intravenous lidocaine administration before surgery (2 mg/kg), and its infusion during surgery (1.5 mg/kg) incision on the reduction of pain intensity in patients undergoing abdominal hysterectomy [11]. In a study conducted by Groudine et al with the aim of evaluating the effectiveness of intravenous lidocaine on the function of intestinal movements, postoperative pain, duration of hospitalization in patients underwent prostatectomy, showed that bolus administration of lidocaine (1.5 mg/kg) and then its infusion (3 mg/min), led to early back of intestines function and reduced postoperative pain [20]. The results of the mentioned research were different from those of our study in terms of comparing the pain intensity in adding lidocaine on postoperative pain intensity. Khoshrang et al., evaluated the effectiveness of 0.25% marcain injection plus lidocaine 2% in the surgical site on the severity of pain in patients undergoing cesarean section with spinal anaesthesia. The results showed that topical injection of marcain (0.25%), compared to lidocaine, in the cesarean section could decrease pain severity in the early hours after surgery and also reduce the need for analgesics [21].

In conclusion, according to the research results, it seems that adding lidocaine to PCA morphine, compared to morphine PCA alone, did not have a significant effect on reducing the postoperative pain intensity after cesarean section with spinal anaesthesia.

## References

1. Rafiei M, Saei Ghare M, Akbari M, Kiani F, Sayehmiri F, Sayehmiri K, et al. Prevalence, causes, and complications of cesarean delivery in Iran: A systematic review and meta-analysis. Int J Reprod Biomed (Yazd). 2018; 16(4):221-234.

https://doi.org/10.29252/ijrm.16.4.221

2. Kintu A, Abdulla S, Lubikire A, Nabukenya MT, Igaga E, Bulamba F, et al. Postoperative pain after cesarean section: assessment and management in a tertiary hospital in a low-income country. BMC Health Serv Res. 2019; 19(1):68. https://doi.org/10.1186/s12913-019-3911-x PMid:30683083 PMCid:PMC6347795

3. Gholipour Baradari A, Firouzian A, Hasanzadeh Kiabi F, Emami Zeydi A, Khademloo M, Nazari Z, et al. Bolus administration of intravenous lidocaine reduces pain after an elective caesarean section: Findings from a randomised, double-blind, placebo-controlled trial. J Obstet Gynaecol. 2017; 37(5):566-570. https://doi.org/10.1080/01443615.2016.1264071 PMid:28604179

4. Lavand'homme P. Postoperative cesarean pain: real but is it preventable? Curr Opin Anaesthesiol. 2018; 31(3):262-267. https://doi.org/10.1097/ACO.0000000000000585 PMid:29521684

5. Unlugenc H, Vardar MA, Tetiker S. A comparative study of the analgesic effect of patient-controlled morphine, pethidine, and tramadol for postoperative pain management after abdominal hysterectomy. Anesth Analg. 2008; 106(1):309-12. https://doi.org/10.1213/01.ane.0000287815.32869.2a PMid:18165596

6. Hasanzadeh Kiabi F, Soleimani A, Habibi MR, Emami Zeydi A. Can vitamin C be used as an adjuvant for managing postoperative pain? A short literature review. Korean J Pain. 2013; 26(2):209-10. https://doi.org/10.3344/kjp.2013.26.2.209 PMid:23614091 PMCid:PMC3629356

7. Pogatzki-Zahn EM, Segelcke D, Schug SA. Postoperative pain-from mechanisms to treatment. Pain Rep. 2017; 2(2):e588. https://doi.org/10.1097/PR9.0000000000000588 PMid:29392204 PMCid:PMC5770176

8. Firouzian A, Alipour A, Rashidian Dezfouli H, Zamani Kiasari A, Gholipour Baradari A, Emami Zeydi A, et al. Does lidocaine as an adjuvant to morphine improve pain relief in patients presenting to the ED with acute renal colic? A double-blind, randomized controlled trial. Am J Emerg Med. 2016; 34(3):443-8. https://doi.org/10.1016/j.ajem.2015.11.062 PMid:26704774

9. Martin F, Cherif K, Gentili ME, Enel D, Abe E, Alvarez JC, et al. Lack of impact of intravenous lidocaine on analgesia, functional recovery, and nociceptive pain threshold after total hip arthroplasty. Anesthesiology. 2008; 109(1):118-23. https://doi.org/10.1097/ALN.0b013e31817b5a9b PMid:18580181 PMCid:PMC2728117

10. Koppert W, Weigand M, Neumann F, Sittl R, Schuettler J, Schmelz M, et al. Perioperative intravenous lidocaine has preventive effects on postoperative pain and morphine consumption after major abdominal surgery. Anesth Analg. 2004; 98(4):1050-5. https://doi.org/10.1213/01.ANE.0000104582.71710.EE PMid:15041597

11. Yardeni IZ, Beilin B, Mayburd E, Levinson Y, Bessler H. The effect of perioperative intravenous lidocaine on postoperative pain and immune function. Anesth Analg. 2009; 109(5):1464-9. https://doi.org/10.1213/ANE.0b013e3181bab1bd PMid:19843784

12. Alebouyeh MR, Imani F, Rahimzadeh P, Entezary SR, Faiz SH, Soraya P. Analgesic effects of adding lidocaine to morphine pumps after orthopedic surgeries. J Res Med Sci. 2014; 19(2):122-7.

13. Choi SJ, Kim MH, Jeong HY, Lee JJ. Effect of intraoperative lidocaine on anesthetic consumption, and bowel function, pain intensity, analgesic consumption and hospital stay after breast surgery. Korean J Anesthesiol. 2012; 62(5):429-34. https://doi.org/10.4097/kjae.2012.62.5.429 PMid:22679539 PMCid:PMC3366309

14. Wuethrich PY, Romero J, Burkhard FC, Curatolo M. No benefit from perioperative intravenous lidocaine in laparoscopic renal surgery: a randomised, placebo-controlled study. Eur J Anaesthesiol. 2012; 29(11):537-43. https://doi.org/10.1097/EJA.0b013e328356bad6 PMid:22907609

15. de Oliveira CM, Issy AM, Sakata RK. Intraoperative intravenous lidocaine. Rev Bras Anestesiol. 2010; 60(3):325-33. https://doi.org/10.1016/S0034-7094(10)70041-6

16. Zeisler JA, Gaarder TD, De Mesquita SA. Lidocaine excretion in breast milk. Drug Intell Clin Pharm. 1986; 20(9):691-3. https://doi.org/10.1177/106002808602000913 PMid:3757781

17. Sachs HC, Committee On Drugs. The transfer of drugs and therapeutics into human breast milk: an update on selected topics. Pediatrics. 2013; 132(3):e796-809. https://doi.org/10.1542/peds.2013-1985 PMid:23979084

18. Moslemi F, Rasooli S, Dehdilani M, Sadeghi-Bazargani H, Dehdilani M. The Effect of Intravenous Infusion of Lidocaine to Control Postoperative Pain After Gynecologic Laparoscopic Surgery: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial. Iran Red Crescent Med J. 2018; 20(7):e14531. https://doi.org/10.5812/ircmj.14531

19. Cepeda MS, Delgado M, Ponce M, Cruz CA, Carr DB. Equivalent outcomes during postoperative patient-controlled intravenous analgesia with lidocaine plus morphine versus morphine alone. Anesth Analg. 1996; 83(1):102-6. https://doi.org/10.1213/00000539-199607000-00018 PMid:8659717

20. Groudine SB, Fisher HA, Kaufman RP Jr, Patel MK, Wilkins LJ, Mehta SA, et al. Intravenous lidocaine speeds the return of bowel function, decreases postoperative pain, and shortens hospital stay in patients undergoing radical retropubic prostatectomy. Anesth Analg. 1998; 86(2):235-9. https://doi.org/10.1213/00000539-199802000-00003

21. Khoshrang H. Comparing the effects of local infiltration of lidocaine 2% and marcaine 0.25% on the severity of pain in patients after cesarean section. Jour Guilan Uni Med Sci. 2012; 21(83):86-94.

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Jun 30; 7(12):1951-1956.
https://doi.org/10.3889/oamjms.2019.587
eISSN: 1857-9655
***Clinical Science***

# Evaluation of Pregnancy Outcomes at Advanced Maternal Age

Małgorzata Radoń-Pokracka, Beata Adrianowicz*, Magdalena Płonka, Paulina Danił, Magdalena Nowak, Hubert Huras

*Department of Obstetrics and Perinatology, Jagiellonian University Medical College, Kraków, Poland*

**Citation:** Radoń-Pokracka M, Adrianowicz B, Płonka M, Danił P, Nowak M, Huras H. Evaluation of Pregnancy Outcomes at Advanced Maternal Age. Open Access Maced J Med Sci. 2019 Jun 30; 7(12):1951-1956. https://doi.org/10.3889/oamjms.2019.587

**Keywords:** advanced maternal age; pregnancy outcomes; perinatology; pregnancy complications

***Correspondence:** Beata Adrianowicz. Department of Obstetrics and Perinatology, University Hospital in Kraków, Ul. Kopernika 23, 31-501 Kraków, Poland. Fax.: +48 12 424 84 12; Tel.: +48 663 892 440. E-mail: beata.adrianowicz@gmail.com

**Received:** 27-May-2019; **Revised:** 28-Jun-2019; **Accepted:** 29-Jun-2019; **Online first:** 30-Jun-2019

**Copyright:** © 2019 Małgorzata Radoń-Pokracka, Beata Adrianowicz, Magdalena Płonka, Paulina Danił, Magdalena Nowak, Hubert Huras. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

**Funding:** This research was financially supported by the Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia

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

**Abstract**

**AIM:** The study aimed to investigate the association between advanced maternal age (AMA) and the risk of adverse maternal, perinatal and neonatal outcomes about parity in singleton pregnancies.

**METHODS:** We retrospectively analysed 950 women who gave birth in the Department of Obstetrics and Perinatology of the University Hospital in Kraków for six months (between 1st January and 30th June 2018). The patients were divided into 3 groups according to their age (30-34 years old, 35-39 years old and over 40 years old). Each of these groups was subsequently subdivided into 2 groups depending on parity (primiparae and multiparae). Maternal, perinatal and neonatal outcomes were compared between the groups and the subgroups.

**RESULTS:** Comparison of the three age groups revealed that advanced maternal age might constitute a predisposing factor for preterm birth, caesarean section and large for gestational age (LGA). From these parameters, statistical significance was reached in case of greater risk of LGA (OR = 2.17), caesarean section (OR = 2.03) and elective C-section (OR = 1.84) in women over 40 years old when compared to the patients aged 30-34. Furthermore, AMA increases the risk of postpartum haemorrhage (OR = 6.43). Additionally, there is a negative correlation between maternal age and gestational age at delivery ($R = -0.106$, $p < 0.05$).

**CONCLUSIONS:** Advanced maternal age can undoubtedly be associated with several adverse perinatal outcomes. At the same time, the risk of perinatal complications begins to increase after the age of 35 but becomes significant in women aged ≥ 40.

## Introduction

Delayed childbearing has become increasingly common in the past decades. Recent years have seen significant growth in mean maternal age at first childbirth as well as in number of pregnancies at advanced maternal age (AMA). In Poland, the percentage of live births to women aged 35 and over increased almost twice - from 9.1% in 2005 to 16.3% in 2016. At the same time, the rates of deliveries among patients over 40 rose by nearly 50% - from 1.8% in 2005 to 2.6% in 2016 [1]. Similar trends have been observed worldwide, in both high- and low-income countries [1], [2], [3], [4].

Various reasons for delayed childbearing can be identified [5]. Among them, the most significant one seems to be a progress in assisted reproductive technologies (ART) (e.g. in vitro fertilisation, oocyte donation), which enables patients in their 40s, 50s or even 60s to become pregnant [6], [7].

Furthermore, recent enormous changes in work and society have been reflected in women's desire to develop their careers, obtain financial security and built a stable relationship with their partner before becoming mothers. Higher educational level among females led to a better knowledge and awareness of different types of contraception and, together with greater access to birth control methods,

constitutes another factor responsible for an increase in maternal age [8], [9].

Historically, advanced maternal age was defined as age ≥ 35 years. However, in many contemporary studies, the cut-off for AMA has been changed to the age of 40 [5], [10], [11]. Access to ARTs and tendency towards postponing childbearing led to the creation of new definitions - very advanced maternal age (VAMA) and extremely advanced maternal age (EAMA) describing women delivering at age 45-49 and ≥ 50, respectively [12].

Delayed childbearing is believed to be associated with an increased rate of obstetrical and perinatal complications as well as adverse pregnancy outcomes. When compared to younger patients, women in advanced maternal age are reported to be at greater risk of congenital disorders, placenta previa, ectopic pregnancy, spontaneous abortion, stillbirth, preterm birth, induction of labour, caesarean delivery and small for gestational age (SGA). Also, the prevalence of chronic medical conditions (e.g. diabetes mellitus, hypertension) and other diseases with a possible influence on a course of pregnancy (such as cancer) are higher among older patients [5]. Multiple studies suggest that the incidence rate of perinatal complications only begins to increase after the age of 35, but the most significant growth can be observed after the age of 40 [9], [11].

Therefore, the objective of the study was to investigate the association between advanced maternal age (35-39 and ≥ 40 separately) and the risk of adverse maternal, perinatal and neonatal outcomes about parity in singleton pregnancies.

## Methods

The retrospective study enrolled 950 women at age ≥ 30 years in singleton pregnancy who gave birth in the Department of Obstetrics and Perinatology of the University Hospital in Kraków during six months (between 1st January 2018 and 30th June 2018). The patients were divided into 3 main groups according to their age: 30-34 years (group 1), 35-39 years (group 2), ≥ 40 years (group 3). Each of these groups was subsequently subdivided into 2 subgroups depending on parity (primiparae and multiparae). The groups and the subgroups were compared about maternal, perinatal and neonatal outcomes.

Data were obtained from the hospital electronic medical records and included demographic features, maternal medical conditions, pregnancy complications, delivery mode (including indications for caesarean section) as well as perinatal and neonatal outcomes. The demographic information consisted of maternal age at delivery, BMI, gravidity and parity, history of caesarean sections and other surgical procedures. Pregnancy complications included pregnancy-induced hypertension (PIH) (defined as a blood pressure >140/90 mmHg measured on 2 separate occasions, > 6 hours apart, without proteinuria, developed after 20 weeks of gestation), preeclampsia (defined as PIH accompanied by proteinuria of >300 mg in a daily urine sample), anaemia (Hb <11 g/dl), hypothyroidism (TSH >2.5 mIU/l), oligohydramnios (defined as AFI ≤ 5 cm), polyhydramnios (defined as AFI >20 cm), preterm rupture of membranes (PROM) (defined as rupture of membranes > 1 hour before the onset of labour) and placenta praevia.

Perinatal and neonatal outcomes included: gestational age at delivery, preterm birth (PTB) (defined as delivery at < 37 weeks of gestation), birth asphyxia, stillbirth, lack of progress of labour, postpartum haemorrhage (PPH) (defined as loss of > 500 ml or > 1000 ml of blood within the first 24 hours after vaginal delivery and caesarean section, respectively), incidence of episiotomy and perineal tears, a 5-minute Apgar score (7-10 was considered normal, 4-6 - intermediate, 0-3 - low), incidence of congenital anomalies, macrosomia (defined as birth weight > 4500 g), small for gestational age (SGA) (defined as a weight <10th percentile for the gestational age), large for gestational age (LGA) (defined as a weight >90th percentile for the gestational age).

All the patients had either a vaginal delivery (VD) (non-induced or induced) or caesarean section (CS) (emergency or elective).

Statistical analysis was performed using *STATISTICA 13.1 (StatSoft®, Poland)* statistical analysis software. The patients' characteristics were presented as medians with interquartile ranges (IQR) or means with standard deviations (SD) for continuous variables and numbers of cases and percentages for categorical data. Comparison of qualitative values was assessed with the Chi-squared test and exact Fisher's test; quantitative variables were compared with the use of the Mann-Whitney U test, p-value < 0.05 was considered statistically significant. Odds ratios (OR) and 95% confidence intervals (CI) were calculated.

## Results

Out of 1500 women who delivered at the Department of Obstetrics and Perinatology of the University Hospital in Kraków during the study period, 950 were ≥30 years old and in singleton pregnancy. General characteristics of the patients, perinatal complications and pregnancy outcomes, about parity, are presented in Table 1, 2 and *3*.

**Table 1: General characteristics, perinatal complications and pregnancy outcomes among the whole study population (*percentage of all the vaginal deliveries)**

| Median (IQR) or n(%) | | Maternal age | | |
|---|---|---|---|---|
| | | 30-34 (n = 594) | 35-39 (n = 298) | ≥40 (n = 58) |
| Demographic features | | | | |
| Pre-pregnancy BMI | | 21.97 (4.53) | 21.61 (4.19) | 24.22 (3.12) |
| Previous caesarean delivery | | 166 (28) | 114 (38.3) | 19 32.8 |
| Gravidity | | | | |
| 1st | | 238 (40) | 54 (18.1) | 9 (15.5) |
| 2nd | | 233 (39) | 119 (40) | 14 (24.1) |
| 3rd | | 78 (13) | 77 (25.8) | 20 (34.5) |
| 4th | | 33 (6) | 27 (9.1) | 9 (15.5) |
| ≥5th | | 12 (2) | 21 (7) | 6 (10.4) |
| Parity | | | | |
| 1st | | 266 (44.8) | 75 (25.2) | 14 (24.1) |
| 2nd | | 273 (46) | 150 (50.3) | 22 (38) |
| 3rd | | 37 (6.2) | 59 (19.8) | 18 (31) |
| 4th | | 12 (2) | 11 (3.7) | 3 (5.2) |
| ≥5th | | 6 (1) | 3 (1) | 1 (1.7) |
| Pregnancy complications | | | | |
| Pregnancy-induced hypertension (PIH) | | 52 (8.8) | 19 (6.4) | 8 (13.8) |
| Hypothyroidism | | 229 (38.6) | 106 (35.6) | 18 (31) |
| Anaemia | | 61 (10.3) | 23 (7.7) | 4 (6.9) |
| Placenta praevia | | 8 (1.3) | 3 (1) | 2 (3.4) |
| PROM | | 24 (4) | 11 (3.7) | 4 (6.9) |
| Oligohydramnios | | 13 (2.2) | 5 (1.7) | 2 (3.4) |
| Polyhydramnios | | 5 (0.8) | 4 (1.3) | 0 (0) |
| Mode of delivery | | | | |
| Caesarean section | | 361 (60.8) | 193 (64.8) | 45 (77.6) |
| Emergency C-section | | 113 (19) | 62 (20.8) | 12 (20.7) |
| Elective C-section | | 248 (41.8) | 131 (44) | 33 (56.9) |
| Non-induced vaginal delivery | | 172 (29) | 87 (29.2) | 11 (19) |
| Induced vaginal delivery | | 61 (10.2) | 18 (6) | 2 (3.4) |
| Perinatal outcome | | | | |
| Gestational age at delivery | | 39 (1+5) | 38+6 (1+2) | 38+4 (1+3) |
| Preterm birth | | 65 (10.9) | 40 (13.4) | 9 (15.5) |
| Birth asphyxia | | 48 (8.1) | 22 (7.4) | 1 (1.7) |
| Lack of progress of labour | | 27 (4.5) | 4 (1.3) | 1 (1.7) |
| Postpartum haemorrhage | | 5 (0.8) | 1 (0.3) | 3 (5.2) |
| Episiotomy | | 104 (44.6*) | 39 (37.1*) | 3 (23.1*) |
| Perineal tears | | 66 (28.3*) | 23 (21.9*) | 5 (38.5*) |
| Neonatal outcome | | | | |
| Birth weight [g] | | 3320 (640) | 3350 (698) | 3275 (510) |
| Macrosomy | | 4 (6.7) | 2 (0.7) | 0 (0) |
| SGA | | 43 (7.2) | 16 (5.4) | 3 (5.2) |
| LGA | | 52 (8.8) | 32 (10.7) | 10 (17.2) |
| APGAR score: | 0-3 | 2 (0.3) | 1 (0.3) | 0 (0) |
| | 4-6 | 4 (6.7) | 1 (0.3) | 2 (3.4) |
| | 7-10 | 588 (99) | 296 (99.3) | 56 (96.6) |
| Stillbirth | | 2 (0.3) | 0 (0) | 0 (0) |
| Congenital anomalies | | 37 (6.2) | 20 (6.7) | 3 (5.2) |

Comparison of the women aged 35-39 and ≥ 40 with the control group (30-34) (Table 4) revealed that advanced maternal age was associated with a lower gestational age at delivery (p = 0.0025, 38+6 vs 39 and p = 0.0003, 38+4 vs 39, respectively), lower incidence of vaginal delivery and elevated rate of caesarean section - both elective and emergency. The results met statistical significance criteria only for the group aged ≥ 40 (p = 0.0238, OR = 2.23 for CS and p = 0.0262, OR = 1.84 for elective CS).

At the same time, AMA seems to reduce the risk of lack of progress of labour (p = 0.0232, OR = 0.29 for the age group 35-39) and, in case of vaginal birth, it increases the probability of no need for episiotomy and lack of perineal tear (p = 0.0167, OR = 1.87 for the age group 35-39). Analysis of the cohorts ≥ 40 and 30-34 revealed that large for gestational age (LGA) occurred statistically more often in advanced maternal age (p = 0.0355, OR = 2.17). Furthermore, delivery among the women aged ≥ 40 was more frequently followed by postpartum haemorrhage than childbirth among younger patients (p = 0.0254, OR = 6.43).

Examination of primiparous women revealed that advanced maternal age in this cohort was associated with a lower gestational age at delivery and a higher incidence of caesarean section (p = 0.0107, OR = 9.03 for CS and p = 0.0187, OR = 3.83 for elective CS among the group aged ≥ 40). In addition, AMA increased risk of postpartum haemorrhage among primiparas (p = 0.0213, OR = 14.61 for the patients ≥ 40).

**Table 2: General characteristics, perinatal complications and pregnancy outcomes among primiparas**

| Median (IQR) or n(%) | | Maternal age | | |
|---|---|---|---|---|
| | | 30-34 (n = 266) | 35-39 (n = 75) | ≥40 (n = 14) |
| Demographic features | | | | |
| Pre-pregnancy BMI | | 20.92 (4.30) | 20.90 (1.57) | 21.56 (0) |
| Previous caesarean delivery | | - | - | - |
| Gravidity | | | | |
| 1st | | 238 (89.5) | 54 (72) | 9 (64.3) |
| 2nd | | 19 (7.1) | 14 (18.7) | 2 (14.3) |
| 3rd | | 7 (2.6) | 3 (4) | 0 (0) |
| 4th | | 2 (0.8) | 3 (4) | 1 (7.1) |
| ≥5th | | 0 (0) | 1 (1.3) | 2 (14.3) |
| Parity | | | | |
| 1st | | 266 (100) | 75 (100) | 14 (100) |
| 2nd | | - | - | - |
| 3rd | | - | - | - |
| 4th | | - | - | - |
| ≥5th | | - | - | - |
| Pregnancy complications | | | | |
| Pregnancy-induced hypertension (PIH) | | 24 (9) | 5 (6.7) | 3 (21.4) |
| Hypothyroidism | | 125 (47) | 21 (28) | 3 (21.4) |
| Anaemia | | 23 (8.6) | 6 (8) | 0 (0) |
| Placenta praevia | | 1 (0.4) | 0 (0) | 1 (7.1) |
| PROM | | 12 (4.5) | 4 (5.3) | 0 (0) |
| Oligohydramnios | | 7 (2.6) | 2 (2.7) | 1 (7.1) |
| Polyhydramnios | | 0 (0) | 1 (1.3) | 0 (0) |
| Mode of delivery | | | | |
| Caesarean section | | 157 (59) | 50 (66.7) | 13 (92.9) |
| Emergency C-section | | 72 (27) | 22 (29.3) | 4 (28.6) |
| Elective C-section | | 85 (32) | 28 (37.3) | 9 (64.3) |
| Non-induced vaginal delivery | | 75 (28.2) | 18 (24) | 1 (7.1) |
| Induced vaginal delivery | | 34 (12.8) | 7 (9.3) | 0 (0) |
| Perinatal outcome | | | | |
| Gestational age at delivery | | 39+2 (1+5) | 39 (1+6) | 38+5 (1+5) |
| Preterm birth | | 29 (10.9) | 9 (12) | 1 (7.1) |
| Birth asphyxia | | 33 (12.4) | 9 (12) | 0 (0) |
| Lack of progress of labour | | 24 (9) | 3 (4) | 1 (7.1) |
| Postpartum haemorrhage | | 3 (1.1) | 0 (0) | 2 (14.3) |
| Episiotomy | | 75 (68.8*) | 18 (72*) | 1 (100*) |
| Perineal tears | | 21 (19.3*) | 2 (8*) | 0 (0*) |
| Neonatal outcome | | | | |
| Birth weight | | 3260 (610) | 3330 (600) | 3265 (258) |
| Macrosomy | | 1 (0.4) | 1 (1.3) | 0 (0) |
| SGA | | 19 (7.1) | 3 (4) | 2 (14.3) |
| LGA | | 26 (9.8) | 9 (12) | 1 (7.1) |
| APGAR score: | 0-3 | 2 (0.8) | 0 (0) | 0 (0) |
| | 4-6 | 2 (0.8) | 0 (0) | 0 (0) |
| | 7-10 | 262 (98.5) | 75 (100) | 14 (100) |
| Stillbirth | | 2 (0.8) | 0 (0) | 0 (0) |
| Congenital anomalies | | 19 (7.1) | 5 (6.7) | 2 (14.3) |

Considering only multiparas, children of older women were more often large for gestational age (LGA) than those born to younger mothers (p = 0.0029, OR = 3.41 for ≥ 40 cohorts). At the same time, labour was more frequently complicated by postpartum haemorrhage. Advanced maternal age in multiparas was also associated with a lower gestational age at delivery (p = 0.0028, 38+3 vs 39 for the patients ≥ 40). Table 5 and 6 show detailed results of a comparison of the age groups about parity.

Further evaluation of the whole study population, as well as primiparous and multiparous women, separately revealed a correlation between maternal age and gestational age at delivery. Nonetheless, the findings met statistical significance criteria only when analysing all the patients irrespective of parity and in the case of multiparas (Figure 1).

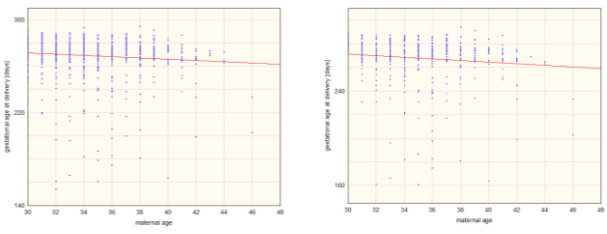


*Figure 1: Correlation between maternal age and gestational age at delivery among the whole study population (p<0.05, r= -0.106) (left); Correlation between maternal age and gestational age at delivery among multiparas (p<0.05, r= -0.107) (right)*

## Discussion

In our study, 77.6% of women over 40 years delivered via caesarean section in comparison to 64.8% women aged 35-39 and 60.8% at age 30-34. Caesarean section rate in the group over 40 was significantly higher than in the control group (women aged 30-34) (p = 0.0238, OR 2.23). Moreover, an elective caesarean section among women aged ≥ 40 was performed almost twice as often as among younger patients (p = 0.0262, OR = 1.84).

**Table 3: General characteristics, perinatal complications and pregnancy outcomes among multiparas**

| Median (IQR) or n(%) | Maternal age | | |
|---|---|---|---|
| | 30-34 (n = 328) | 35-39 (n = 223) | ≥40 (n = 44) |
| Demographic features | | | |
| Pre-pregnancy BMI | 22.68 (4.45) | 22.58 (4.18) | 24.57 (2.62) |
| Previous caesarean delivery | 166 (50.6) | 114 (51.1) | 19 (43.2) |
| Gravidity | | | |
| 1st | - | - | - |
| 2nd | 214 (65.2) | 105 (47.1) | 12 (27.3) |
| 3rd | 71 (21.6) | 74 (33.2) | 20 (45.4) |
| 4th | 31 (9.5) | 24 (10.7) | 8 (18.2) |
| ≥5th | 12 (3.7) | 20 (9) | 4 (9.1) |
| Parity | | | |
| 1st | - | - | - |
| 2nd | 273 (83.2) | 150 (67.3) | 22 (50) |
| 3rd | 37 (11.3) | 59 (26.5) | 18 (40.9) |
| 4th | 12 (3.7) | 11 (4.9) | 3 (6.8) |
| ≥5th | 6 (1.8) | 3 (1.3) | 1 (2.3) |
| Pregnancy complications | | | |
| Pregnancy-induced hypertension (PIH) | 22 (6.7) | 14 (6.3) | 5 (11.4) |
| Hypothyroidism | 84 (25.6) | 85 (38.1) | 15 (34.1) |
| Anaemia | 36 (11) | 17 (7.6) | 4 (9.1) |
| Placenta praevia | 6 (1.8) | 3 (1.3) | 1 (2.3) |
| PROM | 12 (3.7) | 7 (3.1) | 4 (9.1) |
| Oligohydramnios | 6 (1.8) | 3 (1.3) | 1 (2.3) |
| Polyhydramnios | 5 (1.5) | 3 (1.3) | 0 (0) |
| Mode of delivery | | | |
| Caesarean section | 204 (62.2) | 143 (64.1) | 32 (72.7) |
| Emergency C-section | 41 (12.5) | 40 (17.9) | 8 (18.2) |
| Elective C-section | 163 (49.7) | 103 (46.2) | 24 (54.5) |
| Non-induced vaginal delivery | 97 (29.6) | 69 (31) | 10 (22.7) |
| Induced vaginal delivery | 27 (8.2) | 11 (4.9) | 2 (4.6) |
| Perinatal outcome | | | |
| Gestational age at delivery | 39 (1+3) | 38+6 (1+2) | 38+3 (1+2) |
| Preterm birth | 36 (11) | 31 (13.9) | 8 (18.2) |
| Birth asphyxia | 13 (4) | 13 (5.8) | 1 (2.3) |
| Lack of progress of labour | 3 (0.9) | 1 (0.4) | 0 (0) |
| Postpartum haemorrhage | 1 (0.3) | 1 (0.4) | 1 (2.3) |
| Episiotomy | 29 (23.4*) | 21 (26.3*) | 2 (16.7*) |
| Perineal tears | 35 (28.2*) | 21 (26.3*) | 5 (41.7*) |
| Neonatal outcome | | | |
| Birth weight | 3380 (655) | 3350 (730) | 3360 (608) |
| Macrosomy | 3 (0.9) | 1 (0.4) | 0 (0) |
| SGA | 18 (5.5) | 13 (5.8) | 1 (2.3) |
| LGA | 23 (7) | 23 (10.3) | 9 (20.5) |
| APGAR score: 0-3 | 0 (0) | 1 (0.4) | 0 (0) |
| 4-6 | 2 (0.6) | 1 (0.4) | 2 (4.6) |
| 7-10 | 326 (99.4) | 221 (99.1) | 42 (95.4) |
| Stillbirth | 0 (0) | 0 (0) | 0 (0) |
| Congenital anomalies | 14 (4.3) | 15 (6.7) | 1 (2.3) |

Furthermore, when analysing only primiparas, the incidence of cesarean section in the group ≥ 40 was nine times higher than in the control group (p = 0.0107, OR = 9.03). Out of that, elective cesarean section was proposed almost four times more often to the older women (p = 0.0187, OR = 3.38).

Similar to our findings, different studies also report that advanced maternal age predisposes to caesarean delivery. A. Dietl et al. indicated that C-section rate was higher in the study group (> 40 years old) comparing to the control group (< 30 years old) (42.7% vs 24.7%) [6]. Furthermore, according to the study, the percentage of caesarean deliveries was increasing with a growing maternal age at childbirth: 24.7% (< 30 years old), 26.8% (30-34), 34.8% (35-39), up to 42.7% (> 40 years old, p < 0.001). In the group of nulliparous women aged > 40, the percentage of caesarean section was reaching up to 59.1%, out of which 30.7% were the elective ones (p < 0.001) [6]. Similar results have been presented in other studies [1], [13].

**Table 4: Comparison of all the women aged 35-39 and ≥ 40 with the control group (30-34)**

| Outcome | 35-39 | | ≥40 | |
|---|---|---|---|---|
| | p-value | OR (95% CI) | p-value | OR (95% CI) |
| Pregnancy complications | | | | |
| Pregnancy-induced hypertension (PIH) | 0.2158 | 0.71 (0.41-1.22) | 0.2055 | 1.67 (0.75-3.71) |
| Hypothyroidism | 0.3857 | 0.88 (0.66-1.17) | 0.2599 | 0.72 (0.40-1.29) |
| Anaemia | 0.2185 | 0.73 (0.44-1.20) | 0.5560 | 0.65 (0.23-1.86) |
| Placenta praevia | 0.9104 | 0.74 (0.19-2.81) | 0.4944 | 2.62 (0.54-12.64) |
| PROM | 0.8000 | 0.91 (0.44-1.88) | 0.4935 | 1.76 (0.59-5.26) |
| Oligohydramnios | 0.6091 | 0.76 (0.27-2.15) | 0.8792 | 1.60 (0.35-7.27) |
| Polyhydramnios | 0.7261 | 1.60 (0.43-6.00) | - | - |
| Mode of delivery | | | | |
| Caesarean section | 0.3356 | 1.19 (0.89-1.59) | 0.0238 | 2.23 (1.18-4.22) |
| Emergency C-section | 0.5273 | 1.12 (0.79-1.58) | 0.7583 | 1.11 (0.57-2.16) |
| Elective C-section | 0.5290 | 1.09 (0.82-1.44) | 0.0262 | 1.84 (1.07-3.17) |
| Non-induced vaginal delivery | 0.9410 | 1.01 (0.74-1.37) | 0.1060 | 0.57 (0.29-1.26) |
| Induced vaginal delivery | 0.0360 | 0.56 (0.32-0.97) | 0.1483 | 0.31 (0.07-1.30) |
| Perinatal outcome | | | | |
| Gestational age at delivery | 0.0025 | - | 0.0003 | - |
| Preterm birth | 0.2783 | 1.26 (0.83-1.92) | 0.2949 | 1.49 (0.70-3.17) |
| Birth asphyxia | 0.7145 | 0.91 (0.54-1.54) | 0.1358 | 0.20 (0.03-1.48) |
| Lack of progress of labour | 0.0232 | 0.29 (0.10-0.84) | 0.5014 | 0.37 (0.05-2.77) |
| Postpartum haemorrhage | 0.6613 | 0.40 (0.05-3.44) | 0.0254 | 6.43 (1.50-27.63) |
| Episiotomy | 0.2344 | 0.73 (0.46-1.17) | 0.1573 | 0.37 (0.10-1.38) |
| Perineal tears | 0.2420 | 0.71 (0.41-1.22) | 0.5297 | 1.58 (0.50-5.01) |
| Neonatal outcome | | | | |
| Birth weight [g] | 0.9030 | - | 0.9305 | - |
| Macrosomy | - | - | - | - |
| SGA | 0.2892 | 0.73 (0.40-1.32) | 0.7505 | 0.70 (0.21-2.33) |
| LGA | 0.3386 | 1.25 (0.79-1.99) | 0.0355 | 2.17 (1.04-4.54) |
| APGAR score: 0-3 | 0.5417 | 1.00 (0.09-11.07) | - | - |
| 4-6 | 0.8713 | 0.50 (0.06-4.49) | 0.1639 | 5.27 (0.94-29.41) |
| 7-10 | 0.8966 | 1.51 (0.30-7.53) | 0.3246 | 0.29 (0.06-1.47) |
| Stillbirth | - | - | - | - |
| Congenital anomalies | 0.7811 | 1.08 (0.62-1.90) | 0.9733 | 0.82 (0.24-2.75) |

Advanced maternal age is known as an independent risk factor for delivery via cesarean section. Several hypotheses for the increased need for cesarean sections among women at advanced maternal age were proposed, including atherosclerotic changes in uterine arteries [14], lower contraction potential and decreased oxytocin receptor level [15] as well as generally longer labour duration [16]. There is a persistent negative relationship between the age of pregnant women and the function of the uterus [17].

**Table 5: Comparison of the women 35-39 and ≥ 40 with the control group among primiparas (**one-tailed Mann-Whitney U test)**

| Outcome | 35-39 | | ≥40 | |
|---|---|---|---|---|
| | p-value | OR (95% CI) | p-value | OR (95% CI) |
| Pregnancy complications | | | | |
| Pregnancy-induced hypertension (PIH) | 0.5189 | 0.72 (0.26-1.96) | 0.1416 | 2.75 (0.72-10.54) |
| Hypothyroidism | 0.0033 | 0.44 (0.25-0.77) | 0.0960 | 0.31 (0.08-1.14) |
| Anaemia | 0.8595 | 0.92 (0.36-2.35) | - | - |
| Placenta praevia | - | - | 0.0977 | 20.38 (1.21-344.39) |
| PROM | 0.9906 | 1.19 (0.37-3.80) | - | - |
| Oligohydramnios | 0.6958 | 1.01 (0.21-4.97) | 0.3401 | 2.85 (0.33-24.91) |
| Polyhydramnios | - | - | - | - |
| Mode of delivery | | | | |
| Caesarean section | 0.5697 | 1.39 (0.81-2.38) | 0.0107 | 9.03 (1.16-70.05) |
| Emergency C-section | 0.6982 | 1.12 (0.64-1.97) | 1.0000 | 1.08 (0.33-3.55) |
| Elective C-section | 0.3821 | 1.27 (0.74-2.17) | 0.0187 | 3.83 (1.25-11.78) |
| Non-induced vaginal delivery | 0.4712 | 0.80 (0.44-1.45) | 0.1219 | 0.20 (0.03-1.56) |
| Induced vaginal delivery | 0.4180 | 0.70 (0.30-1.65) | - | - |
| Perinatal outcome | | | | |
| Gestational age at delivery | 0.0700 | - | 0.0498** | - |
| Preterm birth | 0.7899 | 1.11 (0.50-2.46) | 1.0000 | 0.63 (0.08-5.00) |
| Birth asphyxia | 0.9248 | 0.96 (0.44-2.11) | - | - |
| Lack of progress of labour | 0.2378 | 0.42 (0.12-1.44) | 1.0000 | 0.78 (0.10-6.22) |
| Postpartum haemorrhage | - | - | 0.0213 | 14.61 (2.23-95.79) |
| Episiotomy | 0.9427 | 1.17 (0.45-3.06) | - | - |
| Perineal tears | 0.2449 | 0.36 (0.08-1.65) | - | - |
| Neonatal outcome | | | | |
| Birth weight [g] | 0.5890 | - | 0.6750 | - |
| Macrosomy | 0.9180 | 3.58 (0.22-57.93) | - | - |
| SGA | 0.4762 | 0.54 (0.16-1.88) | 0.2829 | 2.17 (0.45-10.41) |
| LGA | 0.5754 | 1.26 (0.56-2.82) | 1.0000 | 0.71 (0.09-5.65) |
| APGAR score: 0-3 | - | - | - | - |
| 4-6 | - | - | - | - |
| 7-10 | - | - | - | - |
| Stillbirth | - | - | - | - |
| Congenital anomalies | 0.8869 | 0.93 (0.36-2.58) | 0.2829 | 2.17 (0.45-10.41) |

Other reasons for such a high proportion of cesarean sections among older patients might be an increased occurrence of medical conditions (both pre-

existing and gestational), induction of labour, fetal malposition and maternal request for cesarean section [18].

**Table 6: Comparison of the women 35-39 and ≥ 40 with the control group among multiparas**

| Outcome | 35-39 | | ≥40 | |
|---|---|---|---|---|
| | p-value | OR (95% CI) | p-value | OR (95% CI) |
| Pregnancy complications | | | | |
| Pregnancy-induced hypertension (PIH) | 0.8414 | 0.93 (0.47-1.86) | 0.2643 | 1.78 (0.64-4.97) |
| Hypothyroidism | 0.0018 | 1.79 (1.24-2.58) | 0.2320 | 1.50 (0.77-2.93) |
| Anaemia | 0.1902 | 0.67 (0.37-1.23) | 0.9046 | 0.81 (0.27-2.40) |
| Placenta praevia | 0.9223 | 0.73 (0.18-2.95) | 0.6984 | 1.25 (0.15-10.63) |
| PROM | 0.7431 | 0.85 (0.33-2.19) | 0.2033 | 2.63 (0.81-8.55) |
| Oligohydramnios | 0.9223 | 0.73 (0.18-2.95) | 0.6984 | 1.25 (0.15-10.63) |
| Polyhydramnios | 0.8491 | 0.88 (0.21-3.72) | - | - |
| Mode of delivery | | | | |
| Caesarean section | 0.5699 | 1.09 (0.77-1.55) | 0.2862 | 1.62 (0.80-3.26) |
| Emergency C-section | 0.0769 | 1.53 (0.95-2.46) | 0.2960 | 1.56 (0.68-3.59) |
| Elective C-section | 0.4188 | 0.87 (0.62-1.22) | 0.5457 | 1.21 (0.64-2.28) |
| Non-induced vaginal delivery | 0.7311 | 1.07 (0.74-1.55) | 0.3462 | 0.70 (0.33-1.47) |
| Induced vaginal delivery | 0.1336 | 0.58 (0.28-1.19) | 0.5775 | 0.53 (0.12-2.31) |
| Perinatal outcome | | | | |
| Gestational age at delivery | 0.0880 | - | 0.0028 | - |
| Preterm birth | 0.3023 | 1.31 (0.78-2.19) | 0.1651 | 1.80 (0.78-4.17) |
| Birth asphyxia | 0.3160 | 1.50 (0.68-3.30) | 0.8954 | 0.56 (0.07-4.39) |
| Lack of progress of labour | 0.9033 | 0.49 (0.05-4.74) | - | - |
| Postpartum haemorrhage | 0.6552 | 1.47 (0.09-23.63) | 0.5630 | 7.60 (0.47-123.74) |
| Episiotomy | 0.8988 | 1.17 (0.61-2.24) | 0.7337 | 0.66 (0.14-3.19) |
| Perineal tears | 0.5006 | 0.91 (0.48-1.71) | 0.7591 | 1.25 (0.37-4.17) |
| Neonatal outcome | | | | |
| Birth weight [g] | 0.3420 | - | 0.5050 | - |
| Macrosomy | 0.9033 | 0.49 (0.05-4.74) | - | - |
| SGA | 0.8643 | 1.07 (0.51-2.23) | 0.5858 | 0.40 (0.05-3.07) |
| LGA | 0.1691 | 1.53 (0.84-2.80) | 0.0029 | 3.41 (1.46-7.95) |
| APGAR score: 0-3 | - | - | - | - |
| 4-6 | 0.7360 | 0.73 (0.07-8.10) | 0.1099 | 7.76 (1.06-56.55) |
| 7-10 | 0.9033 | 0.68 (0.10-4.86) | 0.1099 | 0.13 (0.02-0.95) |
| Stillbirth | - | - | - | - |
| Congenital anomalies | 0.2047 | 1.62 (0.77-3.43) | 0.8229 | 0.52 (0.07-4.05) |

As mentioned above, the rate of CCs among AMA women was significantly increased, thus the percentage of vaginal delivery was lower (39.2% for 30-34, 35.2% for 35-39 and 22.4% for ≥ 40 in total, both induced and non-induced). We obtained a result of a decreased episiotomy rate and an increased perineal tears rate in the whole group of women aged ≥40. Furthermore, the highest rate of episiotomy was observed among 35-39 primiparous women (72%) and the gap between episiotomy and perineal tear percentages was the biggest for ≥ 40 multiparas (16.7% vs 41.7%). Simultaneously, in the group of primiparas, the perineal tears rate was quite low (8%). Considering general indications for episiotomy, risk factors for perineal tears (such as primiparity or fetal weight) and the outcomes of the group aged 35-39, we suppose that episiotomy was accurately performed as prevention of unintended laceration of peritoneum [19].

In the whole group of women aged ≥ 40 as well as in the cohort of primiparas ≥ 40 years old, the incidence of postpartum haemorrhage (PPH) was nearly 7 and 14 times higher than in the relevant control groups, respectively. The literature confirms these results, listing the age of 40 and obesity as risk factors for postpartum bleeding [20]. Therefore, we strongly believe that appropriate prophylaxis of PPH, as well as strict control of anaemia parameters, should be performed in this group of the patients [24].

Our analysis revealed that the incidence of large for gestational age (LGA) was significantly higher in the whole group of women aged ≥ 40 when compared to the control group (p = 0.0355, OR = 2.17). Similar results were observed among multiparas aged ≥ 40 (p = 0.029, OR = 3.41). Other studies also report the LGA rate to be increased among mothers giving birth at an advanced age [6]. M.S. Schimmel at al. reported the twice more frequent occurrence of LGA among AMA mothers (OR = 1.64 p = 0.0001) and, what is more, the weights of newborns were simultaneously increasing with the growth of maternal age [21]. For primiparas, such a correlation has not been observed, similar to our study. Although LGA may result from maternal diabetes and multiparity, the bigger tendency for LGA among AMA women prevailed after calibrating for the two latter variables in the multivariable analysis.

In our study, the higher maternal age, the lower gestational age at delivery was: control group delivered at 39 (1+5) weeks of gestation, the group aged 35-39 at 38+6 (1+2) weeks of gestation and women aged ≥ 40 at 38+4 (1+3) gestational weeks. Both relationships met statistical significance criteria (p = 0.0025 and p = 0.0003, respectively). Our results confirm the conclusions from other studies [12].

Moreover, we observed a negative correlation between maternal age and gestational age at delivery. The correlation was statistically significant for the whole study population as well as for multiparous women (p = 0.003, r = -0.0949 and p = 0.004, r = -0.1168, respectively).

As for preterm delivery (PTB), several researchers reported that its incidence was dramatically increasing with maternal age (9.4 vs 19.1, p < 0.001) [22]. Nonetheless, our study revealed no statistically significant difference between the preterm birth rate among younger women and AMA patients.

Finally, in general population of pregnant women, the normal ratio of pregnancy-induced hypertension (PIH) ranges from 6-10 % and is one of the main causes of perinatal morbidity and mortality of both women and the newborns [23]. In our study the rates for the women aged 30-39 fitted in that range (8.8% for 30-34 and 6.1% for 35-39 considering whole study population), but in the group aged ≥40 it was much higher – 13.8% for the whole population of the age ≥ 40, 11.4% for the multiparas and even 21.4% for primiparas. Even though these results weren't statistically significant, they might confirm the immunological hypothesis of PIH hazard decreasing with each subsequent pregnancy with the same partner [Dudenhausen]. Dietl et al. investigated similar outcomes (AMA women more likely evolving PIH). However, the percentages in his study were remarkably lower (2.0% for women aged > 40 and 0.9% for 30-34) comparing to our study [6].

In conclusion, in addition to observing the increased frequency of pregnancy-induced hypertension, lower gestational age at delivery, increased cesarean section rate and higher incidence of LGA were observed among advanced maternal age patients. What is more, we showed that these adverse effects were proceeding with age. Most studies

compare only two groups of mothers, above 40 years old and younger. Our research is uncommon since we have divided the advanced maternal age group into two subgroups (≥ 40 and 35-39) and compared them with the control group aged 30-34. Furthermore, each group was further subdivided into primiparas and multiparas for a better evaluation of results. Based on the study, delayed child-bearing seems to be associated with an increased rate of obstetrical and perinatal complications. Care providers need to be aware of these complications and adapt obstetrical supervision for better pregnancy outcomes.

## Acknowledgements

The paper was awarded the Best Oral Presentation at the 42. International Medical Congress, May 27-29, 2019, Ohrid, the Republic of Macedonia by the Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia.

## References

1. Ben-David A, Glasser S, Schiff E, Zahav AS, Boyko V, Lerner-Geva L. Pregnancy and Birth Outcomes Among Primiparae at Very Advanced Maternal Age: At What Price? Maternal and Child Health Journal. 2016; 20: 833-842. https://doi.org/10.1007/s10995-015-1914-8 PMid:26686195

2. Ogawa K, Urayama KY, Tanigaki S. et al. Association between very advanced maternal age and adverse pregnancy outcomes: A cross sectional Japanese study. BMC Pregnancy and Childbirth 17, 2017. https://doi.org/10.1186/s12884-017-1540-0 PMid:29017467 PMCid:PMC5635576

3. Almeida NKO, Almeida RMVR, Pedreira CE. Adverse perinatal outcomes for advanced maternal age: A cross-sectional study of Brazilian births. Jornal de Pediatria. 2015; 91:493-498. https://doi.org/10.1016/j.jped.2014.12.002 PMid:26054772

4. Wang Y, Tanbo T, Åbyholm T, Henriksen T. The impact of advanced maternal age and parity on obstetric and perinatal outcomes in singleton gestations. Archives of gynecology and obstetrics. 2011; 284(1):31-7. https://doi.org/10.1007/s00404-010-1587-x PMid:20632182 PMCid:PMC3112324

5. Johnson JA, Tough S, Wilson RD, Audibert F, Blight C, BrockS JA, Cartier L, Desilets VA, Gagnon A, Langlois S, Murphy-Kaulbeck L. Delayed child-bearing. Journal of obstetrics and gynaecology Canada. 2012; 34(1):80-93. https://doi.org/10.1016/S1701-2163(16)35138-6

6. Dietl A, Cupisti S, Beckmann MW, Schwab M, Zollner U. Pregnancy and obstetrical outcomes in women over 40 years of age. Geburtshilfe und Frauenheilkunde. 2015; 75(08):827-32. https://doi.org/10.1055/s-0035-1546109 PMid:26366002 PMCid:PMC4554509

7. Ciancimino L, Lagana AS, Chiofalo B, Granese R, Grasso R, Triolo O. Would it be too late? A retrospective case-control analysis to evaluate maternal-fetal outcomes in advanced maternal age. Archives of gynecology and obstetrics. 2014; 290(6):1109-14. https://doi.org/10.1007/s00404-014-3367-5 PMid:25027820

8. Kalayci H, Ozdemir H, Alkas D, Cok T, Tarim E. Is primiparity a risk factor for advanced maternal age pregnancies? The Journal Of Maternal-fetal & Neonatal Medicine. 2017; 30(11):1283-7. https://doi.org/10.1080/14767058.2016.1211633 PMid:27406982

9. Wielgos A, Szymusik I, Bartnik P, Kacperczyk J, Kosinska-Kaczynska K, Pietrzak B. Pregnancy beyond the age of 40-the influence of parity on perinatal outcome. Neuroendocrinology Letters. 2015; 36(4):101-7.

10. Chan BC, Lao TT. Effect of parity and advanced maternal age on obstetric outcome. International Journal of Gynecology & Obstetrics. 2008; 102(3):237-41. https://doi.org/10.1016/j.ijgo.2008.05.004 PMid:18606410

11. Kenny LC, Lavender T, McNamee R, O'Neill SM, Mills T, Khashan AS. Advanced maternal age and adverse pregnancy outcome: evidence from a large contemporary cohort. PloS one. 2013; 8(2):e56583. https://doi.org/10.1371/journal.pone.0056583 PMid:23437176 PMCid:PMC3577849

12. Osmundson SS, Gould JB, Butwick AJ, Yeaton-Massey A, El-Sayed YY. Labor outcome at extremely advanced maternal age. American journal of obstetrics and gynecology. 2016; 214(3):362-e1. https://doi.org/10.1016/j.ajog.2015.09.103 PMid:26454124

13. Karlström A, Lindgren H, Hildingsson I. Maternal and infant outcome after caesarean section without recorded medical indication: findings from a Swedish case-control study. BJOG: An International Journal of Obstetrics & Gynaecology. 2013; 120(4):479-86. https://doi.org/10.1111/1471-0528.12129 PMid:23316937

14. Crawford BS, Davis J, Harrigill K. Uterine artery atherosclerotic disease: histologic features and clinical correlation. Obstetrics & Gynecology. 1997; 90(2):210-5. https://doi.org/10.1016/S0029-7844(97)00225-1

15. Bayrampour H, Heaman M. Advanced maternal age and the risk of cesarean birth: a systematic review. Birth. 2010; 37(3):219-26. https://doi.org/10.1111/j.1523-536X.2010.00409.x PMid:20887538

16. Vahratian A, Zhang J, Troendle JF, Savitz DA, Siega-Riz AM. Maternal prepregnancy overweight and obesity and the pattern of labor progression in term nulliparous women. Obstetrics & Gynecology. 2004; 104(5):943-51. https://doi.org/10.1097/01.AOG.0000142713.53197.91 PMid:15516383

17. Main DM, Main EK, Moore II DH. The relationship between maternal age and uterine dysfunction: a continuous effect throughout reproductive life. American journal of obstetrics and gynecology. 2000; 182(6):1312-20. https://doi.org/10.1067/mob.2000.106249 PMid:10871444

18. Callaway LK, Lust K, McIntyre HD. Pregnancy outcomes in women of very advanced maternal age. Australian and New Zealand journal of obstetrics and gynaecology. 2005; 45(1):12-6. https://doi.org/10.1111/j.1479-828X.2005.00333.x PMid:15730358

19. Corrêa Junior MD, Passini Júnior R. Selective episiotomy: indications, techinique, and association with severe perineal lacerations. Revista Brasileira de Ginecologia e Obstetrícia. 2016; 38(6):301-7. https://doi.org/10.1055/s-0036-1584942 PMid:27399925

20. Weeks A. The prevention and treatment of postpartum haemorrhage: what do we know, and where do we go to next?. BJOG: An International Journal of Obstetrics & Gynaecology. 2015; 122(2):202-10. https://doi.org/10.1111/1471-0528.13098 PMid:25289730

21. Schimmel MS, Bromiker R, Hammerman C, Chertman L, Ioscovich A, Granovsky-Grisaru S, Samueloff A, Elstein D. The effects of maternal age and parity on maternal and neonatal outcome. Archives of gynecology and obstetrics. 2015; 291(4):793-8. https://doi.org/10.1007/s00404-014-3469-0 PMid:25227657

22. Yaniv SS, Levy A, Wiznitzer A, Holcberg G, Mazor M, Sheiner E. A significant linear association exists between advanced maternal age and adverse perinatal outcome. Archives of gynecology and obstetrics. 2011; 283(4):755-9. https://doi.org/10.1007/s00404-010-1459-4 PMid:20376672

23. Kintiraki E, Papakatsika S, Kotronis G, Goulis DG, Kotsis V. Pregnancy-Induced hypertension. Hormones (Athens). 2015; 14:211-223. https://doi.org/10.14310/horm.2002.1582 PMid:26158653

24. Prevention and Management of Postpartum Haemorrhage: BJOG: An International Journal of Obstetrics & Gynaecology. 2017; 124(5): e106-e149. https://doi.org/10.1111/1471-0528.14178 PMid:27981719

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Jun 30; 7(12):1957-1961.
https://doi.org/10.3889/oamjms.2019.550
eISSN: 1857-9655
***Clinical Science***

# Brain-Derived Neurotropic Factor Serum Level and Severity Symptom of Bataknese Male Patients with Schizophrenia in North Sumatera, Indonesia

Deasy Hendriati*, Elmeida Effendy, Mustafa Mahmud Amin, Vita Camellia, Muhammad Surya Husada

*Department of Psychiatry, Faculty of Medicine, Universitas Sumatera Utara, Medan 20155, Indonesia*

**Citation:** Hendriati D, Effendy E, Amin MM, Camellia V, Husada MS. Brain-Derived Neurotropic Factor Serum Level and Severity Symptom of Bataknese Male Patients with Schizophrenia in North Sumatera, Indonesia. Open Access Maced J Med Sci. 2019 Jun 30; 7(12):1957-1961. https://doi.org/10.3889/oamjms.2019.550

**Keywords:** Schizophrenia; Brain-derived neurotrophic factor; Severity symptom; Bataknese

***Correspondence:** Deasy Hendriati. Department of Psychiatry, Faculty of Medicine, Universitas Sumatera Utara, Medan 20155, Indonesia. E-mail: deasyhendriati@gmail.com

**Received:** 02-May-2019; **Revised:** 11-Jun-2019; **Accepted:** 12-Jun-2019; **Online first:** 30-Jun-2019

**Copyright:** © 2019 Deasy Hendriati, Elmeida Effendy, Mustafa Mahmud Amin, Vita Camellia, Muhammad Surya Husada. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

**Funding:** This research did not receive any financial support

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

**Abstract**

**BACKGROUND:** Schizophrenia is a severe mental disorder that is multi-causative and multi-factor, generally affecting about 1% of the population. The elevation level of brain-derived neurotrophic factor (BDNF) offers several protections from other neurodegenerative processes that occur in schizophrenia since this deficit of neurotrophic factors can contribute to changes in brain structure and function that underlie the schizophrenia psychopathology.

**AIM:** To analyse the correlation between BDNF serum levels and symptom severity by using the Positive and Negative Syndrome Scale (PANSS) instrument in Bataknese male patients with schizophrenia

**METHODS:** This study was a correlative analytical study with a cross-sectional approach using the Positive and Negative Syndrome Scale (PANSS) instrument to assess symptom severity with 60 subjects of Bataknese male patients with chronic schizophrenia. Moreover, this research was conducted at the Psychiatric Hospital of Prof. Dr M. Ildrem Medan, Indonesia. BDNF serum was analysed with the Quantitative sandwich enzyme immunoassay technique by via Quantikine ELISA Human CXCL8/IL-8 HS. Also, the data analysis was performed through Spearman's correlative bivariate analytics using SPSS software.

**RESULTS:** A negative correlation between the BDNF serum level and the negative scale PANSS score in men with schizophrenia ($r = -0.820$, $p < 0.001$) was found. Moreover, there is a negative correlation between BDNF serum levels and PANSS total scores in men with schizophrenia ($r = -0.648$, $p < 0.001$)

**CONCLUSION:** BDNF serum level in Bataknese male patients with schizophrenia has a relationship that affects the severity of symptoms in schizophrenic patients, especially for negative symptoms.

## Introduction

Schizophrenia is a common psychotic disorder, with a risk of around 1%. The most frequent initial onset of this disorder is the age of 15-30 years and is a chronic disease that disrupts patients and their families. Moreover, it has a major impact on society and economy [1]. Schizophrenia patients experience complex mental deterioration disorders. The mechanism that causes this disorder is still unclear, but some evidence shows that the cause is multi-causal and multi-factors. These factors include genetic, neuro-developmental, social [2], [3], [4], [5], [6] and immune factors [7]. On the other hand, schizophrenia is not a static mental disorder but a dynamic process that causes dysregulation of various pathways [8]. Other factors that are suspected of having a role in the presence of a degenerative central nervous system (neuro-degeneration) [9].

The aetiology and pathophysiology of schizophrenia have not been explained so far. Various changes in the central nervous system can cause clinical manifestations of the disease. Neurotransmitter deficits are considered as epiphenomena underlying the disorganisation of neurotropins [10]. Brain-Derived Neurotropic Factor (BDNF), a member of the neurotropic derivative, is often found in adult mammalian brains and plays an important role in the development, regeneration, survival, maintenance and function of neurons [11], [12]. During the development, BDNF plays an important role in maintaining proper axonal growth. BDNF is also essential for the development and

survival of dopaminergic, serotonergic, GABAergic, and cholinergic neurons. Also, there are significant roles in the pathophysiology of psychiatric diseases, including schizophrenia [13].

Furthermore, BDNF plays an important role in the mesolimbic dopaminergic system and regulates the expression of D3 dopamine receptors [14]. Finally, BDNF is produced by immune cells in response to neuroimmune and inflammatory response to protect the brain from any damage. Based on this evidence, BDNF turns into a potentially useful biomarker to study the inflammatory features of schizophrenia, which has a role in the persistent negative symptoms and cognitive features in schizophrenia [15]. This suggests that a relationship between BDNF properties with dopamine pathway in schizophrenia [16].

Some studies performed sub-categories of schizophrenia symptoms into 5 parts, positive symptoms, negative symptoms, cognitive symptoms, aggressive symptoms and depression/anxiety symptoms [1]. Negative symptoms of schizophrenia are characterised by the deficits in normal emotions and social functions that can be primary or secondary for the treatment of diseases or other manifestations. These symptoms are strongly associated with long-term paralysis that tends to worsen over time and negatively affect the patient's quality life. Despite this burden, negative symptoms are more resistant to treatment compared to positive symptoms, and existing antipsychotics are not able to reduce the negative symptoms of schizophrenia [17]. In general, Indonesian population is determined to follow the paternalistic line (father/male), for example, the Javanese and Bataknese tribes. In this case, Bataknese male will give offspring who are also Bataknese. The total number of ethnic groups in Indonesia as a whole reaches more than 1,300 ethnic groups. In addition to the various types, the number or size of the population of each type of ethnic group is also very varied [18]. In this study, the subjects chosen were Bataknese. The author was interested in choosing the Bataknese because the majority of schizophrenia patients who were hospitalised in the psychiatric hospital, Prof. Dr M. Ildrem in North Sumatra is Batak tribe.

To sum up, this study is aimed to investigate the relationship between BDNF serum levels and symptom severity measured using the PANSS instrument in Batak male patients with chronic schizophrenia.

## Methods

This study performed a cross-sectional study design to evaluate the relationship of BDNF serum level as of symptoms severity as measured by PANSS in Bataknese male patients with schizophrenia. The total sample of this study was 60 people, collected using the consecutive sampling method. Furthermore, this study has followed the Medical Ethics Committee of Universitas Sumatera Utara, Medan, Indonesia.

*Participants:* A total of 60 subjects with schizophrenia were established by structured interviews using the MINI ICD-10, which was hospitalised at the Psychiatric Hospital of Prof. dr. M. Ildrem, Medan, Indonesia.

*Inclusion criteria:* Age between 20-60 years, bataknese male, smoking, chronic schizophrenia patients for 2 years with stabilisation phase (PANSS 60-80 score), understanding Indonesian language, willing to be a respondent and able to be interviewed.

*Exclusion Criteria:* Having other mental disorders, suffered from neurologic diseases, history of alcohol use and other substances except for tobacco.

Data collection was preceded by screening using inclusion and exclusion criteria. Individuals who met the inclusion criteria and exclusion criteria were asked for approval to take part in the study after obtaining informed consent. Then, the demographic data of the subject were filled PANSS score assessment on the research subject was performed. The next stage was as much as 5 ml of the subject's blood samples were taken by laboratory officers who will then be examined in the laboratory to obtain the result of BDNF serum level.

### *Measurement*

The severity was measured through the Positive and Negative Syndrome Scale (PANSS). It was developed in the late 1980s aimed at assessing clinical symptoms of schizophrenia. PANSS contains 30 items in three subscales, seven items include positive symptoms (for example, delusions and hallucinations), seven items include negative symptoms (for example, social withdrawal, flat affect, lack of motivation), and 16 items include general psychopathology (for example, anxiety and depression). Assessment can be completed in 30 to 40 minutes. The reliability is good, and the validity is very good [19]. In previous PANSS examination, the former of the researcher was trained with an interpreter, which then PANSS score will be conducted with a suitability test between the researcher and the interpreter.

BDNF was assessed by taking 5 ml of the subject's blood samples by laboratory personnel in the morning at around 8 am, which will then be examined in the laboratory to obtain the results of BDNF levels. BDNF serum levels were analysed with the Quantitative sandwich enzyme immunoassay technique by the use of Quantikine ELISA Human

CXCL8/IL-8 HS.

The sample used was a human serum. The standard calibration range is 62.5-4000 pg / mL. The limit of detection is 20 pg / mL, and the dilution factor is 20 times. The results have been multiplied by a dilution factor. Measurements using the Microplate Reader Biorad model 680 instruments (Bio-rad Laboratories Inc., CA, USA) with the Microplate Manager version 5.2.1 software (Bio-rad Laboratories Inc., CA, USA).

### *Data Analysis*

The normal distribution test was performed through the Kolgomorov-Smirnov test. Moreover, the analysis between BDNF serum levels and each PANSS score item was conducted using the Spearman's correlative bivariate analytic (N = 60, $Z\alpha$ 5%, $Z_\beta$ 20%) with SPSS 22 software (SPSS Inc, Chicago, Illinois, USA), and $p < 0.05$ was considered as statistically significant.

## Results

A total of 60 research subjects were analysed. In Table 1, the demographic characteristics of the research subjects are described. The average age of the study subjects was 37.65 years with a standard deviation of 6.58 years.

**Table 1: The demographic characteristic of the research subject**

| Variable | Mean/f | SD/% |
|---|---|---|
| Age | 37.650 | 6.581 |
| Educational Background | | |
| Junior High school | 17 | 28.3% |
| Senior High school | 41 | 68.3% |
| Diploma | 2 | 3.3% |
| Marital Status | | |
| Not married | 19 | 31.7% |
| Married | 41 | 68.3% |
| Smoking Level | | |
| Current smokers | 31 | 51.7% |
| Former smokers | 21 | 35.0% |
| Never smokers | 8 | 13.3% |
| Relapse | 3.417 | 0.497 |
| Body Mass Index | 22.232 | 1.543 |

Mean/f: Mean/Frequency; SD/%: Standard Deviation/Percentage.

The highest level of education is a senior high school with 41 people (68.3%). Most subjects are married (68.3%) and 51.7% of samples are a current smoker. Furthermore, the mean of relapse was 3.417 (SD = 0.497), and the average Body Mass Index is 22.232 (SD = 1.543).

**Table 2: The BDNF serum level and PANSS score**

| Variable | Mean ± SD |
|---|---|
| BDNF serum | 24.573 ± 4.035 |
| PANSS Positive score | 11.050 ± 2.118 |
| PANSS negative score | 26.783 ± 3.923 |
| Psychopathology general PANSS score | 31.466 ± 3.202 |
| Total PANSS score | 69.300 ± 6.181 |

In Table 3, it can be seen that a negative correlation between BDNF level and PANSS scores is discovered. There was a strong and significant correlation between BDNF serum levels and negative PANSS scores ($r = -0.820$, $p < 0.001$) and between BDNF serum levels and PANSS total scores ($r = -0.664$, $p < 0.001$). Moreover, a very weak correlation between BDNF serum level and positive PANSS score and PANSS score general psychopathology is recorded.

**Table 3: The correlation between BDNF and PANSS**

| | PANSS Score | Coefficient Correlation (r) | *p* |
|---|---|---|---|
| BDNF | PANSS Positive score | -0.155 | 0.237 |
| | PANSS negative score | -0.820 | < 0.001* |
| | Psychopathology general PANSS score | -0.140 | 0.287 |
| | Total PANSS score | -0.648 | < 0.001* |

*Spearman correlation ($p < 0.05$).

## Discussion

The main finding in this cross-sectional study was a negative correlation between BDNF serum levels and negative scale PANSS scores in Bataknese male patients with schizophrenia and a negative correlation between BDNF serum levels and PANSS total scores. This study is by Akyo et al., (2015) which concluded that there were negative correlations between BDNF peripheral measured levels and the symptomatology of schizophrenia. BDNF plays an important role in the development of the central nervous system. It has an impact on the serotonergic signalling, glial cells, hippocampus neurons and the brain cortex. Moreover, BDNF, in contrast to other neurotrophins, is secreted in response to neuron excitation and releasing dopamine and glutamate from the hippocampal cells. BDNF expression in the frontal cortex can be regulated via dopaminergic receptors [20,21]. Koeva (2014) discovered that changes in the neurotrophic factor system were one of the factors considered in the pathological cascade of schizophrenic psychosis. The decreased BDNF serum levels show a potential deficit in the release of neurotrophic factors in patients with schizophrenia. The results of this study support the view that BDNF is related to schizophrenia [10].

Furthermore, this study is also by the study of Sasha (2018), found that the levels of BDNF serum were associated with negative symptoms in older adults with schizophrenia. She found that the higher BDNF serum level and greater severity of negative symptom items [13]. Recently, Niitsu et al. (2014) investigated BDNF levels in patients with schizophrenia. They reported that the serum was mature and had a positive correlation between BDNF serum levels and negative symptoms. They concluded that the mature BDNF might not be a good candidate as a biomarker in schizophrenia [22].

According to Zhang et al., (2010), an increase in BDNF levels protects the neurodegenerative processes that occur in schizophrenia, since this deficit of neurotrophic factors can contribute to changes in brain structure and function underlying the psychopathology of schizophrenia [11]. The nervous development of abnormalities and dopamine dysregulation systems have been implicated in the pathophysiology of schizophrenia. Therefore, BDNF can be a marker of abnormal nerve development and neurotransmission in schizophrenia [23]. The BDNF serum levels are widely measured in numerous psychiatric disorders, and the use of BDNF plays a role in the treatment of many psychiatric disorders [20].

In contrast to Fernandez et al. (2015), peripheral BDNF levels in serum and plasma were slightly reduced in Schizophrenia compared to controls. In particular, this decline is emphasised by the duration of the disease. However, the rate of decrease in peripheral BDNF levels did not correlate with the severity of positive and negative symptoms. In plasma, but not serum, peripheral BDNF levels consistently increase after the antipsychotic treatment regardless of the patient's response to treatment [24].

For BDNF itself, there are many factors that contribute to influence, such as gender, smoking habit, body mass index, etc. In this study, the recruited subjects were male patients, where previous studies have stated that gender affects serum BDNF levels. Estrogens have multiple functions in the brain. Pluchino et al. (2013) showed that estrogen could regulate the expression of BDNF via the estrogen response element on the BDNF gene. Another group also indicated that BDNF mediated the effects of testosterone on neuronal survival [25]. In the baseline data, it is found that the most current smokers are 31 people (51.7%) as in the study of Zhang and colleagues found a significant association between BDNF levels and negative symptoms of schizophrenia with a further link to nicotine. Because the negative symptoms of schizophrenia are associated with hypoactivity of the dopaminergic system, smoking can reduce negative symptoms by increasing dopamine in the nucleus accumbency. Also, BDNF increases the release of dopamine in the mesolimbic dopamine system and induces dopamine-related behaviours [11].

For Body Mass Index, the subjects selected were in normal-weight categorisation, considering previous studies by Lommatzsch et al., (2005) shown that plasma BDNF levels in people health decrease significantly with weight gain [26]. Araya et al., (2008) also emphasized the relationship between weight and plasma BDNF levels in overweight and obese people who had gone on a diet, where BDNF levels increased after 3 months on a diet [27].

This study has decided that Batak tribes were chosen. Whether there is a connection between the Bataknese male patients and BDNF serum levels, it still needs further and deeper research. Previous research stated that there were 2 types of sequences of Batak ethnics especially in BP 113-116 were observed. Also, it was expected that Batak ethnicity had a tradition to keep their purity by marrying their relative would show a similar sequence. However, no similarity was found. The reason of these phenomena could be resulted by some Bataknese man married with other race, but they adopted their wife or husband into ethnic Batak by adding a Batak's surname. Interestingly, batak ethnic with ATCG sequences were categorized to have higher risk for having schizophrenia [28].

In this study, a strong and significant correlation has been discovered. There is a negative correlation between BDNF serum level and negative PANSS score in Batak male schizophrenia patients ($r = -0.820$, $p < 0.001$) in the sense that the higher the severity of negative symptoms in Schizophrenic patients, the lower serum BDNF levels. In this study there was also a negative correlation between BDNF serum levels and PANSS total score in Bataknese male patients with schizophrenia ($r = -0,648$, $p < 0.001$).

## Acknowledgement

To this opportunity, authors would like to send a gratitude to Psychiatric Hospital Prof. Dr M. Ildrem, Medan, Indonesia for providing the research facilities.

## Ethical Aspects

Authors state that there is no conflict of interest to this research and the procedure conducted has followed the ethics regulated by Universitas Sumatera Utara.

## References

1. Sadock BJ, Sadock VA. Kaplan and Sadock's Synopsis of Psychiatry: Behavioral Sciences/Clinical Psychiatry. 10th ed. Philadelphia: Lippincott Williams & Wilkins; 2007.

2. Lewis DA, Lieberman JA. Catching up on schizophrenia: natural history and neurobiology. Neuron. 2000; 28:325-34. https://doi.org/10.1016/S0896-6273(00)00111-2

3. Frankle WG, Lerma J, Laruelle M. The synaptic hypothesis of schizophrenia. Neuron. 2003; 39:205-16. https://doi.org/10.1016/S0896-6273(03)00423-9

4. Heinz A, Romero B, Gallinat J, Juckel G, Weinberger DR. Molecular Brain Imaging and the Neurobiology and Genetics of Schizophrenia. Pharmacopsychiatry. 2003; 36:152-7. https://doi.org/10.1055/s-2003-45123 PMid:14677072

5. Mueser KT, McGurk SR. Schizophrenia. Lancet. 2004; 363:2063-72. https://doi.org/10.1016/S0140-6736(04)16458-1

6. Harrison PJ, Weinberger DR. Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. Mol Psychiatry. 2005; 10:40-68. https://doi.org/10.1038/sj.mp.4001558 PMid:15263907

7. Brown AS. Prenatal Infection as a Risk Factor for Schizophrenia. Schizophr Bull. 2006; 32:200-2. https://doi.org/10.1093/schbul/sbj052 PMid:16469941 PMCid:PMC2632220

8. Lang UE, Puls I, Müller DJ, Strutz-Seebohm N, Gallinat J. Molecular Mechanisms of Schizophrenia. Cell Physiol Biochem. 2007; 20:687-702. https://doi.org/10.1159/000110430 PMid:17982252

9. Kulhara P, Gupta S. What is schizophrenia: A neurodevelopmental or neurodegenerative disorder or a combination of both? A critical analysis. Indian J Psychiatry. 2010; 52:21. https://doi.org/10.4103/0019-5545.58891 PMid:20174514 PMCid:PMC2824976

10. Koeva YA, Sivkov ST, Akabaliev VH, Ivanova RY, Deneva TI, Grozlekova LS, Georgieva V. Brain-derived neurotrophic factor and its serum levels in schizophrenic patients. Folia medica. 2014; 56(1):20-3. https://doi.org/10.2478/folmed-2014-0003 PMid:24812918

11. Zhang XY, Xiu MH, Chen DC, De Yang F, Wu GY, Lu L, et al. Nicotine dependence and serum BDNF levels in male patients with schizophrenia. Psychopharmacology (Berl). 2010; 212:301-7. https://doi.org/10.1007/s00213-010-1956-y PMid:20661552

12. Zhang XY, Chen D-C, Tan Y-L, Luo X, Zuo L, Lv M-H, et al. Smoking and BDNF Val66Met polymorphism in male schizophrenia: A case-control study. J Psychiatr Res. 2015; 60:49-55. https://doi.org/10.1016/j.jpsychires.2014.09.023 PMid:25455509

13. Binford SS, Hubbard EM, Flowers E, Miller BL, Leutwyler H. Serum BDNF Is Positively Associated With Negative Symptoms in Older Adults With Schizophrenia. Biol Res Nurs. 2018; 20:63-9. https://doi.org/10.1177/1099800417735634 PMid:29050493 PMCid:PMC5942501

14. Gourion D, Goldberger C, Leroy S, Bourdel M-C, Olié J-P, Krebs M-O. Age at onset of schizophrenia: interaction between brain-derived neurotrophic factor and dopamine D3 receptor gene variants. Neuroreport. 2005; 16:1407-10. https://doi.org/10.1097/01.wnr.0000175245.58708.6b PMid:16056149

15. Nurjono M, Lee J, Chong S-A. A Review of Brain-derived Neurotrophic Factor as a Candidate Biomarker in Schizophrenia. Clin Psychopharmacol Neurosci. 2012; 10:61-70. https://doi.org/10.9758/cpn.2012.10.2.61 PMid:23431036 PMCid:PMC3569148

16. Guillin O, Demily C, Thibaut F. Brain-Derived Neurotrophic Factor in Schizophrenia and Its Relation With Dopamine. Int Rev Neurobiol. 2007; 78:377-95. https://doi.org/10.1016/S0074-7742(06)78012-6

17. Farokhnia M, Azarkolah A, Adinehfar F, Khodaie-Ardakani M-R, Hosseini S-M-R, Yekehtaz H, et al. N-Acetylcysteine as an Adjunct to Risperidone for Treatment of Negative Symptoms in Patients With Chronic Schizophrenia. Clin Neuropharmacol. 2013; 36:185-92. https://doi.org/10.1097/WNF.0000000000000001 PMid:24201233

18. Heriawan R. Kewarganegaraan, Suku Bangsa, Agama dan bahasa sehari-hari Penduduk Indonesia. 1st ed. Jakarta: Badan Pusat Statistik; 2011.

19. Gottlieb J, Fan X, Goff DC. Rating Scales in Schizophrenia. Handbook of Clinical Rating Scales and Assessment in Psychiatry and Mental Health. New York: Humana Press; 2010. https://doi.org/10.1007/978-1-59745-387-5_10

20. Akyol ES, Albayrak Y, Beyazyüz M, Aksoy N, Kuloglu M, Hashimoto K. Decreased serum levels of brain-derived neurotrophic factor in schizophrenic patients with deficit syndrome. Neuropsychiatr Dis Treat. 2015; 11:865-72. https://doi.org/10.2147/NDT.S79444 PMid:25848285 PMCid:PMC4386764

21. Libman-Sokołowska M, Drozdowicz E, Nasierowski T. BDNF as a biomarker in the course and treatment of schizophrenia. Psychiatr Pol. 2015; 49:1149-58. https://doi.org/10.12740/PP/37705 PMid:26909392

22. Niitsu T, Ishima T, Yoshida T, Hashimoto T, Matsuzawa D, Shirayama Y, et al. A positive correlation between serum levels of mature brain-derived neurotrophic factor and negative symptoms in schizophrenia. Psychiatry Res. 2014; 215:268-73. https://doi.org/10.1016/j.psychres.2013.12.009 PMid:24377440

23. Rowbotham IM, Orsucci FF, Mansour MF, Chamberlain SR, Raja HY. Relevance of Brain-derived Neurotrophic Factor Levels in Schizophrenia: A Systematic Review and Meta-Analysis. AIMS Neurosci. 2015; 2:280-93. https://doi.org/10.3934/Neuroscience.2015.4.280

24. Fernandes BS, Steiner J, Berk M, Molendijk ML, Gonzalez-Pinto A, Turck CW, et al. Peripheral brain-derived neurotrophic factor in schizophrenia and the role of antipsychotics: meta-analysis and implications. Mol Psychiatry. 2015; 20:1108-19. https://doi.org/10.1038/mp.2014.117 PMid:25266124

25. Pluchino N, Russo M, Santoro AN, Litta P, Cela V, Genazzani AR. Steroid hormones and BDNF. Neuroscience. 2013; 239:271-9. https://doi.org/10.1016/j.neuroscience.2013.01.025 PMid:23380505

26. Lommatzsch M, Zingler D, Schuhbaeck K, Schloetcke K, Zingler C, Schuff-Werner P, et al. The impact of age, weight and gender on BDNF levels in human platelets and plasma. Neurobiol Aging. 2005; 26:115-23. https://doi.org/10.1016/j.neurobiolaging.2004.03.002 PMid:15585351

27. Araya AV, Orellana X, Espinoza J. Evaluation of the effect of caloric restriction on serum BDNF in overweight and obese subjects: preliminary evidences. Endocrine. 2008; 33:300-4. https://doi.org/10.1007/s12020-008-9090-x PMid:19012000

28. Effendy E, Loebis B, Amir N, Siregar AY. SNP8NRG433E1006 Neuregulin-1 genetic variation in bataks ethnic with schizophrenia paranoid and healthy control. Bali Med J. 2014; 3. https://doi.org/10.15562/bmj.v3i2.75

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Jun 30; 7(12):1962-1967.
https://doi.org/10.3889/oamjms.2019.466
eISSN: 1857-9655
***Clinical Science***

# Child Blood Pressure Profile in Bali, Indonesia

Dyah Kanya Wati[1*], Putu Cintya Denny Yuliyatni[2], I Made Krisna Dinata[3], Gusti Ayu Putu Nilawati[1], I Gede Raka Widiana[4], Ida Bagus Ramajaya Sutawan[1], I Gusti Ngurah Putu Mandela Agatha Sunantara

*[1]Department of Child Health, School of Medicine Udayana University, Sanglah Denpasar Hospital, Denpasar, Indonesia; [2]Department of Public Health, School of Medicine Udayana University, Denpasar, Indonesia; [3]Department of Physiology, School of Medicine Udayana University, Denpasar, Indonesia; [4]Department of Internal Medicine, School of Medicine Udayana University , Sanglah Denpasar Hospital, Denpasar, Indonesia*

**Citation:** Wati DK, Yuliyatni PCD, Dinata IMK, Nilawati GAP, Widiana IGR, Sutawan IBR, Sunantara IGNPMA. Child Blood Pressure Profile in Bali, Indonesia. Open Access Maced J Med Sci. 2019 Jun 30; 7(12):1962-1967. https://doi.org/10.3889/oamjms.2019.466

**Keywords:** Child hypertension; Bali; Age; Nutrition; Family history

***Correspondence:** Dyah Kanya Wati. Department of Child Health, School of Medicine Udayana University - Sanglah Denpasar Hospital, Denpasar, Indonesia. E-mail: dyahkanyawati@unud.ac.id

**Received:** 04-Mar-2019; **Revised:** 01-May-2019; **Accepted:** 02-May-2019; **Online first:** 30-Jun-2019

**Copyright:** © 2019 Dyah Kanya Wati, Putu Cintya Denny Yuliyatni, I Made Krisna Dinata, Gusti Ayu Putu Nilawati, I Gede Raka Widiana, Ida Bagus Ramajaya Sutawan, I Gusti Ngurah Putu Mandela Agatha Sunantara. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

**Funding:** This research did not receive any financial support

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

**Abstract**

**BACKGROUND:** Mortality and morbidity in an adult will be reduced by controlling hypertension from an early age. Uncontrolled blood pressure since children can contribute to diseases such as heart disease, organ damage, and decreased quality of life. As changes in lifestyle, it is estimated that hypertension in children will continue to increase. Until now, data regarding the profile of blood pressure in children in Indonesia is still lacking.

**AIM:** The purpose of this study was to determine the prevalence of increased blood pressure and hypertension in children in Bali.

**METHODS:** This study was a cross-sectional study. The sampling technique in this study was multistage random sampling, that is, from 9 regencies in Bali, the selection of 3 regencies to be sampled according to socio-economic stratification based on regional economic growth and regional per capita income in Bali Province.

**RESULTS:** From 1257, samples examined the prevalence of increased blood pressure, and hypertension was 689 children (54.8%). From the age group, the prevalence of an increase in blood pressure and hypertension in the age group ≤ , 12 years was 47.3%, and in the age group > 12 years was 62.2%. Increased blood pressure in nutritional status including Obesity 51.4%, Nutrition More 52.9%, Good Nutrition 42.2%, Nutrition Less 43.9%, Malnutrition 50.0%. In families with a history of hypertension, the prevalence of increased blood pressure and hypertension in subjects was 60.3% and in families without a history of hypertension was 43.4%.

**CONCLUSION:** It can be concluded that there is still a prevalence of hypertension in children in Bali. Health efforts are needed so that they can minimise the further health impact that might occur. It should also be noted that various factors can influence the prevalence of increased blood pressure and hypertension in children.

## Introduction

Hypertension still becomes the world's concern as this disease could occur at such a young age with its continuous risks until adult and contribute to heart disease, organs damage, and decreased quality of life [1], [2]. Early management of hypertension could decrease mortality and morbidity related to this disease. Its percentage in children varies one another. A study by Meng L et al. showed the increased blood pressure percentage in children aged 3 – 18 years as 3.1% [3]. Also reported that between 2011 to 2014, the prevalence of children hypertension aged 8 – 17 years old was 2.2% [2]. A study by Gupta et al. found the aetiology of hypertension itself, of which 423 children aged > 5 years who had hypertension were 275 children (65%) with essential hypertension of 43% and secondary by 57%. It was also found that the most age experienced hypertension ranged from 13 to 19 years old [4].

Blood pressure measurement in children is more complicated than the adults, because of children, blood pressure values need to be seen according to each normal percentile based on their age, sex, and height. Also, children are harder to cooperate in the measurements, often because they were scared to the examiner; therefore, it could bias the results [5], [6].

Heretofore, there is still a little of profile data of children blood pressure in Indonesia. Moreover, there were only some studies related to children blood pressure as the lack of concern since the difficulty of the measurement and probably also due to small prevalence that could be such a lack in its urgency.

Therefore, the primary objective of this study was to find out the hypertension prevalence of children in Bali. Secondary, it aims to prove the relation of some factors such as age, nutrition status and family history to children blood pressure in Bali. The obtained profile data is expected, contributing to the improvement of screening and prevention management, which can earlier be done in hypertension of children.

## Material and Methods

It was a descriptive, cross-sectional study using a questionnaire and direct examination of the samples. All of the children from elementary and junior high school in Bali Province were included in this study. The inclusion criteria included children aged 6 to 18 years when examination conducted, willing to participate in this study, students, of elementary or junior high school in Bali Province, and live with both biological parents. The exclusion criteria included children with corticosteroid history of > 2 weeks or other drugs impacted blood pressure, children were absent when examination conducted, and research questionnaires were not filled.

The sampling technique is using multistage random sampling, which is from 9 regencies/municipalities in Bali, it was chosen 3 regencies/municipalities to become sampling sites according to socio-economic stratification based on regional economic growth and regional per capita income. In each stratification area in Bali Province, 1 regency/ municipality was taken using the stratified random sampling method, 9 districts/municipalities were designated as sampling locations using cluster sampling method, distribution of 9 sub-districts was carried out proportionally based on proportions the number of elementary and junior high school students in each selected district/municipality. Then, from 9 selected sub-districts, 18 elementary or junior high schools are designated as sampling locations using the cluster sampling method. Determination of students/children selected as samples in each primary school is done by simple random sampling method.

Blood pressure was determined using a sphygmomanometer to get designated samples' blood pressure. The measurement was conducted according to Flynn TJ et al., algorithm both in procedure and hypertension determination [6], i.e.: (1) percentile < 90: normotension; (2) percentile ≥ 90 to < 95 or 120/80 mmHg to percentile < 95: prehypertension; (3) percentile ≥ 95: hypertension, as a nominal measurement scale. Age was defined as chronological age at the time the sample examined. Samples were chosen at the age group of 28 days to 18 years, as a numeric measurement scale. The familial factor was a factor of father or mother who has prior hypertension history. The familial factor was determined by interview and direct examination to the parents; then the results would be categorised into the group "yes" or "no", as a nominal measurement scale. Exclusive breastfeeding was defined as giving only breast milk to the babies without infant formula, or other food and drink for 6 months, the results were obtained from the interview to parent and would be categorised into the group "yes" or "no", as a nominal measurement scale.

Nutrition status was determined accrding anthropometry status, i.e. body weight (BW) to length or height (L/H). Samples aged ≤ 5 years used Z-Score BW/H based on WHO Anthro Chart with interpretation: (1) Z-score BW/H > 3 SD: obese; (2) Z-score BW/H > 2 SD: overweight; (3) Z-score BW/H > 1 SD: potential risk of overweight; (4) Z-score BW/H < -2 SD: wasted; (5) Z-score BW/H < -3 SD: severely wasted. While patients aged > 5 years, it used BW/H based on The Centers for Disease Control and Prevention (CDC) 2000 and then classified to Waterlow criteria (BW/ideal body weight) as following: (1) Obesity: > 120%; (2) More nutrition: > 110 – 120%; (3) Good nutrition: 90 – 110%; (4) Less nutrition: 70% to 90%; (5) Malnutrition: < 70%, as a nominal measurement scale.

The diagnosis was established through anamnesis, physical examination, work up, and a decision made by the physician. The diagnosis of essential hypertension was established if: (1) increased blood pressure ≥ 95 in 3 times measurements; (2) no other causes leading to secondary hypertension; and (3) no influence of drugs that potentially increase blood pressure. While secondary hypertension evaluated by a physician.

Calculation of the estimated sample size required, using the sample size formula [7] by setting the significance level at 1.96, mean of standard deviation in the previous references was 25.3% [8], as well as the degree of precision was set at 2.5%, the number of samples from each group needed was 1161 children.

The obtained then collected and processed into software, to be analysed using the program. Descriptive analysis to determine the prevalence of hypertension in children, childhood obesity, and parental history of hypertension. Bivariate test (chi-square test) and calculation of prevalence ratio (PR) used to assess the relationship between hypertension and related factors.

All parents/guardians who will be included in this study have obtained and approved oral and written explanation regarding the purpose and procedure of the study. This research has received approval from the Ethics Committee of Medicine Faculty of Udayana University-Sanglah Denpasar Hospital.

## Results

This study has been running for 6 months, and the sample was 1257 children from 1161 planned samples. Stratified random sampling used had resulted in three selected districts, namely: Denpasar, Karangasem and Singaraja. In those cities, the selection of elementary and junior high schools was obtained based on cluster sampling 1 and 2 with the distribution of the number of students whose number of students was quite balanced from the entire study sample. This study included an exclusive breastfeeding history and a parental history of hypertension. All canteens studied in the school were categorised as "Red" category (not in the table), which meant that almost all food sold in the canteen in this school is unhealthy or has given no effect on the health of the subject. Certain foods became the risk factors for the incidence of hypertension in children.

Table 1 showed the distribution of sample characteristics. The sex proportion in this research was not much different. In the age category, the age of children included this study was at balanced composition for ages ≤ 12 years and ages > 12 years. The highest proportion of students used as research subjects was 7th-grade students, in the amount of 22.9% while the lowest was 3rd-grade students, which 4.7%. While students from Bali are 96.8% of all students studied.

**Table 1: Distribution of sample chracteristics**

| No | Characteristic | Frequency | Pecentage |
|---|---|---|---|
| 1 | Sex | | |
| | Male | 633 | 50.4% |
| | Female | 624 | 49.6% |
| 2 | Age | | |
| | Median (IQR), years | 12.03 (3.40) | |
| | Minimum-Maximum | 5.80 – 17.37 | |
| | Age category | | |
| | ≤ 12 years old | 620 | 49.3% |
| | > 12 years old | 637 | 50.7% |
| 3 | Grade of school | | |
| | 1 | 71 | 5.6% |
| | 2 | 78 | 6.2% |
| | 3 | 59 | 4.7% |
| | 4 | 138 | 11.0% |
| | 5 | 123 | 9.8% |
| | 6 | 131 | 10.4% |
| | 7 | 288 | 22.9% |
| | 8 | 133 | 10.6% |
| | 9 | 236 | 18.8% |
| 4 | Level | | |
| | Elementary | 600 | 47.7% |
| | Junior High School | 657 | 52.3% |
| 5 | District of School | | |
| | Denpasar | 418 | 33.3% |
| | Singaraja | 440 | 35.0% |
| | Karangasem | 399 | 31.7% |
| 6 | Origin | | |
| | Bali | 1217 | 96.8% |
| | Outside Bali | 40 | 3.2% |
| | Total | 1257 | 100% |

Table 2 is a distribution of subject based on their clinical conditions. The median of waist circumference obtained was 65 cm. Nutritional status of good nutrition was 38.8%, more nutrition was 12.6%, and obesity was 30.2%. Samples who experienced hypertension grade 1 were 22.2%, and grade 2 was 14.1% while samples who began to experience increased blood pressure were elevated in the amount of 18.5%.

**Table 2: Distribution of samples based on the clinical condition**

| No | Clinical condition (n = 1257) | Median (IQR) | Min-Max |
|---|---|---|---|
| 1 | Weight (kg) | 40.0 (21.0) | 15.0 – 105.0 |
| 2 | Height (cm) | 146.0 (21.5) | 104.0 – 245.0 |
| 3 | Waist circumference (cm) | 65.0 (17.0) | 40.0 – 118.0 |
| 4 | Body mass index (kg/m2) | 18.6 (6.3) | 10.5 –36.8 |
| 5 | Sistole (mmHg) | 120.0 (10.0) | 70.0 – 180.0 |
| 6 | Diastole (mmHg) | 70.0 (10.0) | 40.0 – 130.0) |
| 7 | Nutritional status | Frequency | Percentage |
| | Obesity | 379 | 30.2% |
| | More nutrition | 159 | 12.6% |
| | Good nutrition | 488 | 38.8% |
| | Less nutrition | 221 | 17.6% |
| | Malnutrition | 10 | 0.8% |
| 8 | Blood pressure | | |
| | Normal | 568 | 45.2% |
| | Elevated | 233 | 18.5% |
| | Hypertension grade 1 | 279 | 22.2% |
| | Hypertension grade 2 | 177 | 14.1% |
| 9 | Exclusive breastfeeding (n = 528) | | |
| | Yes | 379 | 71.8% |
| | No | 149 | 28.2% |
| 10 | Parental history of hypertension (n = 528) | | |
| | Yes | 46 | 8.7% |
| | No | 482 | 91.3% |
| 11 | Hospitalization history (n = 528) | | |
| | Yes | 60 | 11.4% |
| | No | 468 | 88.6% |

Historical data of exclusive breastfeeding, parental history of hypertension and hospitalisation history were not obtained 100% of the data because most parents did not remember.

**Table 3: Distribution of blood pressure based on sample characteristic**

| No | Characteristic | Blood Pressure | | | | |
|---|---|---|---|---|---|---|
| | | Normal | Elevated | Hyper-tension 1 | Hyper-tension 2 | Total |
| 1 | Sex | | | | | |
| | Male | 280 (44.2%) | 111 (17.5%) | 140 (22.1%) | 402 (16.1%) | 633 (100%) |
| | Female | 288 (46.2%) | 122 (19.6%) | 139 (22.3%) | 75 (12.0%) | 624 (100%) |
| 2 | Age | | | | | |
| | Frequency | 568 | 233 | 279 | 177 | 1257 |
| | Median (IQR) | 11.41 (3.43) | 13.61 (2.37) | 12.17 (2.58) | 11.37 (2.95) | 12.03 (3.40) |
| | Mean Rank | 560.45 | 847.01 | 651.19 | 527.03 | |
| | Age group | | | | | |
| | ≤ 12 years old | 327 (52.7%) | 57 (9.2%) | 127 (20.5%) | 109 (17.6%) | 620 (100%) |
| | > 12 years old | 241 (37.8%) | 176 (27.6%) | 152 (23.9%) | 68 (10.7%) | 637 (100%) |
| 3 | Level | | | | | |
| | Elementary | 322 (53.7%) | 55 (9.2%) | 119 (19.8%) | 104 (17.3%) | 600 (100%) |
| | Junior High School | 246 (37.4%) | 178 (27.1%) | 160 (24.4%) | 73 (11.1%) | 657 (100%) |
| 4 | Schools | | | | | |
| | Denpasar | 275 (65.8%) | 74 (17.7%) | 60 (14.4%) | 9 (2.2%) | 418 (100%) |
| | Singaraja | 108 (24.5%) | 49 (11.1%) | 145 (33.0%) | 138 (31.4%) | 440 (100%) |
| | Karangasem | 185 (46.4%) | 110 (27.6%) | 74 (18.5%) | 30 (7.5%) | 399 (100%) |
| 5 | Origin | | | | | |
| | Bali | 543 (44.6%) | 228 (18.7%) | 274 (22.5%) | 172 (14.1%) | 1217 (100%) |
| | Outside Bali | 25 (62.5%) | 5 (12.5%) | 5 (12.5%) | 5 (12.5%) | 40 (100%) |
| 6 | Nutritional status | | | | | |
| | Obesity | 163 (43.0%) | 63 (16.6%) | 87 (23.0%) | 66 (17.4%) | 379 (100%) |
| | More nutrition | 50 (31.4%) | 52 (32.7%) | 41 (25.8%) | 16 (10.1%) | 159 (100%) |
| | Good nutrition | 239 (49.0%) | 88 (18.0%) | 102 (20.9%) | 59 (12.1%) | 488 (100%) |
| | Less nutrition | 110 (49.8%) | 29 (13.1%) | 48 (21.7%) | 34 (15.4%) | 221 (100%) |
| | Malnutrition | 6 (60.0%) | 1 (10.0%) | 1 (10.0%) | 2 (20.0%) | 10 (100%) |
| 7 | Exclusive breastfeeding | | | | | |
| | Yes | 201 (53.0%) | 99 (26.1%) | 59 (15.6%) | 20 (5.3%) | 379 (100%) |
| | No | 86 (57.7%) | 31 (20.8%) | 25 (16.8%) | 7 (4.7%) | 149 (100%) |
| 8 | Parental history of hypertension | | | | | |
| | Yes | 18 (39.1%) | 15 (32.6%) | 8 (17.4%) | 5 (10.9%) | 46 (100%) |
| | No | 269 (55.8%) | 115 (23.9%) | 76 (15.8%) | 22 (4.6%) | 482 (100%) |
| 9 | History of hospitalised | | | | | |
| | Yes | 30 (50.0%) | 18 (30.0%) | 12 (20.0%) | 0 (0%) | 60 (100%) |
| | No | 257 (54.9%) | 112 (23.9%) | 72 (15.4%) | 27 (5.8%) | 468 (100%) |

The distribution of blood pressure based on sample characteristics can be seen in Table 4. Differences in the composition of subjects experiencing hypertension were found in the subject's composition in blood pressure with hypertension grade 2, which was 16.1% in male compared to 12% in female.

**Table 4: Data Distribution of Sistole and Diastole based on age and sex**

| Percentile | Sistole | | | | | | | Diastole | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 5% | 10% | 25% | 50% | 75% | 90% | 95% | 5% | 10% | 25% | 50% | 75% | 90% | 95% |
| Sex | | | | | | | | | | | | | | |
| Male | 90 | 100 | 110 | 120 | 125 | 130 | 140 | 60 | 60 | 70 | 80 | 80 | 90 | 90 |
| Female | 90 | 100 | 110 | 120 | 120 | 130 | 140 | 60 | 60 | 70 | 70 | 80 | 90 | 90 |
| Age | | | | | | | | | | | | | | |
| 6 | 90 | 98 | 110 | 120 | 120 | 130 | 136 | 54 | 68 | 70 | 70 | 80 | 90 | 90 |
| 7 | 93,5 | 100 | 110 | 120 | 130 | 133 | 140 | 60 | 60 | 70 | 80 | 80 | 90 | 96 |
| 8 | 100 | 100 | 110 | 120 | 120 | 140 | 140 | 60 | 60 | 70 | 70 | 80 | 90 | 100 |
| 9 | 100 | 100 | 110 | 120 | 120 | 130 | 130 | 60 | 60 | 70 | 70 | 80 | 90 | 90 |
| 10 | 100 | 100 | 110 | 110 | 130 | 130 | 140 | 60 | 70 | 70 | 70 | 80 | 90 | 90 |
| 11 | 90 | 90 | 100 | 110 | 120 | 130 | 140 | 50 | 60 | 60 | 70 | 80 | 90 | 90 |
| 12 | 90 | 100 | 100 | 110 | 120 | 130 | 130 | 50 | 60 | 60 | 70 | 80 | 80 | 90 |
| >=13 | 100 | 100 | 110 | 120 | 130 | 130 | 140 | 60 | 60 | 70 | 80 | 80 | 90 | 90 |

| Age | Male | | Female | |
|---|---|---|---|---|
| | Sistole (Mean ± SD) | Diastole (Mean ± SD) | Sistole (Mean ± SD) | Diastole (Mean ± SD) |
| 6 | 116,6 ± 12,9 | 76,0 ± 7,4 | 106,4 ± 18,6 | 67,9 ± 8,9 |
| 7 | 118,7 ± 15,1 | 77,3 ± 11,8 | 104,4 ± 16,7 | 68,1 ± 15,7 |
| 8 | 115,2 ± 11,5 | 74,5 ± 8,5 | 107,5 ± 16,9 | 67,1 ± 12,7 |
| 9 | 115,2 ± 13,0 | 72,7 ± 10,9 | 112,8 ± 16,4 | 71,2 ± 11,4 |
| 10 | 117,7 ± 15,2 | 77,1 ± 11,9 | 109,7 ± 14,4 | 70,4 ± 12,5 |
| 11 | 112,6 ± 17,8 | 71,6 ± 14,1 | 114,8 ± 13,2 | 72,7 ± 11,0 |
| 12 | 113,9 ± 12,0 | 72,8 ± 10,5 | 120,4 ± 12,0 | 78,3 ± 10,9 |
| >=13 | 117,6 ± 14,0 | 75,2 ± 11,1 | 118,4 ± 10,0 | 76,1 ± 8,2 |

The subject's distribution with hypertension grade 1 was the largest distribution (28.4%) of children who experienced increased blood pressure. Whereas the highest region experiencing increased blood pressure was Singaraja (75.5%). In obese children, 57% of children experienced increased blood pressure and 17.4% with grade 2 hypertension. While in children with more nutrition, 68.6% experienced increased blood pressure, with 10.1% with grade 2 hypertension.

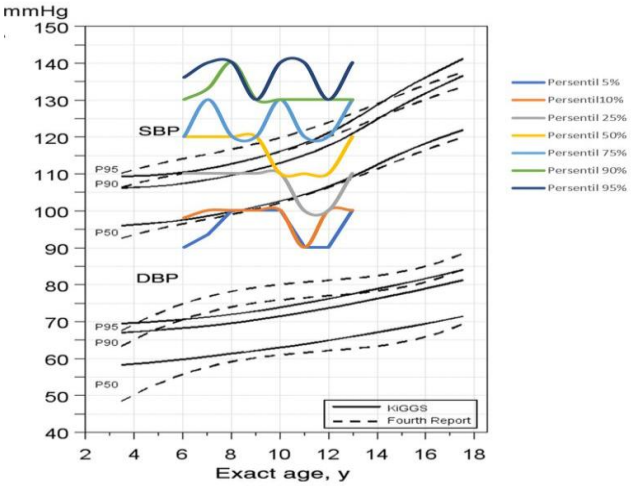



*Figure 1: Study result curve to KIGGS and Fourth Report curve based on systolic blood pressure*

Table 4 shows the data distribution of systole and diastole based on age and sex. The increase in age has a higher profile of mean systole and diastole. The male children have a higher profile of systole and diastole.

Figures 1 and 2 show study result curve to KIGGS and Fourth Report curve based on systolic and diastolic blood pressure, both curves show that this study has a different result than KIGGS and Fourth Report.

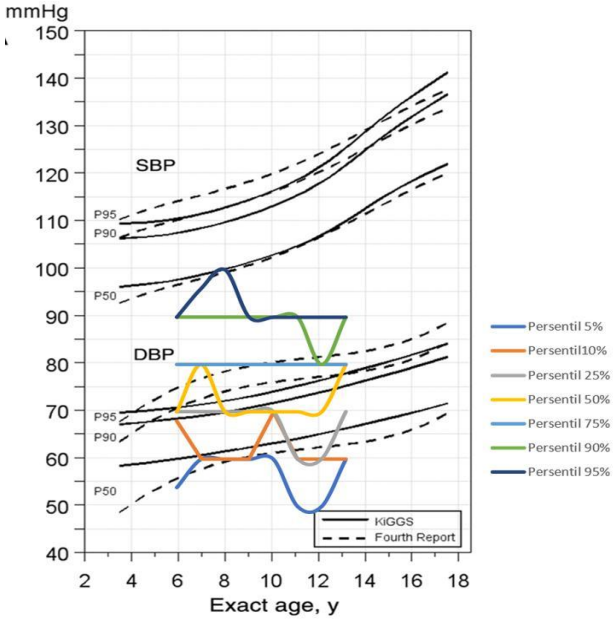



*Figure 2: Study result curve to KIGGS and Fourth Report curve based on diastolic blood pressure*

## Discussion

In this study, the prevalence of hypertension was 36.3%, in which hypertension grade 1 was 22.2%, and grade 2 was 14.1%. This prevalence resembled results obtained by Fuiano *et al.,* [9] in 2006 in Foggia-Italy, which found 35.1% of boys and 40.2% in girls aged 3 to 16 years. This result was higher than the prevalence of 24.2% in children aged 7 to 11 years obtained by Mohkam *et al.,* [10] in 2011 in Tehran-Iran, and also the prevalence of 20.6% in children aged 8 to 13 years obtained by Urrutia-Rojas, *et al.,* [11] in Fort Worth Texas-USA in 2006. This prevalence was also higher than study in Khartoum-Sudan in 2010 by Salman, *et al.,* [12] which found of 4.9% in children aged 6 to 12 years, prevalence in Sabinas Hidalgo-Mexico in 2009 by Aregullin-Eligio and Alcorta-Garza [13] of 4.9% in children aged 6 to with 12 years, and prevalence of 13.8% in children aged 5 to 17 years by Moore, *et al.,* [14] in Oklahoma-USA in 2009.

The differences in the prevalence of hypertension from various studies could be caused by several factors including the differences in the

definition of hypertension in each country, the difference of race or ethnicity, culture, lifestyle, type of food, the prevalence of obesity, and genetic factors of each. Other factors that affect the prevalence of hypertension were the differences in study design, methods of examination of blood pressure, age range, and the number of samples [15].

The prevalence of hypertension in this study was high, reaching 36.3%, this result was similar to the prevalence of hypertension in the adult population in Indonesia in 2007 by 31.7% [16] and much higher than expected by Falkner, *et al.,* [17] of 3 to 5% in children. The high prevalence of hypertension in this study was probably caused by the high prevalence of obesity in the population. This study found obesity was 30.2% and including the rural and urban area. This condition probably caused by all the school of the subject have red canteen category, there for the pattern of food habit at school is not healthy and the student stays at school 6-8 hours-per-day. Quality and quantity persuasive education are needed in the future to make correction of food habits on children at school.

Obesity in children continues to be on the rise, with a predicted increase of 40% in the next decade [18]. The National Health and Nutrition Examination Survey data showed that since 1976–1980, obesity has doubled among preschool children 2-5 years of age and tripled among children and adolescents 6-19 years of age [19], [20]. The most recent National Health and Nutrition Examination Survey data from 2007 to 2008 showed 10% of infants and toddlers < 2 years of age had a weight-for-height ≥ 95th percentile, and 17% of children aged 2 – 19 years had a weight-for-height ≥ 95th percentile [21], [22], [23]. In the USA, the prevalence of obesity was 17% from 2011 to 2014 in the pediatric population [24]. In Europe, an estimated prevalence of 20% of children and adolescents are overweight, with one-third of these being considered as obese [25]. Obesity, or increased BMI, can now be considered as a risk factor not just for cardiovascular disease and diabetes, but also for CKD [18], [19], [20], [21], [22], [23], [24], [25], [26]. The increase in CKD in those who are obese is thought to be in part due to increased metabolic demands which, in turn, lead to a compensatory glomerular hyperfiltration injury in the kidney [18], [19], [20], [21], [22], [23], [24], [25], [26], [27]. This is now termed obesity-related glomerulopathy, and it has been speculated that a decrease in the number of functional nephrons might also be implicated in the pathogenesis [27], [28]. Obesity can also lead to RAAS activation along with many other metabolic pathways leading to HTN and the metabolic syndrome [29], [30]**.**

Metabolic syndrome is defined by three metabolic abnormalities such as obesity, elevated BP, low high-density lipoprotein cholesterol, hypertriglyceridemia, and hyperglycemia. 68 The prevalence of metabolic syndrome in adolescents was 4.5% from 1994 to 2004, as reported in the US National Health and Nutrition Survey data. It is estimated from the CKiD study that 13% of children with CKD had metabolic syndrome [30], [31]. The strong relationship between metabolic syndrome and CKD has become increasingly identified [32], [33], [34]

The high prevalence of hypertension in primary school children required the attention to prevent in the short- and long-term complications of this disease. This result proved that the government needed to do early detection and intervention for hypertension in children in Bali. Also, the school could work together with the government health centre to perform blood pressure checks routinely for the students and provide education to parents. These results could also be basic data for a general practitioner or paediatrician to perform blood pressure checks routinely in pediatric patients.

This study also found a high prevalence of obesity in primary and junior high school children in Bali; the prevalence was 30.2%. Based on this study result and the results of previous studies, blood pressure checks should be done periodically in children with obesity to detect hypertension. Also, the more important thing was how the government, in this case, the health department, the school, and parents play a role to control obesity in children. Early detection Program of obesity in children, education about obesity and lifestyle risk for a parent, and parent awareness of the dangers of obesity would play a role in efforts to control the disease.

## References

1. Essouma M, Noubiap JJN, Bigna JJR, Nansseu JRN, Jinggi AM, Aminde LN, et al. Hypertension prevalence, incidence and risk factors among children and adolescents in Africa: a systematic review and metaanalysis protocol. BMJ Open. 2015; 5. https://doi.org/10.1136/bmjopen-2015-008472 PMid:26373403 PMCid:PMC4577976

2. Lewis MN, Shatat IF, Philips SM. Screening for hypertension in children and adolescents: methodology and current practice recommendations. Front. Pediatr. 2017; 5:51. https://doi.org/10.3389/fped.2017.00051 PMid:28361048 PMCid:PMC5350116

3. Meng L, Liang Y, Liu J, Hu Y, Yan Y, Mi J. Prevalence and risk factors of hypertension based on repeated measurements in Chinese children and adolescents. Blood Pressure. 2013; 22:57-64. https://doi.org/10.3109/08037051.2012.701790 PMid:22853559

4. Gupta-Malhotra M, Banker A, Shete S, Hashmi SS, Tyson JE, Barrat MS, et al. Essential hypertension vs. secondary hypertension among children. American Journal of Hypertension. 2015; 28(1). https://doi.org/10.1093/ajh/hpu083 PMid:24842390 PMCid:PMC4318949

5. Falkner B, Flynn JT, Gidding S, Green LA, Ingelfinger JR, Lauer RM, et al. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescent. USA: U.S. Department of Health and Human Service; 2005.

6. Flynn JT, Kaelber DC, Baker-Smith CM, Blowey D, Carroll AE, Daniels SR, et al. Clinical practice guideline for screening and

management of high blood pressure in children and adolescents. Pediatrics. 2017; 140(3). https://doi.org/10.1542/peds.2017-3035 PMid:29192011

7. Sastroasmoro S. Pemilihan Subyek Penelitian. Dalam: Sastroasmoro, S., Ismael, S., ed. Dasar-dasar metodologi penelitian klinis. 2008.

8. Sutawan IBR. Prevalensi dan hubungan hipertensi dengan obesitas anak dan riwayat hipertensi orang tua pada anak sekolah dasar di Bali. Thesis. 2015.

9. Fuiano N, Luciano A, Pilotto L, Pietrobelli A. Overweight and hypertension: longitudinal study in school-aged children. Minerva Pediatrica. 2006; 58: 451-459.

10. Mohkam M, Karimi A, Khatami NEA, Fallah F, Maham S, Gorji FJFA. Blood pressure screening in school-aged children in Tehran. Iranian Journal of Kidney Diseases. 2011; 5: 229-233.

11. Urrutia-Rojas X, Egbuchunam CU, Bae S, Menchaca J, Bayona M, Rivers PA, et al. High blood pressure in school children: prevalence and risk factors. BMC Pediatrics. 2006; 6. https://doi.org/10.1186/1471-2431-6-32 PMid:17109750 PMCid:PMC1657006

12. Salman Z, Kirk GD, DeBoer MD. High rate of obesity-associated hypertension among primary schoolchildren in Sudan. International Journal of Hypertension. 2010; 11: 1-5. https://doi.org/10.4061/2011/629492 PMid:21234364 PMCid:PMC3014717

13. Aregullin-Eligio EO, Alcorta-Garza MC. Prevalencia y factores de riesgo de hipertensión arterial en escolares Mexicanos. Salud Pública De México. 2009; 51: 14-18. https://doi.org/10.1590/S0036-36342009000100005 PMid:19180308

14. Moore WE, Eichner JE, Cohn EM, Thompson DM, Kobza CE, Abbott KE. Blood pressure screening of school children in a multiracial school district: the healthy kids project. American Journal of Hypertension. 2009; 22: 351-356. https://doi.org/10.1038/ajh.2009.13 PMid:19214168

15. Subhi MD. Blood pressure profiles and hypertension in iraqi primary school children. Saudi Med J. 2006; 27: 482-486.

16. Ministry of Health Republic of Indonesia. The 2007 Report of National Basic Health Survey. Jakarta: Research and Development Board Ministry of Health Republic of Indonesia; 2008.

17. Falkner B, Lurbe E, Schaefer F. High blood pressure in children: clinical and health policy implications. The Journal of Clinical Hypertension. 2010; 12:261-276. https://doi.org/10.1111/j.1751-7176.2009.00245.x PMid:20433547

18. Tkaczyk M, Nowicki M, BałaszChmielewska I, et al. Hypertension in dialysed children: the prevalence and therapeutic approach in Poland-a nationwide survey. Nephrol Dial Transplant. 2006; 21:736-742. https://doi.org/10.1093/ndt/gfi280 PMid:16303782

19. Kramer AM, van Stralen KJ, Jager KJ, et al. Demographics of blood pressure and hypertension in children on renal replacement therapy in Europe. Kidney Int. 2011; 80:1092-1098. https://doi.org/10.1038/ki.2011.232 PMid:21814180

20. Halbach SM, Martz K, Mattoo T, Flynn J. Predictors of blood pressure and its control in pediatric patients receiving dialysis. J Pediatr. 2012; 160:621- 625. https://doi.org/10.1016/j.jpeds.2011.09.046 PMid:22056352 PMCid:PMC3409690

21. Kaelber DC. IBM explorys cohort discovery tool, 2019. Available at: www.ibm. com/watson/health/explorys. Accessed March 3, 2019.

22. Barker DJ. The fetal and infant origins of adult disease. BMJ. 1990;301:1111. https://doi.org/10.1136/bmj.301.6761.1111 PMid:2252919 PMCid:PMC1664286

23. Edvardsson VO, Steinthorsdottir SD, Eliasdottir SB, Indridason OS, Palsson R. Birth weight and childhood blood pressure. Curr Hypertens Rep. 2012; 14:596-602. https://doi.org/10.1007/s11906-012-0311-6 PMid:23054892

24. Mhanna MJ, Iqbal AM, Kaelber DC. Weight gain and hypertension at three years of age and older in extremely low birth weight infants. J Neonatal Perinatal Med. 2015; 8:363-369. https://doi.org/10.3233/NPM-15814080 PMid:26836822

25. Di Salvo G, Castaldi B, Baldini L, et al. Masked hypertension in young patients after successful aortic coarctation repair: impact on left ventricular geometry and function. J Hum Hypertens. 2011; 25:739-745. https://doi.org/10.1038/jhh.2010.118 PMid:21228825

26. Falkner B, Gidding SS, Portman R, Rosner B. Blood pressure variability and classification of prehypertension and hypertension in adolescence. Pediatrics. 2008; 122:238-242. https://doi.org/10.1542/peds.2007-2776 PMid:18676538

27. Tracy RE, Newman WP III, Wattigney WA, Srinivasan SR, Strong JP, Berenson GS. Histologic features of atherosclerosis and hypertension from autopsies of young individuals in a defined geographic population: the Bogalusa Heart Study. Atherosclerosis. 1995; 116:163-179. https://doi.org/10.1016/0021-9150(95)05525-2

28. Urbina EM, Khoury PR, McCoy C, Daniels SR, Kimball TR, Dolan LM. Cardiac and vascular consequences of prehypertension in youth. J Clin Hypertens (Greenwich). 2011; 13:332-342. https://doi.org/10.1111/j.1751-7176.2011.00471.x PMid:21545394 PMCid:PMC3092159

29. de Simone G, Devereux RB, Daniels SR, Koren MJ, Meyer RA, Laragh JH. Effect of growth on variability of left ventricular mass: assessment of allometric signals in adults and children and their capacity to predict cardiovascular risk. J Am Coll Cardiol. 1995; 25:1056-1062. https://doi.org/10.1016/0735-1097(94)00540-7

30. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK Jr; Cardiovascular Health Study Collaborative Research Group. Carotidartery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. N Engl J Med. 1999; 340:14-22. https://doi.org/10.1056/NEJM199901073400103 PMid:9878640

31. Mitchell GF, Hwang SJ, Vasan RS, et al. Arterial stiffness and cardiovascular events: the Framingham Heart Study. Circulation. 2010; 121:505-511. https://doi.org/10.1161/CIRCULATIONAHA.109.886655 PMid:20083680 PMCid:PMC2836717

32. Lloyd-Jones DM, Hong Y, Labarthe D, et al; American Heart Association Strategic Planning Task Force and Statistics Committee. Defining and setting national goals for cardiovascular health promotion and disease reduction: the American Heart Association's strategic impact goal through 2020 and beyond. Circulation. 2010; 121:586-613. https://doi.org/10.1161/CIRCULATIONAHA.109.192703 PMid:20089546

33. Ning H, Labarthe DR, Shay CM, et al. Status of cardiovascular health in US children up to 11 years of age: the National Health and Nutrition Examination Surveys 2003-2010. Circ Cardiovasc Qual Outcomes. 2015; 8:164-171. https://doi.org/10.1161/CIRCOUTCOMES.114.001274 PMid:25782775

34. Steinberger J, Daniels SR, Hagberg N, et al; American Heart Association Atherosclerosis, Hypertension, and Obesity in the Young Committee of the Council on Cardiovascular Disease in the Young; Council on Cardiovascular and Stroke Nursing; Council on Epidemiology and Prevention; Council on Functional Genomics and Translational Biology; Stroke Council. Cardiovascular health promotion in children: challenges and opportunities for 2020 and beyond: a scientific statement from the American Heart Association. Circulation. 2016; 134:e236-e255.

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Jun 30; 7(12):1968-1973.
https://doi.org/10.3889/oamjms.2019.477
eISSN: 1857-9655
***Clinical Science***

# Assessment of Allergic Rhinitis among Children after Low-Level Laser Therapy

Yousry Moustafa[1], Hala G. El Nady[2], Maha M. Saber[3], Ola A. Dabbous[1], Terez Boshra Kamel[4], Khaled G. Abel-Wahhab[5], Sara F. Sallam[2*], Dina A. Zaki[2]

*[1]Department of Medical Applications of Laser, National Institute of Laser Enhanced Sciences (NILES), Cairo University, Cairo, Egypt; [2]Child Health Department, National Research Centre, Cairo, Egypt; [3]Complementary Medicine Department, National Research Centre, Cairo, Egypt; [4]Pediatric Hospitals, Ain Shams University, Cairo, Egypt; [5]Physiology Department, National Research Centre, Cairo, Egypt*

**Citation:** Moustafa Y, El Nady HG, Saber MM, Dabbous OA, Kamel TB, G. Abel-Wahhab KG, Sallam SF, Zaki DA. Assessment of Allergic Rhinitis among Children after Low-Level Laser Therapy. Open Access Maced J Med Sci. 2019 Jun 30; 7(12):1968-1973. https://doi.org/10.3889/oamjms.2019.477

**Keywords:** Allergic rhinitis (AR); C-reactive protein (CRP); National Institute of Laser Enhanced Sciences (NILES); 5-point scale (FPS); High-sensitivity-C-reactive protein (hs-CRP)

***Correspondence:** Sara F. Sallam. Child Health Department, National Research Centre, Cairo, Egypt. E-mail: lara26sara@yahoo.com

**Received:** 11-Mar-2019; **Revised:** 22-May-2019; **Accepted:** 23-May-2019; **Online first:** 30-Jun-2019

**Copyright:** © 2019 Yousry Moustafa, Hala G. El Nady, Maha M. Saber, Ola A. Dabbous, Terez Boshra Kamel, Khaled G. Abel-Wahhab, Sara F. Sallam, Dina A. Zaki. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

**Funding:** This research did not receive any financial support

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

**Abstract**

**BACKGROUND:** Allergic rhinitis (AR) represents one of the most common global health problems with seriously increasing incidence over the last decades. The goal of the treatment of rhinitis is to prevent or reduce the symptoms caused by the inflammation of affected tissues. Intranasal steroids and oral antihistamines are recommended as first lines of treatment. Acupuncture had reported a significant improvement in daily symptoms and an increase of symptom-free days in many studies enrolling adults' patients.

**AIM:** This study aimed to evaluate the laser acupuncture effect on the treatment of children AR in comparison to the effect of the medication and to assess the anti-inflammatory effect of laser acupuncture through measurement of serum inflammatory marker (hs-CRP).

**METHODS:** Sixty patients with allergic rhinitis their age group ranged from 4 to 18 years were divided randomly into two groups. Group 1 enrolled 30 patient that received AR in the form of intranasal steroids, antihistaminic, leukotriene antagonists while group2 thirty patients received 12 laser acupuncture sessions (2 sessions a \ week) on specific traditional Chinese acupuncture points.

**RESULTS:** There was a significant improvement in the severity score symptoms in both groups through and by the end of the study. High Significant improvement in the levels of the inflammatory marker in both groups, especially in the group which receive laser acupuncture sessions.

**CONCLUSION:** Laser acupuncture is a reliable, painless and non-invasive successful technique, which may be used as a complementary treatment for pediatric allergic rhinitis.

## Introduction

Childhood Allergic Rhinitis (AR) is a very common allergic condition which may pass undiagnosed or untreated [1]. It is the most frequent allergic disease in Western Europe that interferes with school attendance and performance, with a prevalence rate of 20-30% [2]. Very few studies of the epidemiology were done about the prevalence of allergic rhinitis in Egypt. In the Middle East, Egypt, the AR prevalence was 9 % [3], [4]. The management plan of AR is quite the same in both adult and children [5] including allergen avoidance, Pharmacotherapy, acupuncture, soft laser phototherapy and immune-therapy [6]. Oral H1 antihistamines and intranasal corticosteroids are the two major pharmacological agents used in the management of allergic rhinitis as monotherapy or in combination, depending on the predominant symptoms and the patient's response to therapy [7]. AR may also be associated with co-existing conditions as bronchial asthma, sinusitis, upper respiratory tract infection, otitis media with effusion and nasal polyposis, which will require further medications.

C-reactive protein (CRP) is an acute phase

reactant; it is produced by the liver and considers a highly conserved plasma protein which participates in the systemic response to inflammation. Its plasma concentration increases during inflammatory states and is a highly sensitive marker of inflammation, infection and tissue damage, which contributes to host defence against infection by activating the complement pathways [8]. High-sensitivity-C-reactive protein (hs-CRP) is a well-known systemic inflammatory marker that is easy and inexpensive to measure, together with ESR, serum Ig-E and total eosinophil count that measured as laboratory markers in the studied groups. According to the World Health Organization "WHO" reports, acupuncture was ranked among the efficient methods for the treatment of allergic rhinitis and other allergic conditions as bronchial asthma [9], [10]. Acupuncture has an immune-modulator therapeutic technique; and examined for its effect on both cellular and humoral components of the immune system [11], [12]. It can stimulate the release of β endorphin, which, coupled to the release of adrenocorticotrophic hormone (ACTH) that acts on the adrenal cortex to stimulate the release of cortisol, which is responsible for its anti-inflammatory effect [13]. Acupuncture involves the stimulation of specific points located along the lines of meridians corresponding to the flow of energy through the body [14]. Traditionally, these acupoints were stimulated using fine needles. Modern acupuncture has evolved to include the application of pressure, the use of electric current or the use of a low-intensity laser to stimulate these points [15]. Acupuncture has been reported to be beneficial in several clinical studies on allergic rhinitis, which reported a significant improvement in daily symptoms and an increase of symptoms-free days [16], [17].

So this study aimed to compare the effect of Low-Level Laser Therapy (LLLT) versus the effect of medications on symptoms of allergic rhinitis through a sample of Egyptian children.

## Patients and Methods

A prospective comparative study enrolling 60 children diagnosed as allergic rhinitis patients according to ARIA score [18], were recruited from the outpatient laser acupuncture clinic at Centre of Excellency at the National Research Centre and laser clinic at the National Institute of Laser Enhanced Sciences (NILES) of Cairo University.

Children were divided into 2 groups; Group 1was subjected to laser acupuncture therapy (LLLT) while Group 2 enrolling 30 children randomly assigned to the different medical treatments in the form of oral antihistaminic, nasal decongestant, intranasal corticosteroids (ICS) and leukotriene antagonist. Children with chronic inflammatory diseases, congenital malformations of the nasal cavity, acute infection, children receiving antibiotic therapy in the last month and parent refusal to join the study, all were excluded from this study.

After the approval of this study from the Ethical Committee of NILES and NRC, informed written consent was obtained from parents of all enrolled children after explaining the objectives of the current study and the possible side effects of the low-level laser therapy. A full Medical history was taken with special stress on the severity of symptoms and medications received in the last attack.

Symptoms were recorded on a 5-point scale (FPS) [19]. These five points are a 1-Nasal mucosal condition by anterior rhinoscopy. 2-Nasal obstruction 3-Nasal secretion 4-sneezing attacks 5-subjective estimation of therapeutic effect. Scoring system for the anterior rhinoscopy was recorded by the physician according to mucosal reddening and swelling of the inferior turbinate as score: 0 = normal, 1 = slightly changed and slight turbinate hypertrophy, 2 = moderately changed that partially obscuring the middle turbinate, 3 = severely changed and hypertrophy that completely obscures the middle turbinate while 4 = most severely changed. Subjective Symptom Scores (complaints) were determined retrospectively from the patients as follows,(nasal obstruction) congestion and (nasal secretion) rhinorrhea were evaluated using the following scale: 0 = free of symptoms, 1 = slight but noticeable symptoms, not interfering with daily activities, 2 = moderate symptoms, hardly interfering with daily activities and sleep, 3 = severe symptoms, clearly interfering with daily activities and sleep,and4 = most severe symptoms, substantially interfering with daily activities and sleep. Then sneezing attacks were classified into three categories: 0 = no sneezing attacks, 1 = rare sneezing attacks, (1-2 sneezing attacks per day), and 2 = frequent sneezing attacks with more than three attacks per day. Finally, Subjective Estimation of the Therapeutic Effect was recorded as 1 = improved and 2 = unchanged or worsened.

Low-level laser acupuncture sessions were used among group 1 children as follows: The laser applied was diode laser Model "Medical-Italia LIS 1050" (made in Italy) which emits laser at 904 nm wavelength and output power of 100 mw. Each acupuncture point of rhinitis was stimulated for 12 sessions (2 sessions weekly) with calculated energy according to the World Association of Laser Therapy. Both patients and doctor used protective goggles. Technical parameters applied are shown in Table 1.

**Table 1: Technical parameters of laser applied**

| Irradiation parameter | Unit of measurement |
|---|---|
| Energy | 6J |
| Power | 100 mw |
| Beam spot size | 1 $cm^2$ |
| Irradiation time | 1 min. per point |
| Treatment interval | 2 sessions per week |

Nasal mucosal condition by anterior rhinoscopy was done by the otolaryngologist, and its score was sent to the paediatrician who recorded the other subjective points of the 5-point scale (FPS) to avoid any bias. Any child that miss 2 successive laser sessions was excluded from the study and replaced by another until we reached the target number aimed in this study.

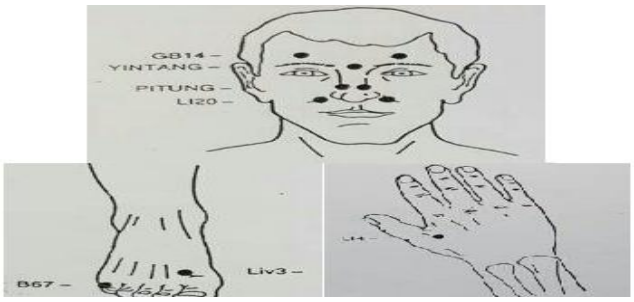



*Figure 1: Acupuncture stimulated points [6], [21]*

To allow standardisation and comparability, all patients were acupunctured at the same points which were chosen by the rules of TCM. Laser acupuncture was inserted bilaterally at the following points: LI 4**,** LI 20 (on the large intestine meridian), GB14, GB 20 on the gall bladder meridian, YIN TANG (at the root of the nose midway between the inner ends of the eyebrow), PITUNG (at the top of the groove above the, nostril), liv3, B67 and EX-HN5) [20], [21]. Reassessment of these patients was done by the end of 12$^{th}$ Low-Level Laser acupuncture session.

Group 2, patients diagnosed as allergic rhinitis and receiving medication were examined as a control group. Medication used was in the form of oral antihistaminic (once daily), intranasal steroids (twice daily), leukotriene antagonist (once daily at night). Both groups gave two blood samples for laboratory investigations that included: ESR, Eosinophil count, Ig-E and hs-CRP.

### *Statistical Methods*

Data were analysed using Med Calc© version 18.2.1 (Med Calc© Software, Ostend, Belgium). Normally distributed numerical variables were presented as mean ± SD and inter-group differences were compared using the unpaired t-test. Nominal variables were presented as number and percentage and differences were compared using the Pearson chi-squared test or Fisher's exact test. Ordinal data were compared using the chi-squared test for trend. Two-sided p-value < 0.05 was considered statistically significant.

## Results

A total of 36 male and 24 female children diagnosed as allergic rhinitis patient were enrolled in this study. Children were grouped according to management regimen into laser acupuncture therapy (group 1) children and Medication therapy (group 2) children, characteristics of each group are shown in Table 2. There were no significant differences between the study groups concerning sex ratio, type of AR, bronchial asthma association or family history of AR.

**Table 2: Characteristics of patients in both study groups**

| Variable | Laser acupuncture therapy (n = 30) | Medication therapy (n = 30) | t(58)/$\chi^2$(1) | p-value* |
|---|---|---|---|---|
| Age in years (mean ± SD) | 8.2 ± 3.3 | 10.2 ± 3.9 | -2.183 | 0.033# |
| Gender | | | | |
| Male | 18 (60.0%) | 18 (60.0%) | 0.000 | 1.000 |
| Female | 12 (40.0%) | 12 (40.0%) | | |
| Type of AR | | 21(70% | | |
| Seasonal | 18 (60%) | 9(30%) | 0.659 | 0.417# |
| Perennial | 12(40%) | | | |
| Associated asthma | | | | |
| AR only | 14 (46.7%) | 15 (50.0%) | 0.067 | 0.796 |
| AR + BA | 16 (53.3%) | 15 (50.0%) | | |
| Family history of AR | | | | |
| *Negative* | 9 (30.0%) | 13 (43.3%) | 1.148 | 0.284 |
| *Positive* | 21 (70.0%) | 17 (56.7%) | | |

Data are mean ± SD or number (%); *Pearson chi-squared test unless otherwise specified; #Unpaired t-test.

As present in Table 3 at the end of laser acupuncture sessions, 20% of group 1 cases become symptom-free of nasal rhinorrhea and secretion; meanwhile, 60% of them reported no sneezing attacks. On the other hand, no more severe nasal rhinorrhea and secretion was found in this group, and only 2 cases stated sneezing attacks after completing the sessions.

**Table 3: Clinical scores of studied groups before and after treatment**

| | Group1=Laser acupuncture therapy (n = 30) | | Group2=Medication therapy (n = 30) | | $\chi^2$ | p-value |
|---|---|---|---|---|---|---|
| | Before laser acupuncture | After laser acupuncture | Before treatment | After treatment | | |
| Rhinorhea | 0 (0) | 6 (20%) | 1 (3.3%) | 5 (16.7) | 0.734* | 0.392* |
| Free | 6 (20) | 12 (40%) | 9 (30%) | 7 (23.3) | | |
| Slight | 8 (26.7) | 8 (26.7%) | 6 (20%) | 9 (30) | | |
| Moderate | 12 (40) | 4 (13.3%) | 10 (33.3%) | 8 (26.7) | 2.486** | 0.115** |
| Severe | 4 (13.3) | 0 (0%) | 4(13.3%) | 1 (3.3) | | |
| Most severe Sneezing | | | | | | |
| NO | 7 (23.3%) | *18 (60%)* | 4 (13.3%) | 11 (36.7%) | 0.153* | 0.696* |
| Rare = (1-2 attack\day) | 13 (43.3%) | 10 (33.3%) | 21 (70.0%) | 17 (56.7%) | | |
| Frequent = > 3 attacks \day | 10 (33.3%) | 2 (6.7%) | 5 (16.7%) | 2 (6.7%) | 2.134** | 0.144** |

*comparing 2 studied groups before treatment; ** Comparing 2 studied groups after treatment.

According to the level of hs-CRP, there were non-significant differences between the studied groups at the beginning of the study while after treatment a considerable statistically significant decrease occurs in laser group hs -CRP level than

group 2 p < 0.0001 (Table 4).

**Table 4: Comparison of the rhinorrhea-congestion score in laser received group before and after laser acupuncture therapy**

| | Rhinorrhea-congestion score after laser acupuncture | | | | | |
|---|---|---|---|---|---|---|
| Rhinorrhea-congestion score before laser acupuncture | Slight to moderate | Severe to most severe | Total | Difference | 95% CI | Significance |
| Slight to moderate | 14 | 0 | 14 (46.7%) | -40.00% | -57.53 to -22.47 | P = 0.0005 |
| Severe and most severe | 12 | 4 | 16 (53.3%) | | | |
| Total | 26 (86.70%) | 4 (13.30%) | 30 | | | |

McNemar test.

As regards the levels of laboratory markers after treatment in both studied groups, there were highly statistically significant differences in the level of hs CRP (p < 0.05) between 2 groups, but there was no statistically significant difference in other laboratory parameters as shown in Table 6.

**Table 5: Comparison of the sneezing score in laser received group before and after laser acupuncture therapy**

| | Sneezing score after laser therapy | | | | | |
|---|---|---|---|---|---|---|
| Sneezing score before laser therapy | No/Rare attacks | Frequent attacks | Total | Difference | 95% CI | Significance |
| No/Rare attacks | 20 | 0 | 20 (66.7%) | -26.67% | -42.49 to -10.84 | P = 0.0078 |
| Frequent attacks | 8 | 2 | 10 (33.3%) | | | |
| Total | 28 (93.30%) | 2 (6.70%) | 30 | | | |

McNemar test.

Table 7 absolute decrease in laser group before and after the sessions, a statistically significant difference after laser acupuncture therapy in the objective scores including ESR level, Ig E, eosinophil count and the level of hs – CRP as regards the absolute decrease in their levels among children receiving laser acupuncture.

**Table 6: Laboratory assays in both studied group**

| | Group1 Mean ± SD | | Group2 Mean ± SD | | Difference | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| | Before laser acupuncture | After laser acupuncture | Before treatment | After treatment | | | |
| ESR (mm/h) | 12.8 ± 8.2 | 7.0 ± 6.7 | 7.2 ± 4.4 | 5.0 ± 2.6 | -5.6* | -9.0 to -2.2* | 0.002* |
| | | | | | -1.9** | -4.5 to 0.7** | 0.145** |
| IgE (IU/ml) | 135.2 ± 209.9 | 93.9 ± 157.2 | 83.1 ± 93.6 | 72.8 ± 85.2 | -52.1* | -136.1 to 31.9* | 0.219* |
| | | | | | -21.1** | -86.5 to 44.3** | 0.521** |
| Eosinophil count (%) | 4.5 ± 2.4 | 2.5 ± 1.5 | 3.7 ± 2.3 | 3.1 ± 2.2 | -0.7* | -1.9 to 0.5* | 0.229* |
| | | | | | 0.6 | -0.3 to 1.6** | 0.196** |
| hsCRP (ng/ml) | 598.4 ± 349 | 292.5 ± 231.4 | 609.8 ± 263.9 | 580.1 ± 262.1 | 11.4 | -148.6 to 171.4* | 0.887* |
| | | | | | 287.7 | 159.9 to 415.4** | < 0.0001** |

*Before sessions or treatment; **After sessions or treatment.

## Discussion

The International Study of Asthma and Allergies in Childhood (ISAAC) phase three studies (1999 – 2004) revealed an average prevalence of rhinitis of 8.5% (range 1.8 – 20.4%) in 6 to 7-year-old children and 14.6% (1.4 – 33.3%) for 13 to14 years old children [22].

**Table 7: Absolute decrease in the levels of markers of inflammation after treatment in laser received groups**

| Variable | Before laser acupuncture Median | IQR | After laser acupuncture Median | IQR | Z | p-value* |
|---|---|---|---|---|---|---|
| ESR (mm/h) | 11 | 7-15 | 7 | 3-8 | -4.629 | < 0.001 |
| IgE (IU/ml) | 44.4 | 10.0-208.0 | 28.4 | 5.4-180.0 | -4.361 | < 0.001 |
| Eosinophil count (%) | 4.2 | 2.1-6.3 | 2.1 | 1.3-3.0 | -4.753 | < 0.001 |
| hsCRP (ng/ml) | 626 | 298-915 | 228 | 115-472 | -3.775 | < 0.001 |

Data are median and interquartile range (IQR); IQR = interquartile range; Z = Z statistic; *Wilcoxon signed ranks test.

Untreated, AR can predispose to adverse consequences as rhino-sinusitis, otitis media up to secondary hearing impairment, which in turn may affect sleep, cognitive and school performance of young children. Achieving adequate symptom control is pivotal to successful AR management. Numerous management options exist for AR; Laser acupuncture provides new non-invasive treatment options in children [23] excluding infection risk caused by needle prick injuries [24].

Asthma frequently co-exists with AR being seen in half to three quarters of children and teenagers with asthma in a range of studies [25]. Allergic rhinitis, but not non-allergic rhinitis, in early childhood, is a risk factor for developing asthma in later childhood and adulthood [26], [27].

Lasmar et al., a study which included 126 asthmatic children and adolescents showed that the prevalence of AR was high in combination with asthma severity, constituted the major risk factor for emergency care attendance [28]. Also, Burgess et al. proved that the occurrence rate of asthma in hyper-responsiveness rhinitis children patients is 2 to 7 times higher than that of common people [29].

In this study 65% of cases were diagnosed as seasonal allergic rhinitis with no significant difference in the type classification between the studied groups, this finding differs from most epidemiologic data that found the prevalence of seasonal AR widely ranges from 1 to 40% [30]. Our study is similar to the study of Crown et al., in which Seventy-nine per cent of the total study sample (80,534 allergy patients) was classified as SAR and 21% as PAR [31].

As regards to AR symptoms, nasal secretion (rhinorrhea) and sneezing attacks were the most frequent complaint in the enrolled children. After the laser acupuncture sessions, 20% of children recorded rhinorrhea free period while 40% mentioned slight attacks of rhinorrhea. This improvement although not significantly differ from group 2 (children using traditional medication), it goes with high quality randomised, sham-controlled trials by Xue et al., which used a total of 16 acupuncture sessions, twice a week for 8 weeks and observed greater decrease rhinorrhea with acupuncture [32]. In the same aspect sneezing attacks disappeared in 60% of children and become rare in about 33% of this group (laser acupuncture – group 1) by the end of their sessions, this is similar to what Zhang et al., mentioned about small but statistically significant improvement in sneezing and quality of life in the acupressure group

after eight weeks therapy, performed in a multicentre randomized controlled trial in which subjects of this group were treated weekly for 5 to 10 in a clinic and perform three times daily therapy at home using a pacifier, for a 10 seconds session [33]. Allergic rhinitis in early childhood is mainly an Ig E-mediated reaction to various allergens in the nasal mucosa while sensitisation to indoor allergens in children less than 2 years old or outdoor allergens in children older than 4-6 years. In this study, as regards the laser acupuncture group, there was a significant decrease in the serum Ig-E level" after the 12 laser acupuncture sessions" ($p < 0.001$). Low-level laser acupuncture involves the application of photic energy to acupuncture points with the objective of augmentation of the normal healing process and pain relief. The usual wavelengths of lasers, which most commonly used in acupuncture are those that penetrate most deeply due to low absorption in the principal constituent in soft tissues and water [34].

Low-level laser photic energy shortens the inflammatory phase, accelerating the repair process, and remodelling after tissue injury [35]. A study found that there are photoreceptors at the molecular level that, when triggered; activate several biological reactions such as DNA/RNA synthesis, increased cAMP levels, protein and collagen synthesis, and cellular proliferation. The result is rapid regeneration, normalisation, and healing of damaged cellular tissue. Thus, light is a trigger for the rearrangement of cellular metabolism [36].

In this study, thirty patients (group 1) were acupunctured at the same points which were chosen in accordance to the rules of traditional Chinese medicine (TCM) twelve sessions of Laser acupuncture was performed 2 sessions per week according to a meta-analysis that expressed as acupuncture was safe and valid for the treatment of allergic rhinitis[37].

Our study goes on accordance to Jung et al. study which suggests an anti-allergic effect as the underlying mechanism of acupuncture in treating allergic rhinitis, based on reducing the expression of substance P, STAT6, NFkB, and iNOS from studying mice models [38]. More studies were advised in this aspect, in both animal and human models [39]. In a double-blind, randomised study, it was found that there was 70% improvement of clinical symptoms of allergic rhinitis after intranasal illumination by low-energy narrow-band phototherapy at 660 nm 3 times a day for 14 consecutive days [40], [41]. High sensitive CRP (Hs CRP) can be used to assess the grade of inflammation in allergic patients, as inflammation is one of the major characteristics of respiratory allergic diseases [42]. In this study, hs-CRP decreased in both groups after treatment, but the laser group children showed lower hs-CRP levels compared by group 2 medication group children. In the same aspect laser group children showed a statistically significant decrease in their serum hs-CRP levels after the laser acupuncture sessions. Other study results demonstrated that children with respiratory allergic diseases had greater concentrations of hs-CRP in serum as compared with healthy children [8].

In conclusion, the application of laser acupuncture may be used as an alternative treatment for pediatric allergic rhinitis. Further studies should be done with a bigger sample size to confirm the effectiveness of laser acupuncture as an alternative treatment in childhood allergic rhinitis, especially incompliant patient to conventional medical therapy.

## References


1. Choi SM, Park JE, Li SS, Jung H, Zi M, Kim TH, Jung S, Kim A, Shin M, Sul JU, Hong Z. A multicentre, randomized, controlled trial testing the effects of acupuncture on allergic rhinitis. Allergy. 2013; 68(3):365-74. https://doi.org/10.1111/all.12053 PMid:23253122

2. Bauchau V, Durham SR. Prevalence and rate of diagnosis of allergic rhinitis in Europe. European Respiratory Journal. 2004; 24(5):758-64. https://doi.org/10.1183/09031936.04.00013904 PMid:15516669

3. The World Bank. Egypt overview. 2005.

4. Abdulrahman H, Hadi U, Tarraf H, Gharagozlou M, Kamel M, Soliman A, Hamad WA, Hanna KM, Mostafa BE, Omrani M, Abdelmotal A. Nasal allergies in the Middle Eastern population: results from the "Allergies in Middle East Survey". American journal of rhinology & allergy. 2012; 26(6_suppl):S3-23. https://doi.org/10.2500/ajra.2012.26.3836

5. Pittsburgh PA. Advances in allergic disease: an update for the new millennium. J Allergy Clin Immunol. 2000; 105:S616-S621. https://doi.org/10.1067/mai.2000.106152

6. Moustafa Y, Kassab AN, el Sharnoubi J, Yehia H. Comparative study in the management of allergic rhinitis in children using LED phototherapy and laser acupuncture. International Journal of Pediatric Otorhinolaryngology. 2013; 77(5):658-65. https://doi.org/10.1016/j.ijporl.2013.01.006 PMid:23394792

7. Rosenwasser M. Intranasal corticosteroids in treatment of pediatric allergic rhinitis. Am J Med. 2002; 113(9A):175-245. https://doi.org/10.1016/S0002-9343(02)01433-X

8. Garba BI, Ibrahim M, Johnson AW, Yahaya IA. Plasma high sensitivity C-reactive protein level in children with asthma seen at Aminu Kano teaching hospital, Kano. Sub-Saharan African Journal of Medicine. 2014; 1(3):148-52. https://doi.org/10.4103/2384-5147.138948

9. World Health Organization. Acupuncture: review and analysis of reports on controlled clinical trials. World Health Organization; 2002.

10. Xue CC, English R, Zhang JJ, Da Costa C, Li CG. Effect of acupuncture in the treatment of seasonal allergic rhinitis: a randomized controlled clinical trial. The American journal of Chinese medicine. 2002; 30(01):1-1. https://doi.org/10.1142/S0192415X0200020X PMid:12067084

11. Joos S, Schott C, Zou H, Daniel V, Martin E. Immunomodulatory effects of acupuncture in the treatment of allergic asthma: a randomized controlled study. The Journal of Alternative and Complementary Medicine. 2000; 6(6):519-25. https://doi.org/10.1089/acm.2000.6.519 PMid:11152056

12. Hauswald B, Dill C, Boxberger J, Kuhlisch E, Zahnert T, Yarin YM. The effectiveness of acupuncture compared to loratadine in patients allergic to house dust mites. Journal of allergy. 2014; 2014. https://doi.org/10.1155/2014/654632 PMid:24995021

PMCid:PMC4068098

13. Xue CC, et al. Acupuncture safe, effective in treating seasonal allergic rhinitis. Ann Allergy Asthma Immunol. 2015.

14. Kim JI, Lee MS, Jung SY, Choi JY, Lee S, Ko JM, Zhao H, Zhao J, Kim AR, Shin MS, Kang KW. Acupuncture for persistent allergic rhinitis: a multi-centre, randomised, controlled trial protocol. Trials. 2009; 10(1):54. https://doi.org/10.1186/1745-6215-10-54 PMid:19602250 PMCid:PMC2715403

15. Stux G, Berman B, Pomeranz B. Basic of Acupuncture. 5th ed., Springer: London, UK, 2003. https://doi.org/10.1007/978-3-642-18988-3

16. Scheewe S, Vogt L, Minakawa S, Eichmann D, Welle S, Stachow R, Banzer W. Acupuncture in children and adolescents with bronchial asthma: a randomised controlled study. Complementary therapies in medicine. 2011; 19(5):239-46. https://doi.org/10.1016/j.ctim.2011.07.002 PMid:21944653

17. Pijnenburg MW, Baraldi E, Brand PLP, et al. Monitoring asthma in children. Eur Respir J. 2015; 45:906-925. https://doi.org/10.1183/09031936.00088814 PMid:25745042

18. ARIA score, 2008.

19. Hauswald B, Dill C, Boxberger J, Kuhlisch E, Zahnert T, Yarin YM. The effectiveness of acupuncture compared to loratadine in patients allergic to house dust mites. Journal of allergy. 2014; 2014. https://doi.org/10.1155/2014/654632 PMid:24995021 PMCid:PMC4068098

20. Maa SH, Sun MF, Hsu KH, Hung TJ, Chen HC, Yu CT, Wang CH, Lin HC. Effect of acupuncture or acupressure on quality of life of patients with chronic obstructive asthma: a pilot study. The Journal of Alternative & Complementary Medicine. 2003; 9(5):659-70. https://doi.org/10.1089/107555303322524517 PMid:14629844

21. Ola A. Dabbous, Mona M. Soliman, Nagwa H. Mohamed, Magda Y. Elseify, Mai S. Elsheikh, Asmaa A. A. Alsharkawy, Manal M. Abd al Aziz. Evaluation of the improvement effect of laser acupuncture biostimulation in asthmatic children by exhaled inflammatory biomarker level of nitric oxide. Lasers Med Sci. 2017; 32(1):53-59. https://doi.org/10.1007/s10103-016-2082-9 PMid:27709308

22. Ant K, Pearce N, Anderson HR, Ellwood P, Montefort S, Shah J. Global map of the prevalence of symptoms of rhinoconjunctivitis in children: The International Study of Asthma and Allergies in Childhood ISAAC Phase Three. Allergy. 2009; 64:123-148. https://doi.org/10.1111/j.1398-9995.2008.01884.x PMid:19132975

23. Raith W, Litscher G, Müller W, Urlesberger B. Laseracupuncture-a possible alternative treatment for agitation and pain in neonates?. Pediatric Anesthesia. 2013; 23(2):205-6. https://doi.org/10.1111/pan.12089 PMid:23289778

24. Jindal V, Ge A, Mansky PJ. Safety and efficacy of acupuncture in children a review of the evidence. Journal of pediatric hematology/oncology. 2008; 30(6):431. https://doi.org/10.1097/MPH.0b013e318165b2cc PMid:18525459 PMCid:PMC2518962

25. Kurukulaaratchy RJ, Raza A, Scott M, Williams P, Ewart S, Matthews S, Roberts G, Arshad SH. Characterisation of asthma that develops during adolescence; findings from the Isle of Wight Birth Cohort. Respiratory medicine. 2012; 106(3):329-37. https://doi.org/10.1016/j.rmed.2011.12.006 PMid:22212639 PMCid:PMC3260349

26. Rochat MK, Illi S, Ege MJ, Lau S, Keil T, Wahn U, von Mutius E. Allergic rhinitis as a predictor for wheezing onset in school-aged children. Journal of Allergy and Clinical Immunology. 2010; 126(6):1170-5. https://doi.org/10.1016/j.jaci.2010.09.008 PMid:21051078

27. Burgess JA, Walters EH, Byrnes GB, Matheson MC, Jenkins MA, Wharton CL, Johns DP, Abramson MJ, Hopper JL, Dharmage SC. Childhood allergic rhinitis predicts asthma incidence and persistence to middle age: a longitudinal study. Journal of Allergy and Clinical Immunology. 2007; 120(4):863-9. https://doi.org/10.1016/j.jaci.2007.07.020 PMid:17825896

28. Lasmar L, Camargos P, Ordones A, Gaspar G, Campos E, Ribeiro G. Prevalence of allergic rhinitis and its impact on the use of emergency care services in a group of children and adolescents with moderate to severe persistent asthma. J Pediatr. 2007; 83:555-561. https://doi.org/10.2223/JPED.1727 PMid:18074061

29. Burgess JA, Walters EH, Byrnes GB, Matheson MC, Jenkins MA, Wharton CL, Johns DP, Abramson MJ, Hopper JL, Dharmage SC. Childhood allergic rhinitis predicts asthma incidence and persistence to middle age: a longitudinal study. Journal of Allergy and Clinical Immunology. 2007; 120(4):863-9. https://doi.org/10.1016/j.jaci.2007.07.020 PMid:17825896

30. Bousquet J, Khaltaev N, Cruz AA, et al. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008. Allergy. 2008; 63(86):8-160. https://doi.org/10.1111/j.1398-9995.2007.01620.x PMid:18331513

31. Crown WH, Olufade A, Smith MW, Nathan R. Seasonal versus perennial allergic rhinitis: drug and medical resource use patterns. Value in health. 2003 Jul 1;6(4):448-56. https://doi.org/10.1046/j.1524-4733.2003.64231.x PMid:12859586

32. Xue CC, An X, Cheung TP, Da Costa C, Lenon GB, Thien FC, Story DF. Acupuncture for persistent allergic rhinitis: a randomised, sham-controlled trial. Medical Journal of Australia. 2007; 187(6):337-41.

33. Zhang CS, Xia J, Zhang AL, Yang AW, Thien F, Li Y, Wu D, Cai J, DaCosta C, Xue CC. Ear acupressure for perennial allergic rhinitis: A multicentre randomized controlled trial. American journal of rhinology & allergy. 2014; 28(4):e152-7. https://doi.org/10.2500/ajra.2014.28.4081 PMid:25197908

34. Avram MR, Friedman PM. Laser and Light Source Treatments for the Skin. JP Medical Ltd; 2014.

35. Sutherland JC. Biological effects of polychromatic light. Photochem Photobiol. 2002; 76:164-70. https://doi.org/10.1562/0031-8655(2002)076<0164:BEOPL>2.0.CO;2

36. Karu TI. The science of low-power laser therapy. Gordon & Breach Science Publishers; 1998.

37. Feng S, Han M, Fan Y, Yang G, Liao Z, et al. Acupuncture for the Treatment of Allergic Rhinitis: A Systematic Review and Meta-Analysis. American Journal of Rhinology & Allergy. 2015; 29:57-62. https://doi.org/10.2500/ajra.2015.29.4116 PMid:25590322

38. Jung D, Lee S, Hong S. Effects of acupuncture and moxibustion in a mouse model of allergic rhinitis. Otolaryngol Head Neck Surg. 2012; 146(1):19-25. https://doi.org/10.1177/0194599811421736 PMid:22020787

39. Pfab F, Schalock PC, Napadow V, Athanasiadis GI, Huss-Marp J, Ring J. Acupuncture for allergic disease therapy-the current state of evidence. Expert review of clinical immunology. 2014; 10(7):831-41. https://doi.org/10.1586/1744666X.2014.924855 PMid:24881629

40. Neuman I, Finkelstein Y. Narrow-band red light phototherapy in perennial allergic rhinitis and nasal polyposis. Ann Allergy Asthma Immunol. 1997; 78(4):399-405. https://doi.org/10.1016/S1081-1206(10)63202-4

41. Emberlin JC, Lewis RA. Pollen challenge study of a phototherapy device for reducing the symptoms of hay fever. Current medical research and opinion. 2009; 25(7):1635-44. https://doi.org/10.1185/03007990903024699 PMid:19476405

42. Kadakal F, Aras G, Kanmaz D, Purisa S, Uzumcu M, Iatk E, Bratfalean D. The assessment of high sensitivity C-reactive protein as a systemic marker in moderate asthma patients and changing levels by inhaled corticosteroids. JPMA. The Journal of the Pakistan Medical Association. 2013; 63(7):893-8.

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Jun 30; 7(12):1974-1978.
https://doi.org/10.3889/oamjms.2019.556
eISSN: 1857-9655
***Clinical Science***

# Role of Perioperative Pregabalin in the Management of Acute and Chronic Post-Thoracotomy Pain

Sara Gaber[1*], Emad Saleh[2], Somaya Elshaikh[2], Rafaat Reyad[2], Mohamed Elramly[2], Ismail Mourad[2], Mohamed Abdel Fattah[2]

*[1]National Heart Institute, Cairo, Egypt; [2]National Cancer Institute, Cairo University, Cairo, Egypt*

**Citation:** Gaber S, Saleh E, Elshaikh S, Reyad R, Elramly M, Mourad I, Fattah MA. Role of Perioperative Pregabalin in the Management of Acute and Chronic Post-Thoracotomy Pain. Open Access Maced J Med Sci. 2019 Jun 30; 7(12):1974-1978. https://doi.org/10.3889/oamjms.2019.556

**Keywords:** Pregabalin; Post-thoracotomy pain syndrome; Thoracic tumour; Neuropathic pain; Thoracotomy

***Correspondence:** Sara Gaber. National Heart Institute, Cairo, Egypt. E-mail: sara.gaber@hotmail.co.uk

**Received:** 14-Apr-2019; **Revised:** 19-Jun-2019; **Accepted:** 20-Jun-2019; **Online first:** 30-Jun-2019

**Copyright:** © 2019 Sara Gaber, Emad Saleh, Somaya Elshaikh, Rafaat Reyad, Mohamed Elramly, Ismail Mourad, Mohamed Abdel Fattah. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

**Funding:** This research did not receive any financial support

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

**Abstract**

**BACKGROUND:** Post-thoracotomy pain syndrome (PTPS) can be challenging to treat.

**AIM:** This study aimed to evaluate the efficacy of perioperative pregabalin in the prevention of acute and chronic post-thoracotomy pain.

**METHODS:** Sixty patients scheduled for thoracotomy for oncologic surgeries were randomly allocated to one of two groups; Pregabalin and Control. In the Pregabalin group, pregabalin 150 mg was administered one hour before thoracotomy and 12 hours later, then every 12 hours for five days. Pain intensity was assessed using the Visual Analogue Scale (VAS) at rest (VAS-R) and dynamic (VAS-D) in the ICU and during the next four days. Morphine consumption and the frequency of side effects were recorded. Assessment of PTPS was done using the Leeds Assessment of Neuropathic Symptoms and Signs (LANSS) scale at 1, 2, and 3 months.

**RESULTS:** The VAS-R and VAS-D scores and the total morphine consumption were significantly lower in Pregabalin group during days 0 through 4. Neuropathic pain, allodynia, and hyperalgesia were significantly lower in Pregabalin group after 1, 2, and 3 months.

**CONCLUSION:** Pregabalin is effective in the reduction of chronic neuropathic pain at 1, 2, and 3 months after thoracotomy and it also reduces pain and opioid consumption during the acute postoperative period with few adverse effects.

## Introduction

Thoracotomy is recognised as one of the most painful surgical procedures [1]. Studies have shown that high levels of acute postoperative pain are associated with an increase in the likelihood of chronic pain [2]. The severity of acute postoperative pain has been linked to the development of persistent postoperative pain [3].

Post-thoracotomy pain syndrome (PTPS) is a common condition; its prevalence has been reported to reach 80% [4]. It is defined as pain that recurs or persists along a thoracotomy scar at least two months after the surgical procedure [5].

In general, postoperative pain after thoracotomy is burning and stabbing pain and shares many features of neuropathic pain [6]. It was found that tissue damage leads to sensitisation of dorsal horn neurons, which causes hyperalgesia or allodynia. This course may contribute to chronification of pain. Therefore, initiating analgesic treatment before tissue damage can reduce hyperexcitability of dorsal horn neurons and central sensitisation [7]. Hence, drugs that reduce postoperative hyperalgesia and control acute postoperative pain may be of clinical interest [8].

Pregabalin is one of these drugs that can reduce the excitability of the dorsal horn neurons. It is a γ-aminobutyric acid analogue that binds to α2-δ subunits of the voltage-gated calcium channels in the central nervous system [9]. It was first introduced as a potent anticonvulsant and anxiolytic drug [10]. Pregabalin is recommended as a first-line treatment for neuropathic pain conditions [11]. Because of its ability to block presynaptic voltage-gated calcium channels implicated in central sensitisation, perioperative use of pregabalin could be valuable in preventing the development of chronic pain [12], [13]. It has been successfully used perioperatively for controlling neuropathic pain during knee and laparoscopic surgery; therefore, similar positive

results are expected for intercostal neuralgia in post-thoracotomy pain [14].

The purpose of this study was to investigate the safety and efficacy of perioperative administration of pregabalin to control intercostal neuralgia after thoracotomy.

## Patients and Methods

The study included 60 ASA physical status I and II patients between 25 and 60 years of age scheduled for elective open thoracotomy for thoracic oncologic surgeries. They were recruited from the National Cancer Institute, Cairo University from January 2017 to February 2018. The study was approved by the local ethical committee, and every patient provided an informed written consent to participate in the study.

Patients with renal insufficiency, history of congestive heart failure, major psychiatric disorder, body mass index > 40 kg/m², weight < 50 kg, pre-existing chronic pain in proposed surgical area or elsewhere requiring chronic analgesic use, hypersensitivity to opioids, tumors extending into chest wall, previous ipsilateral thoracotomy, chest tube in situ at time of surgery, patients on anticonvulsants, patients planned for postoperative ventilation, or those who cannot use patient-controlled analgesia (PCA) were excluded from the study.

Preoperatively patients were instructed how to use a visual analogue scale (VAS) (0 = no pain, 10 = worst pain) to express pain and use of the PCA device. Patients were randomly allocated to one of two equal groups. Pregabalin Group received pregabalin capsules, 150 mg (Lyrica, Pfizer, Egypt) while the Control group received placebo capsules (multivitamin capsules). A treatment schedule in the two groups was one capsule one hour before thoracotomy, 12 hours following operation (day zero postoperative), and then twice a day for 5 postoperative days.

All patients were premedicated with midazolam 0.05 mg/kg IV 30 minutes before the surgical procedure. A crystalloid IV infusion was established, and the baseline means arterial blood pressure (MAP), heart rate (HR), and peripheral oxygen saturation ($SO_2$) were recorded. Anaesthesia was induced with fentanyl 2 µg/kg and propofol 2 mg/kg and atracurium 0.5 mg/kg IV and maintained with sevoflurane (2-3 vol%) and fresh gas flow rate of 2 L/min in combination with 50% oxygen/air. After endotracheal intubation, lungs were mechanically ventilated to maintain the end-expiratory $CO_2$ values between 32-36 mm Hg, then morphine 0.1 mg/kg was administered IV. With skin closure, the residual neuromuscular blockade was antagonised with IV neostigmine 0.04 mg/kg and atropine 0.02 mg/kg. Ondansetron 4 mg IV was given to all patients.

Patients were then transferred to the intensive care unit (ICU) to start using the PCA device for 5 days (Accufuser PLUS M5015L WOO YOUNG MEDICAL CO.LTD, Korea). The PCA device contained a volume of 300 ml (60 mg morphine + 60 mg ketorolac + 2 mg Garnitryl). The basal rate was 5.0 ml/hr, and bolus 1.0 ml (0.2 mg) with a lockout interval of 15 min (limit of 1.8 mg/hr) and total opioid consumption were recorded. The pain was assessed using a VAS score at 0, and after 4, 8, 12, 16, 20, and 24 hours. The mean of VAS at rest (VAS-R) and during a cough or with movement or cough (dynamic) (VAS-D) were recorded. The occurrence of postoperative side effects (confusion, dizziness, headache, dry mouth, nausea, vomiting, and pruritus were recorded at follow up intervals.

Assessment of the incidence of chronic post-thoracotomy pain syndrome and pain quality (neuropathic versus other) using Leeds Assessment of Neuropathic Symptoms and Signs (LANSS) scale was done at 1, 2, and 3 months postoperatively. Patients who suffered from symptoms of neuropathic pain, allodynia or hyperalgesia at first month received pregabalin 150 mg BID and were reassessed at 2$^{nd}$ and 3$^{rd}$ month. The LANSS pain scale consists of five items that document self-reported pain symptoms and two items that document the findings of a simple clinical examination conducted by the healthcare professional to assess the presence of allodynia and pin-prick threshold [15]. A LANSS score of 12 or more is an indication of chronic neuropathic pain; allodynia (assessed by gentle rubbing of the operated site by a piece of cotton) and hyperalgesia (gently applied pressure from the fingertip).

### *Statistical Analysis*

Statistical Analysis was performed using SPSS 15.0 for Windows. Numerical data were expressed as mean and standard deviation or median and range as appropriate. Qualitative data were expressed as frequency and percentage. Chi-square test (Fisher's exact test) was used to examine the relation between qualitative variables. For quantitative data, a comparison between two groups was made using independent sample t-test or Mann-Whitney test. A *p*-value of < 0.05 was accepted as statistically significant.

## Results

Demographic data, duration of anaesthesia and surgery were comparable between groups (Table 1).

**Table 1: Demographic data**

| Variables | Pregabalin (n = 30) | Control (n = 30) | p value |
|---|---|---|---|
| Age (years) | 46.9 ± 10.1 | 41.0 ± 14.5 | 0.072 |
| Weight (kg) | 83.1 ± 9.9 | 77.1 ± 15.5 | 0.078 |
| Height (cm) | 176 ± 9 | 173 ± 11 | 0.246 |
| Gender (M/F) | 22/8 | 21/9 | 1.0 |
| Duration of surgery (minutes) | 188 ± 25 | 178 ± 10 | 0.066 |
| Duration of anesthesia (minutes) | 220 ± 28 | 211 ± 11 | 0.910 |
| Type of surgery | | | |
| Lobectomy | 9 | 3 | 0.117 |
| Pneumonectomy | 11 | 17 | |
| Pleurectomy | 10 | 10 | |

Data are expressed as mean ± SD (standard deviation), or number as appropriate; *P* value < 0.05 is considered significant; There were no statistical differences among groups.

VAS-R and VAS-D were significantly lower in Pregabalin group at 0, 6, 18 and 24 hours but were comparable between groups at 12 hours (Table 2).

**Table 2: VAS-R and VAS-D. Data are expressed as mean ± SD**

| | | Pregabalin (n = 30) | Control (n = 30) | *p* value |
|---|---|---|---|---|
| 0 hour | VAS-R | 2.9 ± 0.7 | 3.5 ± 1.0 | 0.022* |
| | VAS-D | 6.0 ± 0.7 | 6.6 ± 1.0 | 0.016* |
| 6 hours | VAS-R | 3.3 ± 0.9 | 3.8 ± 0.7 | 0.042* |
| | VAS-D | 4.4 ± 0.9 | 5.0 ± 0.7 | 0.023* |
| 12 hours | VAS-R | 2.6 ± 0.8 | 3.0 ± 0.9 | 0.125 |
| | VAS-D | 3.5 ± 0.8 | 3.9 ± 0.9 | 0.209 |
| 18 hours | VAS-R | 2.1 ± 0.6 | 2.6 ± 0.8 | 0.022* |
| | VAS-D | 3.3 ± 0.7 | 3.8 ± 0.9 | 0.020* |
| 24 hours | VAS-R | 1.6 ± 0.6 | 2.0 ± 0.6 | 0.037* |
| | VAS-D | 3.1 ± 0.7 | 3.5 ± 0.8 | 0.039* |

Data are expressed as mean ± SD (standard deviation); VAS-R = visual analogue score at rest; VAS-D = visual analogue score dynamic (during movement); * *P* value < 0.05 is considered significant.

The mean VAS-R and VAS-D during 24 hours on days 1, 2, 3 and 4 were significantly lower in Pregabalin group. There was no significant difference between groups in days 5 and 6 (Table 3).

**Table 3: Mean of VAS-R and VAS-D during postoperative days 1, 2, 3, 4, 5 and 6**

| | | Pregabalin (n = 30) | Control (n = 30) | *p* value |
|---|---|---|---|---|
| Day 1 | VAS-R | 2.1 ± 0.7 | 2.5 ± 0.6 | 0.047* |
| | VAS-D | 4.1 ± 1.0 | 4.8 ± 0.8 | 0.014* |
| Day 2 | VAS-R | 2.0 ± 0.8 | 2.5 ± .8 | 0.031* |
| | VAS-D | 3.1 ± 1.0 | 3.8 ± 0.8 | 0.021* |
| Day 3 | VAS-R | 1.4 ± 0.5 | 1.8 ± 0.6 | 0.027* |
| | VAS-D | 2.5 ± 0.5 | 2.9 ± 0.7 | 0.043* |
| Day 4 | VAS-R | 2.4 ± 0.7 | 2.8 ± 0.8 | 0.022* |
| | VAS-D | 3.3 ± 0.7 | 3.7 ± 0.8 | 0.029* |
| Day 5 | VAS-R | 2.8 ± 0.8 | 2.9 ± 0.8 | 0.981 |
| | VAS-D | 3.6 ± 0.8 | 3.8 ± 0.6 | 0.509 |
| Day 6 | VAS-R | 1.8 ± 0.6 | 2.2 ± 1.1 | 0.163 |
| | VAS-D | 3.0 ± 0.8 | 3.5 ± 1.2 | 0.197 |

Data are expressed as mean ± SD (standard deviation); VAS-R = visual analogue score at rest; VAS-D = visual analogue score dynamic (during movement); * *P* value < 0.05 is considered significant.

Total morphine consumption during the postoperative period was significantly lower in Pregabalin group (Table 4).

**Table 4: Total daily morphine consumption in (mg) with PCA device from day 0 to day 4 in the two studied groups**

| | Pregabalin (n=30) | Control (n=30) | *p* value |
|---|---|---|---|
| Day 1 | 34.8±2.2 | 35.9±1.3 | 0.028* |
| Day 2 | 30.9±2.7 | 32.3±2.2 | 0.033* |
| Day 3 | 28.3±2.2 | 29.6±2.0 | 0.022* |
| Day 4 | 26.4±2.0 | 27.5±1.9 | 0.035* |

Data are expressed as mean ± SD (standard deviation); * *P* value < 0.05 is considered significant.

The frequency of neuropathic pain, allodynia, and hyperalgesia at the site of operation were significantly lower in Pregabalin group after 1, 2- and 3-months post-thoracotomy (Table 5).

**Table 5: The proportion of post-thoracotomy chronic pain after 1, 2 and 3 months**

| | Pregabalin (n = 30) | Control (n = 30) | *p* value |
|---|---|---|---|
| Neuropathic pain | | | |
| At 1 month | 3 (10.0%) | 11 (36.7%) | 0.015* |
| At 2 months | 1 (3.3%) | 8(26.7%) | 0.011* |
| At 3 months | 0 (0.0%) | 5 (16.7%) | 0.052* |
| Allodynia | | | |
| At 1 month | 5 (16.7%) | 13 (43.3%) | 0.024* |
| At 2 months | 2 (6.7%) | 9 (30.0%) | 0.020* |
| At 3 months | 0 (0.0%) | 6 (20.0%) | 0.024* |
| Hyperalgesia | | | |
| At 1 month | 3 (10.0%) | 11 (36.7%) | 0.015* |
| At 2 months | 2 (6.7%) | 9 (30.0%) | 0.020* |
| At 3 months | 0 (0.0%) | 7 (23.3%) | 0.011* |

Data are expressed as number (proportion); * *P* value < 0.05 is considered significant.

Adverse effects were comparable between groups (Table 6). No cases of respiratory depression were recorded.

**Table 6: Incidence of Adverse Effects**

| | Pregabalin (n=30) | Control (n=30) | *p* value |
|---|---|---|---|
| Confusion | 2 (6.7%) | 4 (13.3%) | 0.671 |
| Dizziness | 5 (16.7%) | 8 (26.7%) | 0.347 |
| Headache | 3 (10.0%) | 2 (6.7%) | 1.000 |
| Dry Mouth | 1 (3.3%) | 2 (6.7%) | 1.000 |
| Nausea | 8 (26.7%) | 3 (10.0%) | 0.095 |
| Vomiting | 3 (10.0%) | 1 (3.3%) | 0.612 |
| Pruritus | 3 (10.0%) | 1 (3.3%) | 0.612 |

Data are expressed as number (proportion); * *P* value < 0.05 is considered significant.

## Discussion

The results of this study demonstrated that perioperative administration of pregabalin reduced pain intensity and morphine consumption during the first postoperative 4 days in patients having oncologic surgery through a thoracotomy incision. The frequency of chronic neuropathic pain, allodynia and hyperalgesia was significantly reduced in pregabalin group one, two, and three months after surgery. The frequency of side effects like confusion, dizziness, headache, and dry mouth was not increased in the pregabalin group.

In this study, the first dose of pregabalin 150 mg is given 1 hour before surgery to provide analgesia immediately after surgery. The idea of the study was to test whether pregabalin will have better control of acute postoperative pain aiming at prevention of pain chronification [16]. The proposed action of pregabalin was suppression of hyperexcitability of the dorsal horn neurons and neuroplastic changes after surgery. Pregabalin has predominantly inhibitory effect during the first 24 hours; then it has an excitatory effect for 5 days after surgery [17]. The daily pregabalin dose of 300 mg used in this study was based on previous studies [14], [15].

In the current study, on postoperative day 0, the pain intensity was less in pregabalin group throughout the day. This can be contributed to the synergistic analgesic effect of pregabalin with morphine, which led to a decrease in opioid consumption in the first day. After that, pain intensity was lower in the pregabalin group from day 1 through day 4 with reduced total morphine consumption.

Previous studies reported similar results in different after different surgical procedures. Bornemann-Cimenti et al., [7] found that the preoperative administration of 300 mg pregabalin in patients undergoing transperitoneal nephrectomy reduces postoperative opioid consumption by 33% within the first 48 h. In video-assisted thoracoscopic surgery, a single dose of pregabalin 150 mg preoperatively was effective in reducing the pain intensity and need for additional analgesic drugs [19]. Another study reported adequate postoperative pain control after robot-assisted endoscopic thyroidectomy using one dose of 150 mg 1 h before surgery and another dose 12 hours after [20]. Similar results were reported [21].

In the current study, perioperative administration of pregabalin decreased the frequency of chronic neuropathic pain after one, two and three months after surgery. Similar results were obtained concerning allodynia and hyperalgesia in the pregabalin group.

By these findings, Fawzi and El-Tohamy found that perioperative pregabalin reduces the incidence of chronic post-thoracotomy pain at 3 and 6 months from 60 and 40% to 10 and 6.7%, respectively [22].

On the contrary, Brulotte et al. concluded that pregabalin did not reduce the incidence of PTPS. However, among pregabalin users who developed PTPS, pain intensity was less marked, required significantly fewer analgesics, and presented significantly less neuropathic characteristics compared to patients in the placebo group 3 months after surgery [13].

The limitation of our study is a relatively small sample size and short follow up, which is only three months. In conclusion, perioperative use of pregabalin reduces pain and opioid consumption during the acute postoperative period and the incidence of chronic pain at two and three months after thoracotomy.

## References

1. Peng Z, Li H, Zhang C, Qian X, Feng Z, Zhu S. A retrospective study of chronic post-surgical pain following thoracic surgery: prevalence, risk factors, incidence of neuropathic component, and impact on qualify of life. PLoS ONE. 2014; 9(2):e90014. https://doi.org/10.1371/journal.pone.0090014 PMid:24587187 PMCid:PMC3938555

2. El-Hag-Aly MA, Nashy MR. Double edge closure: a novel technique for reducing post-thoracotomy pain. A randomized control study. Interact Cardiovasc Thorac Surg. 2015; 21(5):630-5. https://doi.org/10.1093/icvts/ivv218 PMid:26254464

3. Maxwell C, Nicoara A. New developments in the treatment of acute pain after thoracic surgery. Curr Opin Anaesthesiol. 2014; 27(1):6-11. https://doi.org/10.1097/ACO.0000000000000029 PMid:24296973

4. Gerner P. Post-thoracotomy Pain Management Problems. Anesthesiol Clin. 2008; 26(2):355-vii. https://doi.org/10.1016/j.anclin.2008.01.007 PMid:18456219 PMCid:PMC2453516

5. Treede R-D, Rief W, Barke A, Aziz Q, Bennett MI, Benoliel R, et al. A classification of chronic pain for ICD-11. Pain. 2015; 156(6):1003-7. https://doi.org/10.1097/j.pain.0000000000000160 PMid:25844555 PMCid:PMC4450869

6. Koehler RP, Keenan RJ. Management of postthoracotomy pain: acute and chronic. Thorac Surg Clin. 2006; 16(3):287-97. https://doi.org/10.1016/j.thorsurg.2006.05.006 PMid:17004557

7. Bornemann-Cimenti H, Lederer AJ, Wejbora M, Michaeli K, Kern-Pirsch C, Archan S, et al. Preoperative pregabalin administration significantly reduces postoperative opioid consumption and mechanical hyperalgesia after transperitoneal nephrectomy. Br J Anaesth. 2012; 108(5):845-9. https://doi.org/10.1093/bja/aes004 PMid:22362672

8. Martinez V, Cymerman A, Ben Ammar S, Fiaud JF, Rapon C, Poindessous F, et al. The analgesic efficiency of combined pregabalin and ketamine for total hip arthroplasty: a randomised, double-blind, controlled study. Anaesthesia. 2014; 69(1):46-52. https://doi.org/10.1111/anae.12495 PMid:24320856

9. Mishriky BM, Waldron NH, Habib AS. Impact of pregabalin on acute and persistent postoperative pain: a systematic review and meta-analysis. Br J Anaesth. 2015; 114(1):10-31. https://doi.org/10.1093/bja/aeu293 PMid:25209095

10. Ogawa S, Satoh J, Arakawa A, Yoshiyama T, Suzuki M. Pregabalin treatment for peripheral neuropathic pain: a review of safety data from randomized controlled trials conducted in Japan and in the west. Drug Saf. 2012; 35(10):793-806. https://doi.org/10.1007/BF03261976 PMid:22967187

11. Freynhagen R, Serpell M, Emir B, Whalen E, Parsons B, Clair A, Latymer M. A comprehensive drug safety evaluation of pregabalin in peripheral neuropathic pain. Pain Pract. 2015; 15(1):47-57. https://doi.org/10.1111/papr.12146 PMid:24279736 PMCid:PMC4320770

12. Katz J, Clarke H, Seltzer Z. Review article: Preventive analgesia: quo vadimus? Anesth Analg. 2011; 113(5):1242-53. https://doi.org/10.1213/ANE.0b013e31822c9a59 PMid:21965352

13. Brulotte V, Ruel MM, Lafontaine E, Chouinard P, Girard F. Impact of pregabalin on the occurrence of postthoracotomy pain syndrome: a randomized trial. Reg Anesth Pain Med. 2015; 40(3):262-9. https://doi.org/10.1097/AAP.0000000000000241 PMid:25899956

14. Buvanendran A, Kroin JS, Della Valle CJ, Kari M, Moric M, Tuman KJ. Perioperative oral pregabalin reduces chronic pain after total knee arthroplasty: a prospective, randomized, controlled trial. Anesth Analg. 2010; 110(1):199-207. https://doi.org/10.1213/ANE.0b013e3181c4273a PMid:19910619

15. Bennett M. The LANSS Pain Scale: the Leeds assessment of neuropathic symptoms and signs. Pain. 2001; 92(1-2):147-57. https://doi.org/10.1016/S0304-3959(00)00482-6

16. Dahl JB, Mathiesen O, Møiniche S. "Protective premedication": an option with gabapentin and related drugs? A review of gabapentin and pregabalin in in the treatment of post-operative pain. Acta Anaesthesiol Scand. 2004; 48(9):1130-6. https://doi.org/10.1111/j.1399-6576.2004.00484.x PMid:15352959

17. Wilder-Smith OHG, Tassonyi E, Arendt-Nielsen L. Preoperative back pain is associated with diverse manifestations of central neuroplasticity. Pain. 2002; 97(3):189-94.

https://doi.org/10.1016/S0304-3959(01)00430-4

18. Singla NK, Chelly JE, Lionberger DR, Gimbel J, Sanin L, Sporn J, et al. Pregabalin for the treatment of postoperative pain: results from three controlled trials using different surgical models. J Pain Res. 2015; 8:9-20. https://doi.org/10.2147/JPR.S67841 PMid:25565885 PMCid:PMC4278776

19. Kim JC, Byun S, Kim S, Lee S-Y, Lee JH, Ahn S. Effect of preoperative pregabalin as an adjunct to a multimodal analgesic regimen in video-assisted thoracoscopic surgery: A randomized controlled trial. Medicine (Baltimore). 2017; 96(49):e8644. https://doi.org/10.1097/MD.0000000000008644 PMid:29245223 PMCid:PMC5728838

20. Kim SY, Jeong JJ, Chung WY, Kim HJ, Nam K-H, Shim YH. Perioperative administration of pregabalin for pain after robot-assisted endoscopic thyroidectomy: a randomized clinical trial. Surg Endosc. 2010; 24(11):2776-81. https://doi.org/10.1007/s00464-010-1045-7 PMid:20376496

21. Chotton T, Singh NR, Singh LC, Laithangbam PS, Singh HS. The effect of pregabalin for relief of postoperative pain after abdominal hysterectomy. Journal of Medical Society. 2014; 28(1):18. https://doi.org/10.4103/0972-4958.135219

22. Fawzi H, El-Tohamy S. Effect of perioperative oral pregabalin on the incidence of post-thoracotomy pain syndrome. Ain-Shams Journal of Anaesthesiology. 2014; 7(2):143. https://doi.org/10.4103/1687-7934.133350

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Jun 30; 7(12):1979-1983.
https://doi.org/10.3889/oamjms.2019.463
eISSN: 1857-9655
***Clinical Science***

# Intrapartum Fetal and Maternal Complications in Low-Risk Pregnancy: Experience of a Tertiary Hospital in Low-Income Countries

Mohamed Alkhatim Alsammani[1,2*], Khalid Nasralla[2], Sumeya A. Khieri[1], Zaheera Saadia[2], Mohamed Abdelgadir Shaaeldin[2], Ali Osman Ali[3]

*[1]Department of Obstetrics and Gynecology, University of Bahri, Khartoum, Sudan; [2]Obstetrics and Gynecology, College of Medicine, Qassim University, Buriadah, Saudi Arabia; [3]Department of Obstetrics and Gynecology, University of Alazhari, Khartoum, Sudan*

**Citation:** Alsammani MA, Nasralla K, Khieri SA, Saadia Z, Shaaeldin MA, Ali AO. Intrapartum Fetal and Maternal Complications in Low-Risk Pregnancy: Experience of a Tertiary Hospital in Low-Income Countries. Open Access Maced J Med Sci. 2019 Jun 30; 7(12):1979-1983. https://doi.org/10.3889/oamjms.2019.463

**Keywords:** Low-risk pregnancy; Labor complications; Pregnancy outcomes

***Correspondence:** Mohamed Alkhatim Alsammani, Department of Obstetrics and Gynecology, University of Bahri, Khartoum, Sudan; Obstetrics and Gynecology, College of Medicine, Qassim University, Buriadah, Saudi Arabia. E-mail: m_sammani@yahoo.com

**Received:** 03-Apr-2019; **Revised:** 27-May-2019; **Accepted:** 28-May-2019; **Online first:** 30-Jun-2019

**Copyright:** © 2019 Mohamed Alkhatim Alsammani, Khalid Nasralla, Sumeya A. Khieri, Zaheera Saadia, Mohamed Abdelgadir Shaaeldin, Ali Osman Ali. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

**Funding:** This research did not receive any financial support

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

**Abstract**

**AIM:** To determine the frequencies of intrapartum fetal and maternal complications in women without the identified prenatal risk factor

**METHODS:** We conducted a prospective cross-sectional investigation from January to June 2017 at Khartoum North Maternity Hospital in women categorised pregnancies as low risk (no prenatal risk factors). We evaluated adverse intrapartum fetal and maternal outcomes.

**RESULTS:** Among 600 pregnancies, of these, 12.5% (n = 75) developed fetal or / and maternal complications. The Frequency of primary cesarean delivery, forceps, and ventose among low-risk pregnancies in this study were 16%, 3%, and 2% respectively. Other adverse pregnancy outcomes were PPH (5%), Blood transfusion (4.5%), admission to ICU (1.8%), while perineal tear, cesarean hysterectomy, and re-laparotomy have equal weight (0.3%). Among all births, the most common adverse fetal outcomes were birth asphyxia (3.8%), low birth weight (2%), admission to the neonatal intensive care unit (1.8%), and fresh stillborn babies (1.3%).

**CONCLUSION:** Of all low-risk pregnancies, 12.5% were reported to have serious obstetrics and neonatal complications. This information is essential for evaluating resources in delivery centres and hospitals and to provide equipment and further training of medical personnel to provide optimal quality care and patient safety.

## Introduction

Pregnancy is considered as a High-risk pregnancy if it is associated with any risk factors about the pregnancy to the mother or the baby. On the other hand, low-risk pregnancy is associated with no identified risk factor for either the mother or in the baby. There is a wide debate in recent years about the benefits and risks of birth in different obstetrics settings [1], [2]. Low-risk mothers are allowed to choose the place of birth freely. In England, births outside an obstetric care unit are relatively uncommon. During 2007, reports have shown that 8% of women in England had delivered the obstetric unit [3]. In one study examining 10,458,616 pregnancies, showed that 29% of low risk had complications that required nonroutine obstetric or neonatal care [4]. The former study reported that the most common complications in the low-risk group were cesarean delivery, meconium staining, and vacuum delivery, 15%, 5%, and 4% respectively [4].

Prior studies on obstetrical risk level and medical outcomes often focused on actual or planned birth location. Furthermore, prior studies assessing the obstetrical risk of the actual or planned birth location showed that home and centres births compared to the obstetrical resource, have decreased in maternal complications such as operative vaginal delivery, cesarean delivery, episiotomy, and epidural use [5], [6]. In another study of 2831 women comparing private and national health system, the obstetric provider reported a rate of cesarean delivery

6.7% and 7.6%, respectively [7]. In the study by de Jonge A [8] and his colleagues identified that for Low-risk women labouring in primary care with planned home birth showed lower rates of manual removal of placenta and postpartum bleeding compared to planned hospital birth [8].

In Sudan, during the antenatal care low-risk mothers are offered to choose the place of birth, and the delivery is usually attended by a midwife or a traditional birth attendant. Before 1920, the home was the main place of birth 0; however, after 1940; the hospital is considered the safest place of delivery [9]. In Sudan, documentation and data on home delivery are scant and insufficient to be analysed. There is a lack of data on pregnancy outcome among low-risk women in low-income countries, and almost all available data were from developed countries. The present study aimed to assess Intrapartum fetal and maternal complications in low-risk pregnancy in a low-income country.

## Material and Methods

This is a perspective cross-sectional study conducted at Khartoum North Maternity Hospital (KNMH) in the period from January to June 2017. KNMH is a referral maternity hospital for women who live in the northern part of the capital as well as for women who live near the hospital. The study was approved by the ethics committee of the Sudanese specialisation board.

### *Study sample*

The study sample was collected from women who were admitted in labour at Khartoum North Maternity Hospital in the period from January to June 2017. All women admitted the labour and delivery suite and who were willing to participate and fulfilled the inclusion criteria were included in the study.

### *Sample size*

A sample of 600 low-risk women was selected to calculate the proportion of women with low risk for obstetrical complications within 3 percentage points of the true proportion, assuming the true proportion was 80% and that 10% of women would not respond.

### *Inclusion criteria*

The primary outcome was maternal and neonatal mortality and morbidities. Women were classified as low-risk pregnancies if they were not known to have any of the medical or obstetric risk factors as defined by An Update on Research Issues in the Assessment of Birth Settings: Workshop Summary [10]. The Update on Research Issues in the Assessment of Birth Settings used these inclusion criteria: “singleton, uncomplicated obstetric history (no stillbirth, neonatal death, consecutive miscarriages, fetal death, preterm birth < 32 weeks, isoimmunization, gestational diabetes), no current pregnancy complications (e.g., fetal anomaly), no precluding medical conditions (no cardiac disease, hypertension, diabetes, epilepsy, severe asthma, substance use, significant psychiatric disorder, BMI > 35 or < 17), and no prior Cesarean”. Both primigravida and grandmultiparous women have been excluded from the study as they have special obstetrics complications, and the study was limited to parity 1 to parity 4.

Socio-demographic characteristics were gathered through structured questionnaires. Maternal characteristics, including maternal age, residence, education, and occupation, were gathered from each participant.

Adverse complications included were medical conditions that arise during pregnancy such as diabetes, hypertension disorders of pregnancy and complications during labour and delivery, including mode of delivery (ventose, forceps or cesarean delivery). Other complications reported such as a tear, transfusion, PPH, hysterectomy.

Fetal complications that were reported included admission to neonatal intensive care unit, birthweight < 2500 g, 5-minute Apgar score 0-3, and early fetal loss.

### *Statistics analysis*

SPSS, version 20.0 was used to record and analyse the data. Descriptive analyses used were the mean, standard deviation, and frequency distribution.

## Results

There were 600 deliveries with live, singleton, cephalic, with term fetuses (9.1% delivered at 37 completed weeks of gestation, 17.6% delivered at 38 weeks, 31.03% delivered at 39 weeks, 31.7% delivered at 40 weeks, and 10.57% delivered at 41 weeks) enrolled in the present study. Of these, 12.5% (n = 75) developed feto-maternal complications. The maternal socio-demographic characteristics are shown in Table 1. The mean maternal age and gestational ages were 28.08 ± 5.681 years and 39.0543 ± .89642 weeks, respectively. The majority of patients 108 (48.4%) were within the age group of 20-25 years. The majority of the studied population were

living in the urban area (67.2%), completed their secondary school (41.5%) and housewives (79.8%).

**Table 1: Basic demographic and obstetrics characteristics**

| Variable | Frequency (%) | *Parity* | | | |
|---|---|---|---|---|---|
| | | Para 1 (n = 223) | Para2 (n = 117) | Para3 (n = 196) | Para4 (n = 64) |
| *Age* | | | | | |
| < 19 | 18 (3) | 18 (8.1) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| 20- 25 | 108 (18) | 108 (48.4) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| 26-30 | 241 (40.2) | 97 (43.5) | 117 (100.0) | 27 (13.8) | 0 (0.0) |
| 31-35 | 184 (30.7) | 0 (0.0) | 0 (0.0) | 169 (86.2) | 15 (23.4) |
| 36-40 | 49 (8.2) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 49 (76.6) |
| *Residence* | | | | | |
| Urban | 403 (67.2) | 223 (55.3) | 117 ((100) | 63 (32.1) | 0 (0.0) |
| Rural | 197 (32.8) | 0 (0) | 0 (0.0) | 133 (67.9) | 64 (32.5) |
| *Education* | | | | | |
| Illiterate | 48 (8) | 48 (100) | 0 (0) | 0 (0) | 0 (0) |
| Primary | 198 (33) | 175 (88.4) | 23 (11.6) | 0 (0) | 0 (0) |
| Secondary | 249 (41.5) | 0 (0) | 94 (37.8) | 155 (62.2) | 0 (0) |
| University and above | 105 (17.5) | 0 (0) | 0 (0) | 41 (39) | 64 (61) |
| *Occupation* | | | | | |
| House wife | 479 (79.8) | 223 (46.6) | 117 (24.4) | 139 (29.0) | 0 (0) |
| Worker | 73 (12.2) | 0 (0.0) | 0 (0.0) | 57 (78.1) | 16 (21.9) |

Data presents as number (%).

Table 2 shows the frequencies of intrapartum maternal complications. The Frequency of primary cesarean delivery, forceps, and ventose among low-risk pregnancies in this study were 16%, 3%, and 2% respectively. The higher rate of interventional delivery was seen among mothers who had 4 previous deliveries while no complications were seen among women with lower parities. Other adverse pregnancy outcomes were PPH (5%), Blood transfusion (4.5%), admission to ICU (1.8%), while perineal tear, cesarean hysterectomy, and re-laparotomy have equal weight (0.3%).

**Table 2: Intrapartum maternal complications**

| Variables | Overall complications (%) | Complications % within parity | | | |
|---|---|---|---|---|---|
| | | Para 1 | Para 2 | Para 3 | Para 4 |
| Cs | 96 (16) | 0 (0) | 0 (0) | 62 (64.6) | 35 (35.4) |
| Forceps delivery | 18 (3) | 0 (0) | 0 (0) | 0 (0) | 18 (100) |
| Ventose delivery | 12 (2) | 0 (0) | 0 (0) | 0 (0) | 12 (100) |
| Perineal tear | 2 (0.33) | 2 (100) | 0 (0) | 0 (0) | 0 (0) |
| Hysterectomy | 2 (0.33) | 2 (100) | 0 (0) | 0 (0) | 0 (0) |
| Relaparotomy | 2 (0.33) | 2 (100) | 0 (0) | 0 (0) | 0 (0) |
| Postpartum hemorrhage | 30 (5) | 30 (100) | 0 (0) | 0 (0) | 0 (0) |
| Blood transfusion | 27 (4.5) | 27 (100) | 0 (0) | 0 (0) | 0 (0) |
| Intensive care unit | 11 (1.8) | 11 (100) | 0 (0) | 0 (0) | 0 (0) |

Data presents as number (%).

Among all births, the most common adverse fetal outcomes were birth asphyxia (3.8%), low birth weight (2%), admission to the neonatal intensive care unit (1.8%), and fresh stillborn babies (1.3%) Table 3.

**Table 3: Intrapartum neonatal complications**

| Fetal complications | Overall complications (%) | Complications % within parity | | | |
|---|---|---|---|---|---|
| | | Para 1 | Para 2 | Para 3 | Para 4 |
| 1-minute apgar score) < 7 | 23 (3.8) | 0 (0) | 0 (0) | 0 (0) | 23 (35.9) |
| 5-minute apgar score) < 7 | 13 (2.2) | 0 (0) | 0 (0) | 0 (0) | 13 (20.3) |
| Fresh stillborn | 8 (1.3) | 0 (0) | 0 (0) | 0 (0) | 8 (12.5) |
| Neonatal intensive care unit | 11 (1.8) | 0 (0) | 0 (0) | 0 (0) | 11 (??) |
| Birth weight < 2500 g | 12 (2) | 12 (100) | 0 (0) | 0 (0) | 0 (0) |

Data presents as number (%).

## Discussion

Low-risk pregnancy is the one in which no identifiable risk factor is discovered through antenatal care. Our antenatal, intranatal and postnatal care is much more focused on the high-risk pregnancies. However, the majority of women progress through pregnancy in an uncomplicated manner. Antenatal care continues to screen women with risk factors like age, medical disorders, and bad obstetric history, and then provide more concentrated care to those high-risk women in whom any risk factor is identified. The number of antenatal visits for high-risk women is more compared to low-risk women. High-risk women are provided with the obstetric care at the consultant level, while the low-risk women are shifted to community-led care by midwives. Much resources, as well as medical efforts, are being utilised for preventing complications among the high-risk group, and these are fruitful as well in reducing the fetomaternal morbidity. However, on the other side, complications continue to prevail in low-risk women.

We noticed a high frequency of primary cesarean section, forceps and ventouse delivery (16%, 3%, and 2% respectively) among low-risk pregnancies (Table 2). This is by the results of Haerskjold who raised concerns over a high rate (8.7%) for the emergency cesarean section in a cohort of 2,748 low-risk pregnancies [11]. The authors analysed that failed progress was the foremost indication for a cesarean section; next was the fetal distress. The study only included women in their first pregnancy. On the other hand, we included women with P1-P4. We found a high rate of cesarean section in P3 and P4 women 64.6% and 35.4% respectively. Our study findings revealed that the need of intervention was highest amongst the P3 and P4 groups, which is mostly the group considered to be low risk as firstly these women had previously experienced pregnancy and labour events and secondly, they are mostly in an age group which is considered to be a low risk (Table 1). Strangely, our study did not find a high rate of intervention in Primigravidas although we think that first pregnancy should be a high-risk pregnancy, and no one can predict how the labour events are going to progress. This aspect needs to be studied further in future. We think that both first times intending mothers and caregivers are more concerned about the first pregnancy as compared to the P3 or P4 therefore, give more attention and time and keep the threshold for intervention very high. Roman et al., conducted a study including all metropolitan units from France and they observed that the rate of elective CS was 2.9% and CS in labour was 4.3% in 5393 women, fitting into the criteria of low-risk pregnancies. Maternal age above 30 and BMI above 25 kg/m2, as well as maternity units with a delivery rate below 100/year, were associated with high CS rate in labour [12].

In our study group, 3% of women had forceps delivery, and 2% underwent ventose delivery. All of these were having parity of 4. In a study conducted by Selvi et al., in 40 patients where 250 were low-risk women, it was observed that in 37 % low-risk women obstetrician was called as compared to 29% of the high-risk group. The need for intervention was 21% vs 12 % respectively for low and high-risk groups. They concluded that even if the pregnancy is a low risk from the beginning, the need for intervention may be there at any time during labour, and it's unpredictable [13]. In another study from Netherland, it was observed that women at low risk who deliver in secondary level care are more liable to get obstetric intervention in the form of cesarean sections, and instrumental delivery as compare to those who are delivered in primary care 8% vs 9% [14]. Published literature favours as well as refute the use of epidural analgesia as one of the major factors increasing the rate of interventional delivery amongst low-risk women [15], [16].

As regards maternal complications, other adverse outcomes were PPH (5%), Blood transfusion (4.5%), admission to ICU (1.8%), while perineal tear, cesarean hysterectomy, and re-laparotomy have equal weight (0.3%). All of these complications were noticed in primigravidas.

Danilack Valery A et al. analysed US Natality data, including 10 million births from 2011-2013. The authors found 38% pregnancies as low risk and 62% as high risk. Out of all birth, 29% low-risk pregnancies ended up in complications that needed extra care. They reported a 15% incidence of CS, 5.5% meconium staining and 4% vacuum delivery. The risk of complication was 27.8% vs 57.35 between low risk and high-risk groups. Therefore, although it was lower, as compared to the high-risk group still, a significant number of unforeseen complications happened in the low-risk group. Forcep0.8vs 0.5 vacuum (3.7 vs 2.3%) delivery, meconium staining (5.5 vs 4.8) and chorioamnionitis (1.4 vs 1.2%) was found to be higher in low-risk women as compared to high-risk women [17].

The observed fetal complications in our study were birth asphyxia (3.8%), low birth weight (2%), admission to the neonatal intensive care unit (1.8%), and fresh stillborn babies (1.3%). All were observed in women with parity of 4 except low birth weight, which was seen amongst primigravidas more as compared to women of higher parity. The low-risk women are often delivered at home care setting, and meta-analysis reveals that home births vs hospital birth have fewer chances of maternal intervention, but it triples the neonatal mortality rate [18]. Home birth also increases the risk of low 5-minute Apgar score and neurological damage [19]. However, some studies report no increased risk of perinatal mortality and morbidity in women delivered at midwifery units rather than obstetric units [20].

We conclude that although low-risk women in whom antenatal care fails to detect any risk to the mother or fetus posed by pregnancy still have a high risk for unforeseen feto-maternal morbidity. Regarding our previous experience of success in reducing the materno-fetal morbidity and mortality in high-risk women by improved obstetric care, it's now needed to concentrate on low-risk pregnancies and develop appropriate plans to reduce the maternal and fetal morbidity in this group as well.

*Limitations:* We did not analyse the indications for modes of delivery and needs for intervention. This needs to be studied in details in our low-risk population.

*Strength:* It is a prospective study, and included a reasonable of cases. Our study found the need of intervention highest amongst the P3 and P4 which is mostly the group considered to be low risk as the women had previously experienced pregnancy and labour events and mostly except few are in an age group considered to be low risk.

## References

1. De Jonge A, van der Goes BY, Ravelli AC, Amelink-Verburg MP, Mol BW, Nijhuis JG, et al. Perinatal mortality and morbidity in a nationwide cohort of 529,688 low-risk planned home and hospital births. BJOG. 2009; 116:1177-84. https://doi.org/10.1111/j.1471-0528.2009.02175.x PMid:19624439

2. Janssen PA, Saxell L, Page LA, Klein MC, Liston RM, Lee SK. Outcomes of planned home birth with registered midwife versus planned hospital birth with midwife or physician. CMAJ. 2009; 181:377-83. https://doi.org/10.1503/cmaj.081869 PMid:19720688 PMCid:PMC2742137

3. Redshaw M, Rowe R, Schroeder L, Puddicombe D, Macfarlane A, Newburn M, et al. Mapping maternity care. The configuration of maternity care in England. Birthplace in England research programme. Final report part 3: NIHR service delivery and organisation programme, 2011.

4. Valery A. Danilack, MPH, PhD, Anthony P. Nunes, MS, PhD, and Maureen G. Phipps, MD, MPH. Unexpected complications of low-risk pregnancies in the United States. Am J Obstet Gynecol. 2015; 212(6):809.e1-809.e6. https://doi.org/10.1016/j.ajog.2015.03.038 PMid:26042957 PMCid:PMC4728153

5. Wax JR, Lucas FL, Lamont M, Pinette MG, Cartin A, Blackstone J. Maternal and newborn outcomes in planned home birth vs planned hospital births: a metaanalysis. Am J Obstet Gynecol. 2010; 203:243.e1-8. https://doi.org/10.1016/j.ajog.2010.05.028 PMid:20598284

6. Overgaard C, Moller AM, Fenger-Gron M, Knudsen LB, Sandall J. Freestanding midwifery unit versus obstetric unit: a matched cohort study of outcomes in low-risk women. BMJ Open. 2011; 1:e000262. https://doi.org/10.1136/bmjopen-2011-000262 PMid:22021892 PMCid:PMC3191606

7. Chiossi G, Palomba S, Balduzzi S, Costantine MM, Falbo AI, La Sala GB. "The More the Better" Paradox of Antenatal Ultrasound Examinations in Low-Risk Pregnancy. Am J Perinatol. 2016; 33(7):646-57. https://doi.org/10.1055/s-0035-1571200 PMid:26862726

8. de Jonge A, Mesman JA, Manniën J, Zwart JJ, van Dillen J, van Roosmalen J. Severe adverse maternal outcomes among low risk

women with planned home versus hospitalbirths in the Netherlands: nationwide cohort study. BMJ. 2013; 346:f3263. https://doi.org/10.1136/bmj.f3263 PMid:23766482 PMCid:PMC3685517

9. Tew M. Understanding intra-natal care through mortality statistics. In: Zander L, Chamberlaing, pregnancy care for the 1990s. London: Royal society of medicine and Macmillan, 1994:105-114. https://doi.org/10.1007/978-1-349-17389-1_12

10. National Research Council. An Update on Research Issues in the Assessment of Birth Settings: Workshop Summary. National Academies Press (US); 2013.

11. Haerskjold A, Hegaard HK, Kjaergaard H. Emergency caesarean section in lowrisk nulliparous women. J ObstetGynaecol. 2012; 32(6):543-7. https://doi.org/10.3109/01443615.2012.689027 PMid:22779958

12. Roman H, Blondel B, Bréart G, Goffinet F. Do risk factors for elective cesarean section differ from those of cesarean section during labor in low risk pregnancies? J Perinat Med. 2008; 36(4):297-305. https://doi.org/10.1515/JPM.2008.044 PMid:18598118

13. SelviDogan F, Calmelet P, Cottenet J, Sagot P, Mace G. Does low-risk delivery exist?. J GynecolObstetBiolReprod. 2013; 42(6):557-63. https://doi.org/10.1016/j.jgyn.2012.12.007 PMid:23332380

14. Maassen MS, Hendrix MJ, Van Vugt HC, Veersema S, Smits F, Nijhuis JG. Operative deliveries in low-risk pregnancies in The Netherlands: primary versussecondary care. Birth. 2008; 35(4):277-82. https://doi.org/10.1111/j.1523-536X.2008.00254.x PMid:19036039

15. Eriksen LM, Nohr EA, Kjaergaard H. Mode of delivery after epidural analgesiain a cohort of low-risk nulliparas. Birth. 2011; 38(4):317-26. https://doi.org/10.1111/j.1523-536X.2011.00486.x PMid:22112332

16. Bakhamees H, Hegazy E. Does epidural increase the incidence of cesarean delivery or instrumental labor in Saudi populations? Middle East J Anaesthesiol. 2007; 19(3):693-704.

17. Danilack VA, Nunes AP, Phipps MG. Unexpected complications of low-risk pregnancies in the United States. Am J Obstet Gynecol. 2015; 212(6):809.e1-6. https://doi.org/10.1016/j.ajog.2015.03.038 PMid:26042957 PMCid:PMC4728153

18. Wax JR, Lucas FL, Lamont M, Pinette MG, Cartin A, Blackstone J. Maternal andnewborn outcomes in planned home birth vs planned hospital births: a metaanalysis. Am J Obstet Gynecol. 2010; 203(3):243.e1-8. https://doi.org/10.1016/j.ajog.2010.05.028 PMid:20598284

19. Grünebaum A, McCullough LB, Sapra KJ, Brent RL, Levene MI, Arabin B, ChervenakFA. Apgar score of 0 at 5 minutes and neonatal seizures or serious neurologicdysfunction in relation to birth setting. Am J Obstet Gynecol. 2013; 209(4):323.e1-6. https://doi.org/10.1016/j.ajog.2013.06.025 PMid:23791692

20. Birthplace in England Collaborative Group, Brocklehurst P, Hardy P, Hollowell J, Linsell L, Macfarlane A, McCourt C, Marlow N, Miller A, Newburn M, PetrouS,Puddicombe D, Redshaw M, Rowe R, Sandall J, Silverton L, Stewart M. Perinatal andmaternal outcomes by planned place of birth for healthy women with low riskpregnancies: the Birthplace in England national prospective cohort study. BMJ. 2011; 343:d7400. https://doi.org/10.1136/bmj.d7400 PMid:22117057 PMCid:PMC3223531

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Jun 30; 7(12):1984-1986.
https://doi.org/10.3889/oamjms.2019.470
eISSN: 1857-9655
***Clinical Science***

# Association between Lactate Dehydrogenase Levels to the Response of Non-Hodgkin Lymphoma in Elderly Patients Who Treated with First-Line Chemotherapy in Sanglah General Hospital

Cokorda Agung Wahyu Purnamasidhi[*], Ketut Suega, I Made Bakta

*Division of Hematology and Medical Oncology, Internal Medicine Department Udayana University, Sanglah General Hospital Denpasar, Denpasar, Bali, Indonesia*

**Citation:** Purnamasidhi CAW, Suega K, Bakta IM. Association between Lactate Dehydrogenase Levels to the Response of Non - Hodgkin Lymphoma in Elderly Patients Who Treated with First - Line Chemotherapy in Sanglah General Hospital. Open Access Maced J Med Sci. 2019 Jun 30; 7(12):1984-1986. https://doi.org/10.3889/oamjms.2019.470

**Keywords:** Prognosis; Non-Hodgkin Lymphoma; Elderly; LDH level

***Correspondence:** Cokorda Agung Wahyu Purnamasidhi. Division of Hematology and Medical Oncology, Internal Medicine Department Udayana University, Sanglah General Hospital Denpasar, Denpasar, Bali, Indonesia. E-mail: purnamasidhi@unud.ac.id

**Received:** 01-Apr-2019; **Revised:** 03-Jun-2019; **Accepted:** 04-Jun-2019; **Online first:** 30-Jun-2019

**Copyright:** © 2019 Cokorda Agung Wahyu Purnamasidhi, Ketut Suega, I Made Bakta. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

**Funding:** This research did not receive any financial support

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

**Abstract**

**BACKGROUND:** Non-Hodgkin Lymphoma (NHL) is a malignant haematological disease originates in the lymphocytes, caused by an abnormality in lymphocytes development which forms a tumour and may become cancer. Chemotherapy is the main treatment modality for aggressive lymphoma, but only a few patients achieve remission. Several factors such as age, clinical stadium, number of extranodal regions, and Lactate Dehydrogenase (LDH) level played a role in determining response to chemotherapy.

**AIM:** To measure the association between LDH levels to prognosis of NHL in elderly patients who treated with rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone chemotherapy in Sanglah General Hospital.

**METHODS:** This study used a retrospective descriptive study on elderly NHL patients in Sanglah General Hospital from January until December 2014. The evaluation was measured using the IPI score to determine the prognosis of patients. Demographic data, the stadium, extranodal region, LDH level, and response to chemotherapy were recorded.

**RESULTS:** Twenty-five patients were included in the study. The age ranged was between 61-76 years old (Mean 65,68 ± 4,7 years; Median 65 years). The number of male patients was 19 (76%). Diffuse Large B-Cell Lymphoma (DLBCL) is the most common histopathological structure observed on the patients (68%). LDH levels were normal in 51.6% of the patients and considered high in the rests (48.4%). Results of the chemotherapy were a good response in 72.2%. Compared to the patients who showed complete response to chemotherapy, patients with no response (partial response and progression) had significantly higher levels of LDH (OR: 13,1; 95%CI: 1,36-126,30; p = 0,001).

**CONCLUSION:** Non-Hodgkin Lymphoma in elderly patients with no response to chemotherapy had significantly higher levels of LDH than patients with complete response.

## Introduction

Lactate dehydrogenase (LDH) is a metabolic enzyme widely expressed in different tissues and is detectable in serum, which catalyses the interconversion of pyruvate and lactate during glycolysis and gluconeogenesis [1]. Since a long time, it has been observed that a high level of serum LDH is seen in patients with different malignancies. Increased LDH levels have been reported in solid tumours, leukaemia and diffuse lymphoma, particularly Burkitt's lymphoma, although correlation has been established with any specific neoplastic disease or with any clinical or histologic parameter. The elevation of LDH in the blood is a relatively nonspecific phenomenon; however, it has been recognized as a tumour marker, as it reflects tumour burden and cellular turnover in several aggressive malignancies, including germ cell tumours [2], sarcomas [3], [4] and non-Hodgkin lymphoma (NHL) [5], [6].

Patients with elevated serum LDH at the time of initial diagnosis have inferior survival outcomes, compared to those with normal LDH levels. Therefore, LDH has been a component of the International Prognostic Index (IPI) [7], a clinical tool for predicting the prognosis of patients with aggressive NHL.

However, the role of serum LDH beyond initial diagnosis, i.e. during active chemotherapy and the post-treatment follow-up period, has not yet been well defined.

In this study, we conducted a retrospective analysis of the prognostic role of serum LDH after the treatment of elderly patients within a homogeneous disease population of NHL, undergoing a standard treatment, three weekly treatments of rituximab with cyclophosphamide, doxorubicin, vincristine and prednisolone (R-CHOP) immunochemotherapy.

## Material and Methods

Among the patients who fulfilled the inclusion criteria, patients who underwent LDH testing after R-CHOP therapy were selected. The LDH level was tested no more than 5 days after therapy. This study used a retrospective descriptive study on elderly NHL patients in Sanglah General Hospital from January until December 2014. The evaluation was measured using the IPI score to determine the prognosis of patients. Demographic data, histopathology, ECOG performance status, standard IPI, LDH level, and response to chemotherapy were recorded. We used RECIST (Response Evaluation Criteria in Solid Tumours) criteria for response therapy result.

## Results

Twenty-five patients were included in the study. The age ranged was between 61-76 years old, with mean 65,68 ± 4,7 years and median 65 years. The number of male patients was 19 (76%), and female patients were 6 (24%). A summary of the baseline characteristics of the entire patient population and the groups is shown in Table 1.

**Table 1. Patient characteristics**

| Characteristics | Number of Patients (n = 25) | Percentage |
|---|---|---|
| Gender | | |
| Male | 19 | 76% |
| Female | 6 | 24% |
| Age, years | | |
| Median (range) | 65 (61-76) | |
| > 65 | 11 | 44% |
| ≤ 65 | 14 | 56% |
| LDH | | |
| Normal | 13 | 51,6% |
| High | 12 | 48,4% |
| ECOG performance status | | |
| 0-1 | 15 | 60% |
| 2-4 | 10 | 40% |
| Standard IPI | | |
| Low | 10 | 40% |
| Low-intermediate | 7 | 28% |
| High-intermediate | 6 | 24% |
| High | 2 | 8% |
| Tumor response | | |
| Complete response | 18 | 72% |
| Partial response | 5 | 20% |
| Progression | 2 | 8% |

ECOG = Eastern cooperative oncology group.

Diffuse Large B-Cell Lymphoma (DLBCL) is the most common histopathological structure observed on the patients (68%), continued with follicular lymphoma (20%), diffuse mixed small and large cell (8%), and large anaplastic cell (4%) (Figure 1).

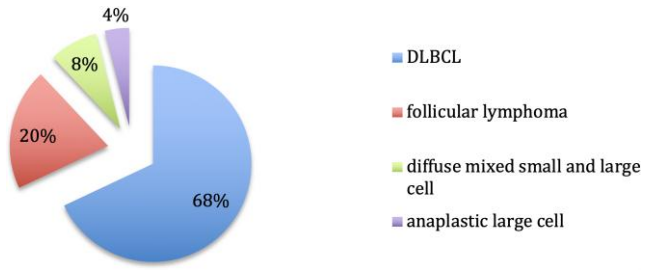



*Figure 1: Histopathology of NHL elderly patients*

LDH levels were normal in 51.6% of the patients and considered high in the rests (48.4%). Results of the chemotherapy were complete response is 72.2%, a partial response is 20%, and progression tumour is 8%. Because we used a t-test, so patient with partial response and progression tumour, we count in one group to no response therapy (Table 2).

**Table 2: Difference of LDH Level between response and no-response patient**

| Groups | N | LDH (Mean ± SD | t-test | |
|---|---|---|---|---|
| | | | t | p |
| No Response | | 7 781.63 ± 25 | | |
| Complete Response | 18 | 444.30 ± 21 | 7.47 | 0.001 |

Compared to the patients who showed good response to chemotherapy, patients with no response had significantly higher levels of LDH (OR: 13,1; 95%CI: 1,36-126,30; p = 0,001) (Table 3).

**Table 3: Association between LDH level and therapy response**

| LDH Level | Therapy Response | | Total |
|---|---|---|---|
| | No Response | Complete Response | |
| High | 6 (42.8%) | 8 (57.7%) | 14 (56%) |
| Normal | 1 (9%) | 10 (91%) | 11 (44%) |
| Total | 7 (27.8%) | 18 (72.2%) | 25 (100%) |

## Discussion

Increased total serum LDH is commonly interpreted as reflecting high tumour burden or tumour aggressiveness. Increased serum LDH has a major prognostic as well as diagnostic significance in a patient with NHL, and total serum LDH is one of the parameters of International Prognostic Index (IPI) used in a patient with NHL [1], [8].

Notably, the prognostic role of serum LDH in oncology has long been recognised. LDH is a key enzyme in the process of energy production in cancer cells; it catalyses the conversion of pyruvate to lactate in hypoxic conditions [9], [10], [11]. Since its function

in anaerobic metabolism, cancer cells grow even after their rapid proliferation that leads to low-oxygen conditions in the tumour microenvironment [12], [13]. Thus, LDH plays an important role in tumour progression and maintenance.

In non-Hodgkin's lymphoma, there has been only a low number of studies on serum LDH as a prognostic factor. Ferraris et al., in a study of 41 patients, reported that an elevated serum LDH was correlated with a shorter surviva1 in al1 the histological types. In another study, Schneider et al. found that pretreatment serum LDH was the single most important prognostic variable for survival in 30 patients with diffuse HL [14], [15]. We analysed 25 consecutive elderly patients with NHL. After the R CHOP Chemotherapy, serum LDH was high in 14 patients (%). Compared to the patients who showed a good response to chemotherapy, patients with no response had significantly higher levels of LDH. In our study, patients with increased LDH (more than 250 U/l) experienced a poorer response to therapy.

This study had several limitations. The enrolled patients were restricted to one local hospital, and the sample was relatively small to justify the effect of multiple clinical features on survival. The prognostic value of LDH level should be evaluated in a larger, multicenter setting.

In conclusion, non-Hodgkin Lymphoma (NHL), a cancer of lymphocytes with a preponderance in sixth to the seventh decade of life range, should be paid on the use of additional parameters like LDH levels, estimation of which can be used for prognostic evaluation of patients with NHL. NHL patients with no response to chemotherapy had significantly higher levels of LDH than patients with good response patient.

## References


1. Schneider RJ, Seibert K, Passes S, Little C, Gee T, Lee BJ, Mike V, Young CW. Prognostic significance of serum lactate dehydrogenase in malignant lymphoma. Cancer. 2006; 46:139-43. https://doi.org/10.1002/1097-0142(19800701)46:1<139::AID-CNCR2820460122>3.0.CO;2-8

2. Schmoll HJ, Souchon R, Krege S, Albers P, Bey- er J, Kollmannsberger C, Fossa SD, Skakkebaek NE, de Wit R, Fizazi K, Droz JP, Pizzocaro G, Daugaard G, de Mulder PH, Horwich A, Oliver T, Huddart R, Rosti G, Paz Ares L, Pont O, Hartmann JT, Aass N, Algaba F, Bamberg M, Bodrogi I, Bokemeyer C, Classen J, Clemm S, Culine S, de Wit M, Derigs HG, Dieckmann KP, Flasshove M, Garcia del Muro X, Gerl A, Germa-Lluch JR, Hartmann M, Heidenreich A, Hoeltl W, Joffe J, Jones W, Kaiser G, Klepp O, Kliesch S, Kisbenedek L, Koehrmann KU, Kuczyk M, Laguna MP, Leiva O, Loy V, Mason MD, Mead GM, Mueller RP, Nicolai N, Ooster- hof GO, Pottek T, Rick O, Schmidberger H, Sedlmayer F, Siegert W, Studer U, Tjulandin S, von der Maase H, Walz P, Weinknecht S, Weissbach L, Winter E, Wittekind C: European consensus on diagnosis and treatment of germ cell cancer: a report of the European Germ Cell Cancer Consensus Group (EGCCCG). Ann Oncol. 2004; 15:1377-1399. https://doi.org/10.1093/annonc/mdh301 PMid:15319245

3. Bacci G, Avella M, McDonald D, Toni A, Or- landi M, Campanacci M: Serum lactate dehy- drogenase (LDH) as a tumor marker in Ew- ing's sarcoma. Tumori. 1988; 74:649-655. https://doi.org/10.1177/030089168807400606 PMid:3232209

4. Gonzalez-Billalabeitia E, Hitt R, Fernandez J, Conde E, Martinez-Tello F, Enriquez de Sala- manca R, Cortes-Funes H: Pre-treatment se- rum lactate dehydrogenase level is an impor- tant prognostic factor in high-grade extremity osteosarcoma. Clin Transl Oncol. 2009; 11:479-483. https://doi.org/10.1007/s12094-009-0388-9

5. International Non-Hodgkin's Lymphoma Prognostic Factors Project. A predictive model for aggressive non-Hodgkin's lymphoma. New England Journal of Medicine. 1993; 329(14):987-94. https://doi.org/10.1056/NEJM199309303291402 PMid:8141877

6. Al-Saadoon EA, Al-Naama LM, Hassan JK. Serum Lactate Dehydrogenase (LDH) Activity in Children with Malignant Diseases. Bahrain Medical Bulletin. 2003; 25(2):1-7.

7. Tilly H, Dreyling M. Diffuse large B-cell non- Hodgkin's lymphoma: ESMO clinical recom- mendations for diagnosis, treatment and follow-up. Ann Oncol. 2009; 20(suppl 4):110-112. https://doi.org/10.1093/annonc/mdp145 PMid:19454426

8. Augoff K, Hryniewicz-Jankowska A, Tobola R. Lactate dehydro-genase 5: an old friend and a new hope in the war on cancer. Cancer Lett. 2015; 358(1):1-7. https://doi.org/10.1016/j.canlet.2014.12.035 PMid:25528630

9. Jurisic V, Konjevic G, Baicevic, Duricic B, Spuzic I. Different alterations in lactate dehydrogenase activity and profile of peripheral blood mononuclear cell in Hodgkin's and non-Hodgkin's lymphoma. Eur J Haematol. 2000; 64:259-66. https://doi.org/10.1034/j.1600-0609.2000.90117.x PMid:10776698

10. El-Sharkawi D, Basu S, Ocampo C, Qian W, D'sa S, Hoskin PJ, Ardeshna KM. Elevated lactate dehydrogenase levels detected during routine follow-up do not predict relapse in patients with diffuse large B-cell lymphoma who achieve complete remission after primary treatment with rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone-like immunochemotherapy. Leukemia & lymphoma. 2012; 53(10):1949-52. https://doi.org/10.3109/10428194.2012.679360 PMid:22462615

11. Cheson BD, Pfistner B, Juweid ME, Gascoyne RD, Specht L, Horning SJ, Coiffier B, Fisher RI, Hagenbeek A, Zucca E, Rosen ST, Stroobants S, Lister TA, Hoppe RT, Dreyling M, Tobinai K, Vose JM, Connors JM, Federi- co M, Diehl V: Revised response criteria for malignant lymphoma. J Clin Oncol. 2007; 25:579-586. https://doi.org/10.1200/JCO.2006.09.2403 PMid:17242396

12. Le A, Cooper CR, Gouw AM, Dinavahi R, Maitra A, Deck LM, et al. Inhibition of lactate dehydrogenase A induces oxidative stress and inhibits tumor progression. Proc Natl Acad Sci USA. 2010; 107:2037-42. https://doi.org/10.1073/pnas.0914433107 PMid:20133848 PMCid:PMC2836706

13. Hong J, Park S, Park J, Kim HS, Kim KH, Ahn JY, Rim MY, Jung M, Sym SJ, Cho EK, Shin DB, Lee JH: Evaluation of prognostic values of clinical and histopathologic characteristics in diffuse large B-cell lymphoma treated with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone therapy. Leuk Lymphoma. 2011; 52:1904-1912. https://doi.org/10.3109/10428194.2011.588761 PMid:21718130

14. Ferraris AM, Giuntini P, Gaetani GF. Serum lactic dehydrogenase as a prog- nostic tool for non-Hodgkin% lymphomas. Blood. 1979, 54:928-932.

15. Schneider RJ, Seibert K, Passes S, Little C, Gee T, Lee BJ, Mike V, Young CW. Prognostic significance of serum lactate dehydrogenase in malignant lymphoma. Cancer. 2006; 46:139-43. https://doi.org/10.1002/1097-0142(19800701)46:1<139::AID-CNCR2820460122>3.0.CO;2-8

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Jun 30; 7(12):1987-1990.
https://doi.org/10.3889/oamjms.2019.471
eISSN: 1857-9655
***Case Report***

# Neuroretinitis Syphilis in Human Immunodeficiency Virus-Infected Patient

AA Mas Putrawati Triningrat*, Ni Made Wasiastiti Budi, IGAM Juliari, Ni Made Ayu Surasmiati, Siska Siska, IGA Ratna Suryaningrum

*Department of Ophthalmology, Faculty of Medicine, Udayana University, Bali, Indonesia*

**Citation:** Triningrat AAMP, Wasiastiti Budi NM, Juliari IGAM, Surasmiati NMA, Siska S, Ratna Suryaningrum IGA. Neuroretinitis Syphilis in Human Immunodeficiency Virus-Infected Patient. Open Access Maced J Med Sci. 2019 Jun 30; 7(12):1987-1990. https://doi.org/10.3889/oamjms.2019.471

**Keywords:** Neuroretinitis; Syphilis; HIV

***Correspondence:** AA Mas Putrawati Triningrat. Department of Ophthalmology, Faculty of Medicine, Udayana University, Bali, Indonesia. E-mail: agrasidi01@gmail.com

**Received:** 10-Apr-2019; **Revised:** 29-May-2019; **Accepted:** 30-May-2019; **Online first:** 25-Jun-2019

**Copyright:** © 2019 AA Mas Putrawati Triningrat, Ni Made Wasiastiti Budi, IGAM Juliari; Ni Made Ayu Surasmiati, Siska Siska, IGA Ratna Suryaningrum. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

**Funding:** This research did not receive any financial support

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

**Abstract**

**BACKGROUND:** In HIV-infected patient who accompanied by syphilis often difficult to diagnose and difficult to treat. The aim is to diagnostics understanding and to optimise the management and response therapy in patients with neuroretinitis syphilis in HIV-infected patients.

**CASE PRESENTATION:** A 53-years old, bisexual, male patient whose initial presentation was a blurry vision on the left eye. History of a painless genital lesion, HIV infection (+) on ARV therapy. The visual acuity of hand movement (HM), RAPD (+), with vitreous opacities and optic disc swelling. The OCT RNFL showed neural layer thickening in all areas. VEP showed increased P100 latency, normal head and orbital CT scan. High VDRL and TPHA titer. Lumbar puncture examination showed non-reactive VDRL. Treated with topical prednisolone eye drops, oral neurotropic vitamin, and intramuscular injection of Benzathine Penicillin G. Diagnosed with OS neuroretinitis *et causa* syphilis infection, HIV stage II on HAART. Follow up in 2 months, the visual acuity improved, and serology post-therapy VDRL was decreased.

**CONCLUSION:** High accuracy is needed for screening signs and symptoms in syphilis patients because of the varied clinical manifestations. Ocular syphilis manifestation in HIV has a higher risk for neurologic complications and the risk of failing treatment with the standard regimen.

## Introduction

Optic neuritis is an inflammation of the optic nerve which is caused by the demyelinating process. Based on its location, optic neuritis can be categorised into retrobulbar neuritis, papillitis, polyneuritis, and neuroretinitis. Neuroretinitis typically occurs in the third or fourth decade with symptoms of blurry vision in one or both eyes [1], [2], [3], [4].

The most common cause of neuroretinitis is cat scratch disease, toxoplasmosis, leptospirosis, mumps, herpes simplex, salmonella, Lyme disease, and syphilis. Syphilis is a well-known infectious and chronic disease caused by Treponema pallidum. Syphilis has numerous presentations and can imitate many other infections, in advanced stages can caused immune-mediated processes. Hence, it has earned the nickname “The Great Imitator” [7], [8].

The incidence of ocular syphilis is 231 cases (0.65%) of all syphilis cases at 2015, with the proportion of men who have sexual intercourse with men and HIV-infected patients which is consistent with syphilis epidemiology in the United States. The incidence of ocular syphilis in Indonesia is very rare; only 0.3% of the total incidence of syphilis. Involvement of ocular in syphilis infection mostly occurs unconsciously, with most often clinical sign is decreased visual acuity [4], [5], [6].

Antibiotic gives better results on neuroretinitis syphilis, but corticosteroids usages have been controversial. The goal of this case report is to understand the diagnosis and management of syphilis neuroretinitis in HIV-infected patients and the results of the therapy given.

## Case Presentation

A 53-years old, bisexual, male patient whose initial presentation was a blurry vision on the left eye for seven months and getting worse since last month.

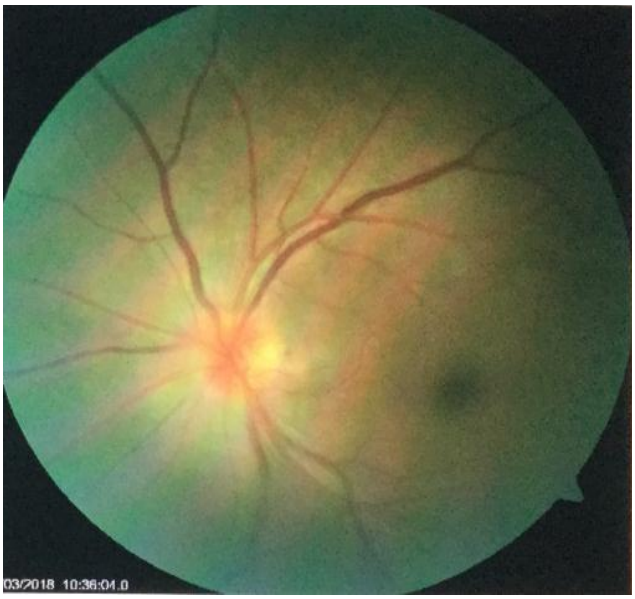



*Figure 1: Fundus photography at first examination on the left eye when the patient first came to the ophthalmology clinic.*

History of the genital wound with discharge three years ago, but resolve without treatment. The last unprotected sex was three years ago. History of decreasing body weight of about ten kilograms in two months. On that time, the patient was diagnosed with HIV infection. The last absolute CD4+ count 220 cells/uL. The patient was on treatment antiretroviral (ARV).

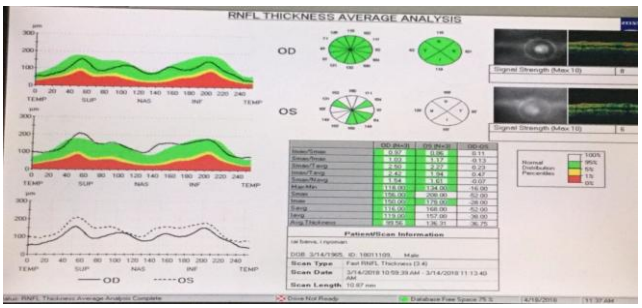



*Figure 2: Optical Coherence Tomography (OCT) on the left eye when the patient first came to the ophthalmology clinic.*

Ophthalmology examinations on the left eye, the visual acuity of hand movement (HM), relative afferent pupillary defect (RAPD) positive, with vitreous opacities, optic disc swelling, and not well-demarcated optic disc border. Cup disc ratio (CDR) difficult to evaluate, the arterio-venous ratio is 2:3. Macula reflex positive. Intraocular pressure was normal. Contrast sensitivity, Visual field, Ishihara test, and Farnsworth can't be evaluated. The Optical Coherence Tomography (OCT) examination showed neural layer thickening in all areas with central macular thickness was 386 µm.

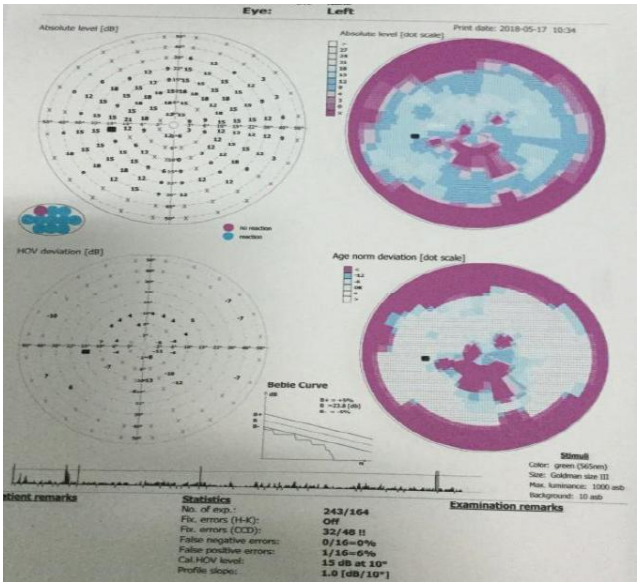



*Figure 3: Visual Field Findings on the left eye when the patient first came to the ophthalmology clinic*

The patient is referring to the Department of Dermatology and Venereology and Department of Neurology. The laboratory results showed the titer of VDRL 1: 2048, TPHA 1: 5120. Visually Evoked Potential (VEP) showed increased P100 latency, normal head and orbital CT scan.

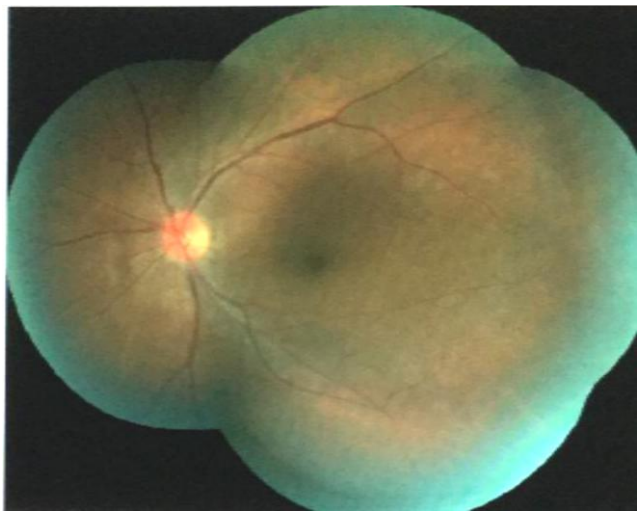


*Figure 4: Fundus photography after two months of treatment on the left eye*

The patient was treated with topical prednisolone eye drops and oral neurotropic vitamin.

The patient has been monitored for the duration of treatment. Follow up in two months showed visual acuity became 6/15 and 6/12 with a pinhole on the left eye, CDR 0.3, the arterio-venous ratio is 2:3. Macula reflex positive. Intraocular

pressure was normal. Contrast sensitivity, visual field, Ishihara test, and Farnsworth were within normal limits. The OCT examination was normal. Central macular thickness had been decreased to 376 µm. Serology VDRL 1:512 after one-month post treatment.

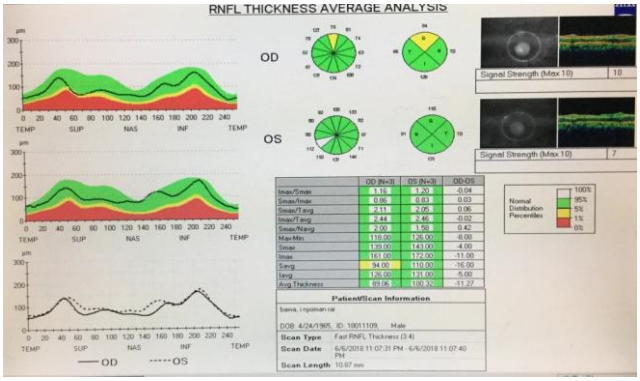



*Figure 5: Optical Coherence Tomography (OCT) retinal nerve fibre layer (RNFL) after two months of treatment on the left eye*

## Discussion

Syphilis is a well-known infectious and chronic disease caused by *Treponema pallidum*. Syphilis has numerous presentations and can imitate many other infections, in advanced stages can caused immune-mediated processes. Hence, it has earned the nickname "*The Great Imitator*" [7], [8].

Men are affected more frequently with primary or secondary syphilis than women. The past decade has found a significant increase in syphilis cases among men, driven mostly by the Men who have sex with men (MSM) community. MSM accounted for 83.7% of all syphilis cases in the United States [9].

Based on its location, optic neuritis can be categorised into retrobulbar neuritis, papillitis, polyneuritis, and neuroretinitis. The most common causes of neuroretinitis are cat scratch disease, toxoplasmosis, leptospirosis, mumps, herpes simplex, salmonella, Lyme disease, and syphilis. Neuroretinitis typically occurs in the third or fourth decade with symptoms of blurry vision in one or both eyes [10].

Decreasing visual acuity mostly followed by visual field disturbance in central scotoma or cecocentral scotoma. The specific sign found in neurosyphilis is weakened of pupillary (pupil response to light slowly disappear) but responds to accommodation, this condition called Argyll Robertson Pupil [10].

A further diagnostic examination that can be done was OCT, perimetry, VEP, fundus fluorescein angiography (FFA), complete laboratory examination. Generally, VEP examination of optic neuritis shows P100 extended, latency, and relatively normal amplitude. P100 extended due to signals interference to the sensory system due to decrease in conduction velocity and the presence of lesions that make deceleration of axon, those conditions were found in 90% of cases [4], [11], [12].

Syphilis neuroretinitis is confirmed by a serological test. Unlike other bacteria, *Treponema pallidum* cannot be cultured. The serological test can be divided into two, which is Non-Treponemal Test and Treponemal Test. Non-Treponemal Test such as Venereal Diseases Research Laboratory test (VDRL) used for screening and monitoring therapy. Treponemal test such as the Treponemal Pallidum Haemagglutination Assay Test (TPHA), used as confirmed test for syphilis because it has a higher sensitivity and specificity, it can detect antibodies in small amounts, and their appearance will last for a lifetime, but this test cannot be used as therapeutic monitoring [13], [14].

The differential diagnosis of neuritis syphilis is papillary oedema and compressive optic neuropathy. Papil oedema is most frequently caused by increased intracranial pressure. Usually patients complain of headache, nausea and vomiting. In acute papillary oedema, optic nerve function, sharp sharpening and colour vision are generally normal. RAPD can be normal, bilateral, with an enlarged blind spot. Compressive optic neuropathy was found in patients with intraorbital or intracranial compression lesions with a sharp decrease in progressive exposure. RAPD is found and loses the monocular field of view [2].

Syphilis patient in HIV-infected has a higher risk to be a neurologist complication and failure outcomes with standard therapy. Some researcher showed that antiretroviral could improve the HIV-infected patient with syphilis [15].

The prognosis of syphilis in HIV-infected patient is influenced by therapy for both syphilis and HIV infections. Neurological monitoring and examination should be done when the patient was on treatment because it can lead to neurosyphilis complications in HIV-infected patient. Recurrency and failure of treatment in HIV-infected patients are higher than patients without HIV. So, clinical and serological monitoring need to be done [13], [16], [17], [18].

Patients were informed to continue the antiretroviral treatment, advised not having sex, especially unprotected sex.

In conclusion, ocular syphilis manifestation in HIV has a higher risk for neurologic complications and the risk of failing treatment with the standard regimen. This patient was treated with Benzathine Penicillin G, topical prednisolone eye drops, oral neurotropic vitamin and antiretroviral. At the end of follow up, showed improvement of visual acuity and decreased of VDRL titer.

## References


1. Renuka DO. Neuroretinitis. Kerala Journal of Ophthalmology. 2006; 17:7-13

2. Hadieh H, Bagherkashi F. Optic neuritis, its Differential. 2012:178-188.

3. Biradar S, Warad VG. Spontaneous resolution of severe neuroretinitis following a febrile illness. Al Ameen J Med Sci. 2015; 8(2):164-167.

4. American Academy of Opthalmology. Staf b. Neuro ophthalmology. Basic and Clinical Science Course. Sec 5. San Fransisco. 2014-2015: 319-353.

5. Parker SE, Pula JH. Neurosyphilis Presenting as Asymptomatic Optic Perineuritis. 2012. https://doi.org/10.1155/2012/621872 PMid:22606498 PMCid:PMC3350282

6. Rodrigues RP, Correia N, Lopes AV. Neurosyphilis with optical involvement in an immunocompetent patient: a case report. International medical case reports journal. 2012; 5:5-11. https://doi.org/10.2147/IMCRJ.S26281 PMid:23754916 PMCid:PMC3658247

7. Cohen SE, Klausner JD, Engelman J, Philip S. Syphilis in the modern era: An update for physicians. Infect Dis Clin N Am. 2014; 27:705-722. https://doi.org/10.1016/j.idc.2013.08.005 PMid:24275265

8. Kats KA. Syphilis. In: Goldsmith L.A, Kats S.I, Gilcherest B.A, Paller A.S, Leffell D.J, eds. Fitzpatrick Dermatology In General medicine. 2012:2367-82.

9. Patton ME, Su JR, Nelson R, Weinstock H. Primary and secondary syphilis-United States, 2005-2013. MMWR. Morbidity and mortality weekly report. 2014; 63(18):402-6.

10. Swallow C. Optic Neuritis, 2013. http://www.emedicine.com/radio/topic 488.hmt.

11. Schiffman. Neuro Ophthalmic Manifestations of Basilar Meningitis, 2015.

12. Tunkel AR, Hartman BJ, Kaplan SL. Practice guidelines for the management of bacterial meningitis. Pada: Clinical Infectious Diseases. Boston: McGraw-hill. 2004:1267-84. https://doi.org/10.1086/425368 PMid:15494903

13. Hicks CB, Hynes NA, Mitty J. Diagnostic testing for syphilis, 2018.

14. Sato NS. Laboratorial diagnosis of syphilis. In Syphilis-recognition, description and diagnosis, 2011. https://doi.org/10.5772/840

15. Workowski KA, Bolan GA. Sexually Transmitted Disease Treatment Guidelines. Centres for Disease Control and Prevention Recommendation and Report. MMWR. 2015; 64(30):34-9.

16. Centres for Disease Control and Prevention. Syphilis - 2015 STD Surveillance, 2016.

17. Ghanem KG, Workowski KA. Management of Adult Syphilis. Clinical Infectious Disease. 2011; 53(3):S110-S128. https://doi.org/10.1093/cid/cir701 PMid:22080265

18. Tuddenham S, Ghanem KG. Ocular Syphilis: opportunities to address important unanswered question. Sex Transm Infect. 2016; 92(8):563-564. https://doi.org/10.1136/sextrans-2016-052570 PMid:27116973 PMCid:PMC5554954

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Jun 30; 7(12):1991-1997.
https://doi.org/10.3889/oamjms.2019.582
eISSN: 1857-9655
***Case Report***

# A Rare Case of Partial Peripheral Thyroid Hormone Resistance Due to a Point Mutation in the Membrane Integrin A(V)B(3) and Concomitant Hashimoto`s Thyroiditis

Martina Savovska[1*], Sinisa Stojanoski[2], Nevena Manevska[2]

*[1]Medical Faculty, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia; [2]Institute for Pathophysiology and Nuclear Medicine "Isak Tadzer", Medical Faculty, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia*

**Citation:** Savovska M, Stojanoski S, Manevska N. A Rare Case of Partial Peripheral Thyroid Hormone Resistance Due to a Point Mutation in the Membrane Integrin A(V)B(3) and Concomitant Hashimoto`S Thyroiditis. Open Access Maced J Med Sci. 2019 Jun 30; 7(12):1991-1997.
https://doi.org/10.3889/oamjms.2019.582

**Keywords:** peripheral resistance; hypothyroidism; secondary hyperthyroidism; Hashimoto's thyroiditis

***Correspondence:** Martina Savovska. Medical Faculty, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia. E-mail: m.savovska98@gmail.com

**Received:** 27-May-2019; **Revised:** 26-Jun-2019; **Accepted:** 27-Jun-2019; **Online first:** 29-Jun-2019

**Copyright:** © 2019 Martina Savovska, Sinisa Stojanoski, Nevena Manevska. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

**Funding:** This research was financially supported by the Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia

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

**Abstract**

**BACKGROUND:** Peripheral resistance to thyroid hormones is a type of unresponsiveness of the peripheral cells or tissues to FT3 and/or FT4. Generalised resistance to thyroid hormones affects the pituitary gland and most of the peripheral tissues. Selective pituitary resistance or central resistance to thyroid hormones include unresponsiveness of the pituitary gland, but the peripheral tissues are responsive. Selective peripheral resistance involves peripheral tissue or cellular resistance to thyroid hormones, but the pituitary gland is responsive.

**CASE PRESENTATION:** We present a rare case of a female patient with partial peripheral resistance to thyroid hormones due to a point mutation coding for the beta subunit of the integrin molecule α(V)β(3) and concomitant Hashimoto`s thyroiditis. Clinically, the patient`s symptoms were in favour of hypothyroidism, and the laboratory results were in favour of the secondary hyperthyroid state. PCR protein amplification detected a point mutation coding for the membrane receptor, which mediates a signal via the MAPK pathway when bonded with thyroid hormones.

**CONCLUSION:** Peripheral resistance to thyroid hormones is a very rare condition and can often be misdiagnosed due to the broad spectrum of clinically similar differential diagnostic entities. Molecular analysis is required to confirm the exact underlying cause for the impaired peripheral sensitivity to thyroid hormones syndrome.

## Introduction

Thyroid hormones, peptide-like in structure but both peptide-like and steroid-like in function, are indispensable for normal fetal development and responsible for the regulation of the tissues basal metabolism. The bioactive form at the cellular level is T3/FT3 (triiodothyronine/free triiodothyronine), a final product of the processes of the outer ring deiodination of the prohormone T4/FT4 (tetraiodothyronine/free tetraiodothyronine). Selenoenzymes (deiodinases) perform this peripheral conversion [1]. Even though the structure of the thyroid hormones is peptide in nature (two iodotyrosine components are fused into one thyronine molecule), the peripheral effects are expressed or mimic the activity of both peptide-like hormones (via the membrane receptors or membrane transporter proteins and consecutive second messenger cascade activation) and steroid-like hormones (via the nuclear receptors and binding to hormone response elements (HREs) with consecutive induction of transcription and gene expression modulation) [2].

The nuclear receptors or thyroid hormones (THR) are presented in two different protein forms α and β, encoded by the THRA and THRB genes located at chromosomes 3 and 17, respectively. Their tissue distribution is not equal, as they undergo splicing at several points and are positioned at different locations in the body. The main isoforms of the THR responsible for mediating cell response when bound to thyroid hormones are THRα1 – widely expressed in the bones, skeletal and cardiac muscles, THRβ1 – expressed in the kidneys, brain, liver, and THRβ2 – expressed in the anterior pituitary, hypothalamus, and retina [3].

Thyroid hormone deficit (hypothyroidism) is not clinically manifested for a few months, even after total thyroidectomy being performed, mostly due to their steroid-like actions (induction of transcription and gene expression modulation) and also due to the release of free hormones fractions from the total hormones fractions bound to the specific hormone carriers: prealbumin, albumin or thyroxine-binding globulin (TBG). The fractions of thyroid hormones bound to their carriers are termed as circulating ``thyroid hormone reserve`` or total hormone fractions (TT4 and TT3), and those that circulate unbound in the bloodstream are referred to as free hormone fractions (FT4 and FT3). The peripheral cellular and tissue effects of thyroid hormones are mostly due to the levels and actions of the free hormone fractions [4].

Peripheral resistance to thyroid hormones is a type of unresponsiveness of the peripheral cells or tissues to FT3 and/or FT4. Thyroid hormone resistance (RTH) in general, or sometimes called as Refetoff syndrome, is a condition where thyroid hormone levels are elevated and fail to suppress the production of TSH (fully or partially) at the level of the pituitary gland. Refetoff et al. suggested using a new nomenclature referring to the condition as "impaired sensitivity to thyroid hormones" [5]. It can occur in several forms. Generalised resistance to thyroid hormones (GRTH), which is affecting the pituitary gland and most of the peripheral tissues, is usually consistent with a clinically euthyroid state but with elevated T3 and T4 levels in the blood, as well as high or normal TSH. Selective pituitary resistance or usually termed as central resistance to thyroid hormone (CRTH or PRTH), includes unresponsiveness of the pituitary gland to thyroid hormones, but the peripheral tissues are responsive. It is manifested with elevated serum thyroid hormone levels, and normal or elevated TSH levels, but the peripheral tissues experience an abnormal influx of thyroid hormones and is clinically associated with symptoms of hyperthyroidism. Selective peripheral resistance to thyroid hormone (PerRTH) involves peripheral tissue or cellular resistance to thyroid hormones, but the pituitary gland is responsive. Clinically the patients present symptoms typical for hypothyroidism, which usually does not correlate with the circulating hormone levels – elevated TSH, FT3, and FT4 [5].

Integrins are part of the transmembrane proteins family that are involved in the processes of adhesion between the cells and the extracellular matrix termed intercellular adhesion molecules (ICAMs). Generally, they recognise a tripeptide Arg – Gly – Asp (RGD) binding site when connected to their ligands. A typical integrin molecule is built by two non covalently connected glycoprotein subunits α and β, respectively [6]. The structure of the integrins encompasses unique heterodimers with an immense variety of ligand recognition, bondage, and response. Overall, they are built by at least eighteen α and eight β subunits, which are afterwards subgrouped depending on their specific role and tissue distribution. The most typical forms of integrin association include the β1 integrins, β2 integrins, and αv-containing integrins [7].

The integrin, α(V)β(3), is a heterodimer made up by an α(V) (also called CD51, MSK8, vitronectin receptor α (VNRα)) 1048 long amino acid sequence located on chromosome 2q31.32, and β(3) (also called CD61, GP3A, GPIIIa, platelet glycoprotein IIIa) with 788 long amino acid sequence, located on chromosome 17q21.32 [7]. Furthermore, the extracellular portion of the integrin consists of multiple domains, including RGD-recognizing site. It is widely distributed in the body, and upon binding to a specific ligand, it mediates a secondary intracellular response that modulates the cellular activity. Ligands for this specific integrin encompass: fibrinogen, vitronectin, vWF, thrombospondin, fibrillin, tenascin, PECAM-1, fibronectin, osteopontin, BSP, MFG-E8, ADAM-15, COMP, Cyr61, ICAM-4, MMP, FGF-2, uPA, uPAR, L1, angiostatin, plasmin, cardiotoxin, LAP-TGF-β, Del-1, including thyroid hormone peripheral recognition and bondage as well [8].

## Case Presentation

A 36 years old female patient, I.I. was referred for evaluation of the function of the thyroid gland. The anamnesis gave information about a high degree of education, no children and a positive family history of hypothyroidism due to Hashimoto`s thyroiditis in mother and sister. She presented symptoms and clinical signs that pointed towards a hypothyroid state such as recent weight gain, constipation, dryness of hair and fickle nails, but mostly, inability to do her daily routine due to feeling tired and sleepy all the time. A recent progressive swelling in the neck that resulted in having swallowing difficulties was also highlighted. There were no previous records of her having any thyroid hormone dysregulation, so a thyroid function laboratory panel was ordered in June 2015. The results presented extremely high levels of TSH, along

with elevated FT3, FT4, TT3, and TT4. A biochemical laboratory panel that was typical for a severe possible secondary hyperthyroid state (Table 1).

**Table 1: Biochemical laboratory results**

| | Reference value | Patients results |
|---|---|---|
| TSH | 0.4 - 4.0 mIU/L | >75 mIU/L |
| FT3 | 3.5 - 7.8 pmol/L | 30.7 pmol/L |
| FT4 | 11 - 25 pmol/L | > 60 pmol/L |
| TT3 | 0.9-1.9 nmol/L | 2.34 nmol/L |
| TT4 | 71-141 nmol/L | 203 nmol/L |
| TGL | <1.7 mmol/L | 3.65 mmol/L |
| Cholesterol | <5.5 mmol/L | 6.6 mmol/L |
| Fe | 17-30 micromol/L | 2.9 micromol/L |
| Hgb | 13.5 - 17.5 g/dL (m) 12 - 15.5 g/dL (f) | 8.6 g/dL |
| Prealbumin | 150 - 360 mg/L | 213 mg/L |
| Albumin | 3.3-5.7 g/dL | 4.1 g/dL |
| TBG | 150-360 nmol/ml | 256 nmol/ml |
| aTPO | >35 IU/mL | >1000 IU/mL |
| aTg | >20 IU/mL | >3000 IU/mL |
| Prolactin | 1.9-25 ng/ml | 14.3 ng/ml |
| Progesterone | Follicular phase: 0,10-1,13; Ovulation: 0.48 – 1,72; Post menopause: 0.36 -1.0; On oral contraceptives: 0.34-0.90 ng/ml | > 0.2 ng/ml |
| SHBG | 15 - 100 nmol/L | 19,57 nmol/L |
| Estrogen | Follicular phase: 26.5-160; Ovulation: 186 – 400; Post menopause: >30; On oral contraceptives: >102 pg/ml | 55.0 pg/ml |
| FSH | Follicular phase: 1.98-11.3; Ovulation: 7.5 – 21; Post menopause: 9.7 - 111; On oral contraceptives: 1.7- 4.9 mIU/L | 3.88 mIU/L |
| LH | Follicular phase: 1,1-11.6; Ovulation: 17-77; Post menopause: 11.3-39.8; On oral contraceptives: 0.5-8.0 mIU/L | 5.57 mIU/L |
| Estradiol | 26.5-160 pg/mL | 55 pg/mL |
| CK | 24-173 U/L | 178 U/L |

Clinically, the patient`s symptoms were in favour of hypothyroidism, which did not correlate with the laboratory results in favour of possible secondary hyperthyroid state or maybe hashitoxicosis.

The ultrasonography of the thyroid gland (performed on Phillips H.D. 6, Version 1.1, probe 7,5 Hz) gave information about an enlarged, nonhomogeneous thyroid gland with hypoechoic structure, initial fibrosis and visible pseudonodules (Fig. 1).

a) b)

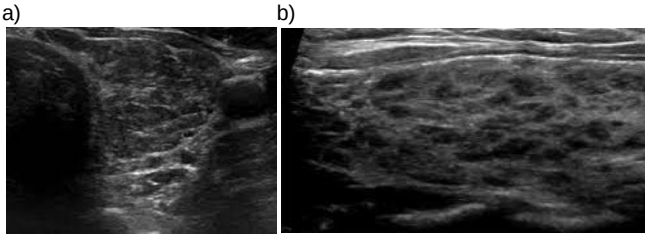


*Figure 1: An ultrasonographic cross-sectional and longitudinal image of the thyroid gland presenting a nonhomogeneous thyroid gland with hypoechoic structure and pseudonodules*

Fine needle aspiration biopsy (FNAB) was conducted for a cytopathological examination (H&E staining) due to the presence of pseudonodules on ultrasonography. The results pointed towards the presence of prominent germinal follicles with active germinal centres, lymphocytic infiltration, oncocytic cells with Hurthle cell metaplasia, and initial fibrosis. These findings in correlation with the high aTPO and aTg laboratory values revealed underlying (previously unsuspected) Hashimoto's thyroiditis – interpreted by the pathologist as group III by the BETHESDA system of classification (Fig. 2).

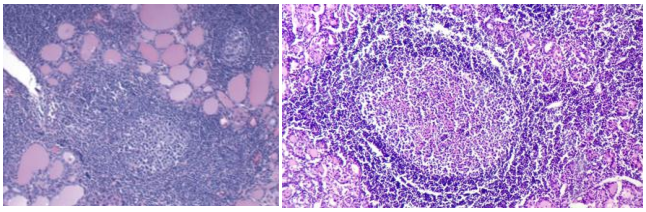


*Figure 2: Microscopic features of the FNAB presenting germinal centres and lymphocytic infiltration - Hashimoto's thyroiditis*

Thyroid scintigraphy performed with $^{99m}$Tc-pertechnetate (185 MBq/5 mCi tracer activity, images obtained 30 min post-injection on dual-headed gamma camera MEDISO DHV Nucline Spirit, with 140 keV photopeak and low-energy, high resolution (LEHR) parallel collimator) was the following step. The patient's thyroid gland was enlarged with increased tracer accumulation intensity in favour of hyperthyroidism caused by the elevated TSH levels (Fig. 3).

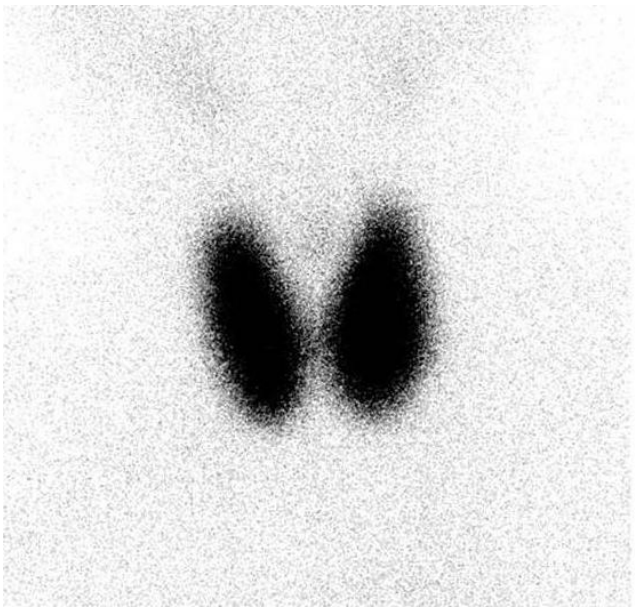


*Figure 3: Thyroid scintigraphy with $^{99m}$Tc-pertechnetate presenting diffusely increased tracer accumulation*

An MRI study followed, and the pituitary gland was visualised as normal, in a standard and additional sequences, in a native and post contrast study series and the suspicion for possible adenoma (TSH-oma) of the pituitary gland was ruled out (Fig. 4).

To combine the anatomical examinations with functional ones, to exclude or to confirm the possible differential diagnosis, a TRH test and T3 suppression test were also conducted.

The TRH stimulation test included administration of small amounts of TRH intravenously,

following which levels of TSH were measured at several subsequent points (15-30 minutes post-injection) using samples of blood taken from a peripheral vein. Before the test, TRH levels were measured and were slightly suppressed. The test was performed to differentiate the possible abnormality in the hypothalamus-pituitary axis. The results presented an increase in TSH levels.

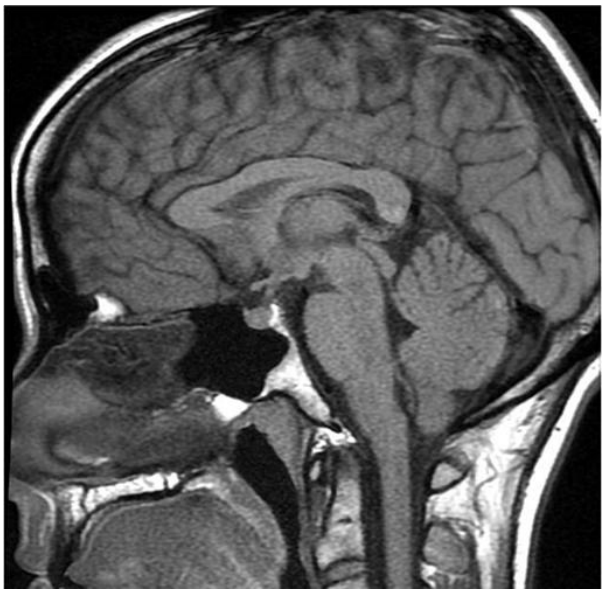


*Figure 4: Normal MRI study of the pituitary gland*

Considering the T3 suppression test, T3 was given to the patient during whom TSH levels were measured. In our case, the levels of TSH remained high.

With rT3 levels within the normal range and a normal FT4 to FT3 conversion, deiodinase enzyme abnormality and selenium deficiency were excluded.

PCR protein amplification was afterwards ordered for the class of integrins α(V)β(3), involved in T3 bondage and signal transduction. It presented a point mutation coding for the beta subunit of the membrane receptor molecule, which was later confirmed to be the exact reason for the impaired sensitivity to thyroid hormones syndrome (ISTHS) in the patient (Fig. 5).

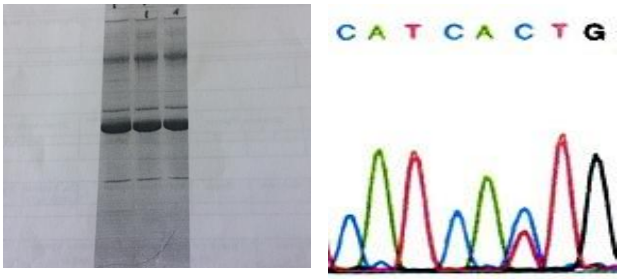



*Figure 5: PCR presenting point mutation*

The patient was prescribed substitution therapy of 50 μg/day of levothyroxine sodium and is regularly followed on check-ups up to date, adjusting the dosage of the therapy depending on the clinical signs and symptomatology of the patient.

## Discussion

The presented case is part of the broad spectrum of impaired sensitivity to thyroid hormones syndrome - ISTHS or to be more precise a rare partial peripheral resistance to thyroid hormones due to a point mutation coding for the beta subunit of the integrin molecule α(V)β(3). The ISTHS has an incidence of 1:40000 and usually the underlying cause is due to different thyroid hormone receptors mutations. Several case reports have been previously published in the literature with various types of mutations in question featuring a broad spectrum of unusual and misleading clinical and biochemical manifestations [9], [10], [11], [12].

Our patient was admitted with a recently formed goitre which in correlation with her ultrasonography findings (enlarged thyroid gland with hypoechoic structure and fibrosis - most likely due to Hashimoto thyroiditis) and the clinical symptoms of hypothyroidism, pointed towards the decreased activity of the thyroid gland as the probable initial diagnosis. A hormone panel typical for secondary hyperthyroidism, with extremely high TSH, FT4, and FT3, also with the accented presence of aTPO and aTg (indicating an autoimmune component to the condition – in favour of Hashimoto thyroiditis) was obtained. Also, the patient had elevated creatine kinase (CK) levels, high cholesterol, and triglyceride levels (pointing towards dyslipidemia), which along with the iron deficiency anaemia (very low iron and haemoglobin levels) indicated a hypothyroid metabolic state. The symptoms presented by the patient, together with the detected dyslipidemia, anaemia and elevated CK levels were in favour of hypothyroidism, which did not correlate with the thyroid biochemical panel which was in favor of the secondary hyperthyroid laboratory state [13], [14], [15].

The cellular architectonic of the tissue provided via the FNAB (due to the pseudonodules detected on the US) correlated with the elevated antibody levels. Adding the information of the positive family history of hypothyroidism in mother and sister (due to Hashimoto thyroiditis) gave rise to the differential diagnosis of a possible primary hypothyroid diagnostic entity with autoimmunity in nature - Hashimoto's thyroiditis in the initial phase of hashitoxicosis.

The diagnostic dilemma of the pathological thyroid hormone panel, which did not correlate with the patient`s symptoms, the underlying Hashimoto

thyroiditis, and the metabolic biomarkers, remained unclear mostly due to the still unexplained reason for elevated TSH levels. Additionally, the results of the thyroid scintigraphy presented information about an enlarged in volume and hyperactive thyroid gland with diffusely increased intensity of tracer accumulation. This result confirmed the prolonged TSH hyperstimulation effect on the thyroid gland tissue, correlated with the elevated FT4, FT3, TT4, and TT3 levels and did not exclude the presence of possible secondary hyperthyroidism [16].

To resolve the underlying reason that might have been responsible for for the high TSH levels, an MRI imaging was performed, focusing on sella turcica and the pituitary gland. Anatomically sound pituitary at native and post-contrast slice series was visualised. The possible presence of autonomous hyperfunctional pituitary adenoma (TSH-oma) was excluded. ISTHS can be misleading and very difficult for diagnostic differentiation from autonomous TSH-producing adenomas of the pituitary gland. Clinically, the patients with TSH-omas are usually hyperthyroid, and the ISTHS patients present euthyroid or hypothyroid clinical features, but both entities have a similar or almost identical thyroid functional laboratory profile. Teng X et al. have published a rare case of concomitant TSH-oma and peripheral resistance to thyroid hormones, which was not the case with our patient [17].

To confirm or exclude the malfunctioning of the negative feedback loop, functional tests were performed. The TRH test gave important and relevant information. It revealed a normally responsive pituitary gland (TSH levels increased) by exogenous stimulation with TRH.

T3 suppression test, on the other hand, proved that neither the hypothalamus nor the pituitary gland responded to the exogenous elevated T3 values with decreasing the secretion of TSH and/or TRH, as normally would have been expected. In turn, TSH levels remained high despite the addition of T3, which indicated for the first time the presence of possible impaired responsiveness of the peripheral tissues to thyroid hormones. This also confirmed that the central regulation of the hypothalamus-pituitary gland-thyroid gland axis was sound.

Thyroid hormones are normally transported in the bloodstream bound with specific hormone carriers such as albumin, prealbumin, and thyroid binding globulin (TBG). Only a small amount of the total hormone release in the circulation is present in its free form, and that fraction is responsible for their peripheral effect manifestations. Abnormal protein bondage could be the reason for a euthyroid hyperthyroxinemia, as seen as in thyroid binding globulin (TBG) excess or familiar dysalbuminemic hyperthyroxinemia (FDH) [18]. But in these conditions, the patients usually have normal TSH levels and are euthyroid considering the clinical symptoms. Furthermore, our patient had normal protein carrier levels. Also, the estrogen levels in the patient were very low, which in turn was contradictory to the normal protein carrier levels since estrogen is the hormone which influences the regulation of their levels.

Thyroid hormone cell membrane transport defect (THCMTD) [19] refers to an abnormality of the MCT8 (monocarboxylate transporter 8), responsible for the cellular transport of the thyroid hormones so that they can express their effect when binding to nuclear receptors. They are mostly found in the brain and liver. But a mutation of the MCT8 generally affects males, since it is X chromosome-linked. Also, patients with MCT8 mutations usually show symptoms of hormone deficiency in the organs in which they are mostly distributed. In our patient, there were neither cognitive nor mental disabilities, which were supported by the fact of her reaching a high level of education. Also, MCT8 abnormality would have presented itself with an elevated T3 level, but low rT3 level, low T4 level and normal or slightly elevated TSH level [20]. Reverse T3 (rT3) levels in our patient were normal, with elevated T4 and TSH, as stated previously.

Thyroid hormone metabolism defect (THMD) refers to the abnormal deiodinase activity, due to a defect in the enzyme itself (qualitative or quantitative) or a condition of selenium deficiency as an essential cofactor of the enzyme. Considering these two, our patient had normal rT3 levels and obvious peripheral conversion of FT4 into FT3. Selenium deficiency presents itself with high TSH and T4 levels, but low T3 levels due to absent conversion [1]. This was not something that we diagnosed in our patient.

There are 3 types of deiodinase enzymes. Deiodinase I is present in the kidney, liver, thyroid and is responsible for 80% of the circulating T3, converted from T4. In this manner, any abnormal deiodinase I activity would have given low T3 levels - also not something we could see in our patient. Deiodinase II is found mostly in the pituitary gland, generally in the central nervous system, and is predominantly found inside the cell. In the pituitary gland, it mediates the negative feedback loop from the thyroid hormone levels. If an abnormality is present in this type of deiodinase, it will give rise to high values of rT3 and TSH, due to a lack of pituitary suppression of TSH production. Deiodinase III, found in CNS, placenta, etc., is responsible for converting T3 and T4 to its inactive form rT3. The decreased activity would result in low rT3 levels - something that was not present in our patient.

Considering the above-mentioned diagnostic analysis performed in our patient, we were able to confirm that the thyroid hormones could enter the cell, had a proper conversion to T3 and rT3 accordingly and that the patient had a weak, but yet an existing response to peripheral effects of the thyroid hormones. Though their effects had been

progressively decreasing as of lately, most probably due to the development of the Hashimoto's thyroiditis (impaired thyroid hormone production at primary level) in the patient, we considered the possibility of present congenital qualitative or quantitative abnormality in the membrane receptor - the integrin α(V)β(3). Therefore this receptor became the target for the PCR amplification technique as the final diagnostic procedure performed to conclude the exact patient diagnosis.

The PCR results revealed the diagnosis of impaired sensitivity to thyroid hormones syndrome (ISTHS). A point mutation coding for the beta subunit of the integrin molecule α(V)β(3) was the reason why the patient's peripheral tissues were metabolically hypothyroid with high T3/FT3 and T4/FT4 circulating levels [21]. This qualitative change in the structure prevented its normal responsiveness to thyroid hormone stimulus, and its peptide-like action, except in very high quantities of the hormones present. It also resulted in absent suppression of TSH secretion from the pituitary - with extremely high TSH levels, but with near normal or slightly suppressed TRH due to the suppression of the hypothalamus from the elevated TSH. The treatment of the patient was by her symptomatology - substitutional therapy, but not focused on lowering the T4 and T3 levels.

In conclusion, peripheral resistance to thyroid hormones is a very rare condition and can often be misdiagnosed due to the broad spectrum of possible similar differential diagnostic entities. Molecular diagnostic procedures are usually required to confirm the exact underlying cause for the ISTHS. The definitive diagnostic algorithm should include not only the biochemical parameters but also the clinical patient`s symptomatology, especially in cases where there is a miss match between those above.

## Acknowledgements

The paper was awarded the Best Case Report Presentation at the 42. International Medical Congress, May 27-29, 2019, Ohrid, the Republic of Macedonia by the Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia.

## References

1. Bianco, AC, Kim BS. Pathophysiological relevance of deiodinase polymorphism. Curr Opin Endocrinol Diabetes Obes. 2018; 25(5):341-346.

2. Singh BK, Sinha RA, Yen PM. Novel transcriptional mechanisms for regulating metabolism by thyroid hormone. Int J Mol Sci. 2018; 19(10): 3284. https://doi.org/10.3390/ijms19103284 PMid:30360449 PMCid:PMC6214012

3. Cyril S. Anyetei-Anum, Vincent R. Roggero, Lizabeth A. Allison. Thyroid hormone receptor localization in target tissues. J Endocrinol. 2018; 237(1):19-34. https://doi.org/10.1530/JOE-17-0708 PMid:29440347 PMCid:PMC5843491

4. Rohit A. Sinha, Brijesh K. Singh, Paul M. Yen. Direct effects of thyroid hormones on hepatic lipid metabolism. Nat Rev Endocrinol. 2018; 14(5):259-269. https://doi.org/10.1038/nrendo.2018.10 PMid:29472712 PMCid:PMC6013028

5. Refetoff S. et al. Classification and Proposed Nomenclature for Inherited Defects of Thyroid Hormone Action, Cell Transport, and Metabolism. Thyroid. 2014; 24(3):407-409. https://doi.org/10.1089/thy.2013.3393.nomen PMid:24588711 PMCid:PMC3950730

6. Takada Y, Ye X, Simon S. The integrins. Genome Biology. 2007; 8:215. https://doi.org/10.1186/gb-2007-8-5-215 PMid:17543136 PMCid:PMC1929136

7. Leith JT, Mousa SA, Hercbergs A, Lin HY, Davis PJ. Radioresistance of cancer cells, integrin αvβ3 and thyroid hormone. Oncotarget. 2018; 9(97):37069. https://doi.org/10.18632/oncotarget.26434 PMid:30651936 PMCid:PMC6319341

8. Krashin E, Piekiełko-Witkowska A, Ellis M, Ashur-Fabian O. Thyroid hormones and cancer: A comprehensive review of preclinical and clinical studies. Frontiers in endocrinology. 2019;10. https://doi.org/10.3389/fendo.2019.00059 PMid:30814976 PMCid:PMC6381772

9. Weiss RE, Weinberg M, Refetoff S. Identical mutations in unrelated families with generalized resistance to thyroid hormone occur in cytosine-guanine-rich areas of the thyroid hormone receptor beta gene. Analysis of 15 families. J Clin Invest. 1993; 91 (6): 2408-2415. https://doi.org/10.1172/JCI116474 PMid:8514853 PMCid:PMC443299

10. McDermott MT, Ridgway EC. Thyroid hormone resistance syndromes. Am J Med. 1993; 94 (4): 424-432. https://doi.org/10.1016/0002-9343(93)90155-I

11. Dumitrescu AM, Refetoff S. The syndromes of reduced sensitivity to thyroid hormone. Biochim Biophys Acta. 2013; 1830 (7): 3987-4003. https://doi.org/10.1016/j.bbagen.2012.08.005 PMid:22986150 PMCid:PMC3528849

12. Gonçalves AP, Aragüés JM, Nobre E, Barbosa AP, Mascarenhas M. A case of thyroid hormone resistance: a rare mutation. Arq Bras Endocrinol Metabol. 2014; 58(9): 962-966. https://doi.org/10.1590/0004-2730000003297 PMid:25627054

13. Fan W, Tandon P, Krishnamurthy M. Oscillating hypothyroidism and hyperthyroidism - a case-based review. 2014. J Community Hosp Intern Med Perspect. 2014; 4 (5): 10. https://doi.org/10.3402/jchimp.v4.25734 PMid:25432652 PMCid:PMC4246140

14. Wong M, Inder W. Alternating hyperthyroidism and hypothyroidism in Graves' disease. Clin Case Rep. 2018 Sep; 6 (9): 1684-1688. https://doi.org/10.1002/ccr3.1700 PMid:30214742 PMCid:PMC6132101

15. Drummond J, Roncaroli F, Grossman AB, Korbonits M. Clinical and pathological aspects of silent pituitary adenomas. The Journal of Clinical Endocrinology & Metabolism. 2018; 104(7):2473-89. https://doi.org/10.1210/jc.2018-00688 PMid:30020466 PMCid:PMC6517166

16. De Leo S, Lee YS, Braverman EL. Hyperthyroidism. Lancet. 2016; 388 (10047): 906-918. https://doi.org/10.1016/S0140-6736(16)00278-6

17. Teng X, Jin T, Brent GA, Wu A, Teng W, Shan Z. A patient with a thyrotropin-secreting microadenoma and resistance to thyroid hormone (P453T) J Clin Endocrinol Metab. 2015; 100(7):2511-2514. https://doi.org/10.1210/jc.2014-3994 PMid:25867808 PMCid:PMC5393528

18. Kobayashi S, Yoshimura Noh J, Shimizu T, Sato T, Kurihara I, Sugino K, Itoh H, Ito K. A case of familial dysalbuminemic

hyperthyroxinemia (FDH) in Japan: FDH as a possible differential diagnosis of syndrome of inappropriate secretion of thyroid-stimulating hormone (SITSH). Endocr J. 2017; 64: 207-212. https://doi.org/10.1507/endocrj.EJ16-0135 PMid:27904073

19. Mullur R, Liu YY, Brent GA. Thyroid hormone regulation of metabolism. Physiological reviews. 2014; 94(2):355-82. https://doi.org/10.1152/physrev.00030.2013 PMid:24692351 PMCid:PMC4044302

20. Jiao Fu, Alexandra M. Dumitrescu. Inherited defects in thyroid hormone cell-membrane transport and metabolism. Best Pract Res Clin Endocrinol Metab. 2014 March; 28 (2): 189-201. https://doi.org/10.1016/j.beem.2013.05.014 PMid:24629861 PMCid:PMC3959653

21. Rivas MA, Lado-Abeal J. Thyroid hormone resistance and its management. Proc (Bayl Univ Med Cent). 2016; 29:209-211. https://doi.org/10.1080/08998280.2016.11929421 PMid:27034574

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Jun 30; 7(12):1998-2001.
https://doi.org/10.3889/oamjms.2019.102
eISSN: 1857-9655
***Case Report***

# An Unusually Large Fibroepithelial Polyp of Uterine Cervix: Case Report and Review of Literature

Meral Rexhepi[1, 3*], Elizabeta Trajkovska[2], Kazimir Koprivnjak[1]

*[1]Department of Obstetrics and Gynecology, Clinical Hospital, Tetovo, Republic of Macedonia; [2]Department of Pathology – Clinical Hospital, Tetovo, Republic of Macedonia; [3]Faculty of Medical Sciences, University of Tetovo, Tetovo, Republic of Macedonia*

**Citation:** Rexhepi M, Trajkovska E, Koprivnjak K. An Unusually Large Fibroepithelial Polyp of Uterine Cervix: Case Report and Review of Literature. Open Access Maced J Med Sci. 2019 Jun 30; 7(12):1998-2001. https://doi.org/10.3889/oamjms.2019.102

**Keywords:** Cervical polyp; Giant fibroepithelial polyp; Uterine cervix

***Correspondence:** Meral Rexhepi. Department of Obstetrics and Gynecology, Clinical Hospital, Tetovo, Republic of Macedonia; Faculty of Medical Sciences, University of Tetovo, Tetovo, Republic of Macedonia. E-mail: dr.meralrexhepi@gmail.com

**Received:** 14-Mar-2019; **Revised:** 07-Jun-2019; **Accepted:** 08-Jun-2019; **Online first:** 30-Jun-2019

**Copyright:** © 2019 Meral Rexhepi, Elizabeta Trajkovska, Kazimir Koprivnjak. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

**Funding:** This research did not receive any financial support

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

**Abstract**

**BACKGROUND:** Fibroepithelial cervical polyps (FEPs) are benign growths protruding from the inner surface of the cervix. They are typically asymptomatic, but a very small minority can undergo malignant change. Giant cervical polyps with a size greater than 4 cm are rare entities with only 23 reported cases in the literature. Cervical polyps develop as a result of focal hyperplasia of the columnar epithelium of the endocervix.

**CASE PRESENTATION:** We present the case of a giant fibroepithelial polyp measuring approximately 11 x 6 x 4 cm which was protruding from the anterior lip of the uterine cervix in a 51-year-old woman who clinically presented vaginal bleeding. She was nulligravida and doesn't have a child. The speculum examination revealed a soft, mobile, painless irregular cerebriform mass on the cervix, protruding through the vagina. The polyp was excised using a scalpel, and the pedicle was sutured using the Vicryl 1-0 under short-term intravenous anaesthesia. Histopathological examination revealed a polypoidal tumour mass composed of cellular fibrovascular stroma covered with stratified squamous epithelium. Three months after the initial surgery, there was no recurrence seen.

**CONCLUSION:** Cervical giant polyps are rare entities and occur mostly in perimenopausal women. Transvaginal polypectomy, as performed for this patient followed by histopathological examination is an adequate procedure for these lesions.

## Introduction

Fibroepithelial cervical polyps are polypoid growths projecting into the cervical canal. They can be one of the most common causes of intermenstrual vaginal bleeding. Polyps are almost always benign. The sparse literature available suggests rates of 0.0 – 1.7% malignant change in cervical polyps [1]. Although fibroepithelial stromal polyps of the lower female genital tract have been well-recognised since their initial description, they still pose diagnostic difficulties mainly owing to their variable histological appearances and rarity [2]. The incidence is about 4 to 10% of all cervical lesions. The polyps usually develop as a result of chronic papillary endocervicitis. They are soft, spherical, glistening red masses and bleed easily when touched. Often, they are friable, and they may be associated with profuse leukorrhea secondary to the underlying endocervicitis [3]. They are most present as an asymptomatic finding upon pelvic examination. They are also found in women who present for intermenstrual or postcoital bleeding, dyspareunia, lower abdominal discomfort and profuse vaginal discharge [4]. Most polyps measure < 1 cm in diameter. Giant cervical polyps measuring > 4 cm are rare, and to date, only 23 cases have been reported [5], [6]. They occur in adult women, rarely are in adolescents and frequently interpreted as malignant neoplasm at the time of the presentation [6]. In this

study, we present a case of a giant fibroepithelial polyp of the uterine cervix in a premenopausal woman.

## Case Presentation

A 51-year-old nulligravida woman presented with three months history of large, soft, painless, pedunculated mass measuring 11 x 6 x 4 cm protruding from the vagina (Figure 1).

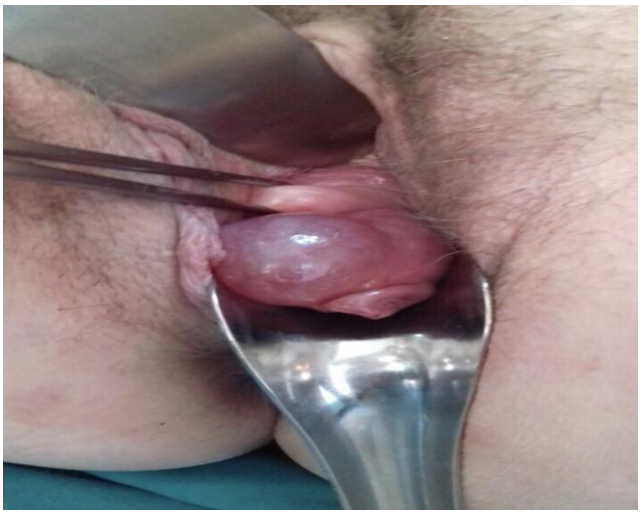


*Figure 1: Cystiform polyp protruding from the vagina*

The pedicle originated from the endocervix. The mass was non-pulsatile, non-reducible, with not prolapsing upon coughing. There was no increase in the size of the mass with Valsalva manoeuvre. There were no signs of ulceration or inflammation. The patient described a menstrual cycle not related to the cycle, and the vaginal bleeding sometimes appeared suddenly. Her menarche was at the age of 13, and the duration of her menstrual cycle was 28 days with 6-days mensis. She never used hormonal therapy and had no past medical or surgical history. She never had been pregnant and had not a child. She is married, sexually active, and her last menstrual period was 5 months before. Medical history and laboratory results were unremarkable. Examination of her breasts, vulva and vagina did not reveal any abnormalities. There was no inguinal lymphadenopathy. The trans-abdominal sonography upon admission, showed an anteverted uterus, with normal age features and thin endometrium. Both ovaries were of normal anatomy. A cytological smear of the cervix showed normal endocervical and ectocervical cells with chronic inflammation. Polypectomy was performed under short intravenous general anaesthesia with the informed consent of the patient and with the procedure details. A Kelly clamp was placed to the tumour base, and the mass was excised (Figure 2).

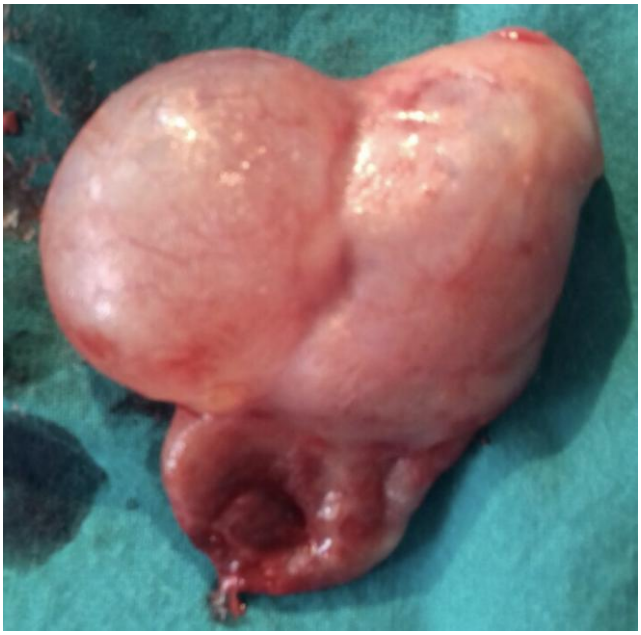


*Figure 2: The polyp after excision*

The basis of the removed tumour was ligated with 1-0 Vicryl suture and hemostasis was achieved. Endometrial curettage was also done. The tumour was sent for pathologic examination, which revealed a polypoid pink mass measuring 11 x 6 x 4 cm. with a macroscopic appearance of three coupled cystic formations of which one was perforated (Figure 3).

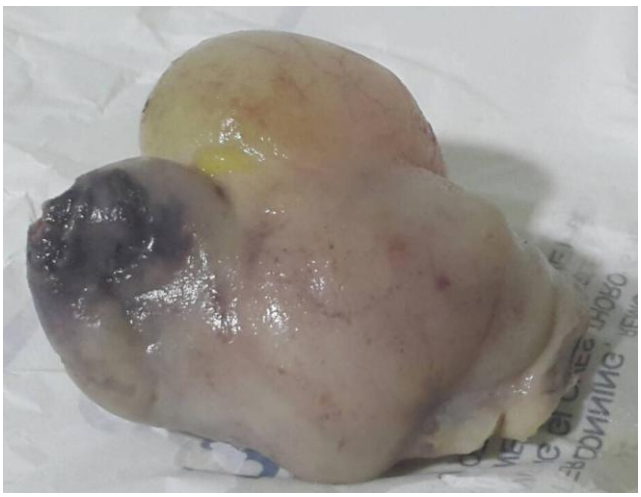


*Figure 3: Three coupled cystic formations*

On the dissection of the tumour, it was seen that the tumour wall was thin and composed of several cavities of different sizes filled with a thick sticky mass (Figure 4).

Microscopically, the most characteristic feature of the fibroepithelial polyp was present. The histopathologic examination revealed a fibro-collagenous tissue in the stroma, thickened blood vessels, fibroblasts, and chronic inflammatory

perivascular infiltrate covered with columnar cervical. All cavities were distended with Nabothian cysts, coated with columnar much-secretory epithelium (Figure 5, A and B).

*Figure 4: The dissection of the tumour*

Uterine curettage confirmed simple columnar epithelium without malignant changes. The patient's postoperative recovery was uneventful, with vaginal bleeding ceasing.

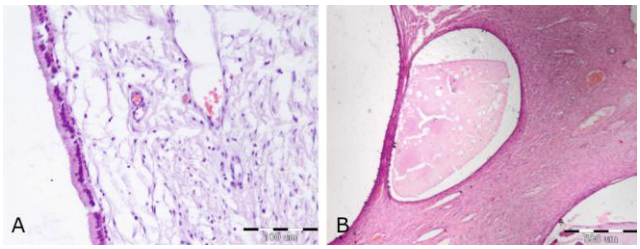



*Figure 5: A) HE x 20. Fibrocollagenous stroma covered; B) He x 40. The stroma of the polyp with a cystic cavity by stratified squamous epithelium*

## Discussion

Polyps are benign lesions and may represent a reactive hyperplastic process of subepithelial myxoid stroma and misdiagnosed as malignant. Polyps are usually small, soft, spherical and asymptomatic lesions [7], but large polyps can present with a lot of discomfort symptoms like a lower abdominal pain, abnormal vaginal discharge, intermittent bleeding and introitus mass [8]. We present the case of a giant cervical polyp was the first case in our centre in the past 20 years; thus, confirming its rarity. The symptoms of a painless mass protruding through the vagina with discharge and bleeding in our case was consistent with that reported in the literature [9], [10]. Only 22 cases of giant cervical polyps have been reported so far [11], and there is no case reported from North Macedonia. All the reported cases of giant cervical polyps were benign and thought to be the result of reactive changes from long-standing chronic inflammation [5], which corresponds with our case. Thus, it has been suggested that biopsy of these tumours before excision may not be necessary [9].

Previous reports indicate that giant cervical polyps originate more often from the ectocervix and rarely from the endocervix [11]. In our patient, the base of the polyp extended from the endocervix and protruding from the vaginal introitus. Most of the reported cases were nulliparous [6], [12] ranging between age 5-61 years old. In our case, the woman was nulliparous, in premenopausal with 5 months period of menstrual missing and above 51 years age.

The treatment of choice is surgical excision of the polyp with carefully obtained hemostasis. Some authors reported undertaking vaginal or abdominal hysterectomy because of endometrial hyperplasia and clinical suspicion of malignancy [5,13]. At histologic analysis, the tumour was diagnosed as a fibroepithelial cystic polyp.

In conclusion, giant cervical fibroepithelial polyps are rare benign tumours, presenting as protruding introital masses with vaginal bleeding in perimenopausal women. Thus, a wide range of morphological appearance of FEPs needs expert pathological interpretation to exclude atypical tumours and malignant pathology. The treatment is surgical and definitive diagnosis is verified by histological analysis.

## References

1. MacKenzie IZ, Naish C, Rees CMP, Manek. Why remove all cervical polyps and examine them histologically? BJOG. 2009; 116(8):1127-9. https://doi.org/10.1111/j.1471-0528.2009.02169.x PMid:19515151

2. Song JS, Song DE, Kim K-R, Ro JY. Cellular Pseudosarcomatous Fibroepithelial Stromal Polyp of the Vagina During Pregnancy: A lesion That Is Overdiagnosed as a Malignant Tumor. Korean J Pathol. 2012; 46(5):494-498. https://doi.org/10.4132/KoreanJPathol.2012.46.5.494 PMid:23136578 PMCid:PMC3490121

3. Khanam SA, Rashid F, Chowdhury S. An Unusually Big Cervical Fibroid Polyp: A Case Report.Chattagram Maa-o-Shishu Hospital Medicall College Journal. 2018; 15(2):65-67. https://doi.org/10.3329/cmoshmcj.v15i2.31810

4. Liu Q, Sun X. Giant fibroepithelial polyp of the uterine cervix. J Obstet Gynaecol. 2012; 32(4):405-6. https://doi.org/10.3109/01443615.2012.658895 PMid:22519500

5. Yadav BS, Nandedkar SS, Malukani K, Agrawal P. Multiple giant cervical polyps: A case report with literature review. Indian Journal of Basic and Applied Medical Research 2014; 3(3):338-343.

6. Ota K, Sato Y, Shiraishi S. Giant Polyp of Uterine Cervix: A

Case Report and Brief Literature Review. Gynecol Obstet Case Rep. 2017; 3(2):49-52. https://doi.org/10.21767/2471-8165.1000049

7. Rexhepi M, Trajkovska E, Besimi F, Rufati N. Giant Fibroepithelial Polyp of Vulva: A Case Report and Review of Literature. Prilozi, 2018; 39(2-3):127-130. https://doi.org/10.2478/prilozi-2018-0051 PMid:30864355

8. Harrison AO. Prolapsed giant cervical fibroid polyp mimicking procidentia - aftermath of traditional therapy for fibroids - a case report and review of the literature. Open Science Journal of Clinical Medicine. 2014; 2(1):15-18.

9. Agarwal M, Singh S, Agarwal R, Jaiswal S. Prolapsed Giant Cervical Fibroid Polyp. J South Asian Feder Obst Gynae. 2016; 8(1):77-78. https://doi.org/10.5005/jp-journals-10006-1391

10. Gupta A, Gupta P, Manaktala U. Varied Clinical Presentations, the Role of Magnetic Resonance Imaging in the Diagnosis and Successful Management of Cervical Leiomyomas: a case-Series and Review of Literature. Cureus. 2018; 10(5):e2653. https://doi.org/10.7759/cureus.2653

11. Palacios-Pereira LT, Cervantes-Ramirez L, Espinal-Rodriguez JM, Espinal-Madrid JM, Sabillon-Vallejo JE, Urquia-Osorio H. Giant cervical polyp long standing. A case report. Archivos de Medicina. 2015; 11(4):5.

12. Simavli S, Kinay T. Giant cervical polyp: A case report and review of the literature.Turkiye Klinikleri J Gynecol Obst. 2013; 23(2):119-22.

13. Massinde AN, Mpogoro F, Rumanyika, RN, Magoma M. Uterine prolapsed complicated with a giant cervical polyp: Journal of lower genital tract disease. 2012; 16:64-65. https://doi.org/10.1097/LGT.0b013e31822b752d PMid:21964209

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Jun 30; 7(12):2002-2005.
https://doi.org/10.3889/oamjms.2019.272
eISSN: 1857-9655
***Case Report***

# Central Nervous System Tuberculoma Complicated with Spinal Arachnoiditis in Immunocompetent Patient

A.A. Ayu Suryapraba[1*], Ni Made Susilawathi[2], I Wayan Niryana[3]

*[1]Neurology Department, Faculty of Medicine, Udayana University, Sanglah Hospital Denpasar, Denpasar, Bali, Indonesia; [2]Neurology Department, Faculty of Medicine, Udayana University, Udayana University Hospital Jimbaran, Jimbaran, Bali, Indonesia; [3]Neurosurgery Departement Faculty of Medicine Udayana University, Sanglah Hospital Denpasar, Bali, Indonesia*

**Citation:** Suryapraba AAA, Susilawathi, NM, Niryana IW. Central Nervous System Tuberculoma Complicated with Spinal Arachnoiditis in Immunocompetent Patient. Open Access Maced J Med Sci. 2019 Jun 30; 7(12):2002-2005. https://doi.org/10.3889/oamjms.2019.272

**Keywords:** Tuberculoma; Central nervous system; Arachnoiditis; Immunocompetent

***Correspondence:** A.A. Ayu Suryapraba. Neurology Department Faculty of Medicine Udayana University/Sanglah Hospital Denpasar, Denpasar, Bali, Indonesia. E-mail: ayu.suryapraba@gmail.com

**Received:** 04-Apr-2019; **Revised:** 07-Jun-2019; **Accepted:** 08-Jun-2019; **Online first:** 30-Jun-2019

**Copyright:** © 2019 A.A. Ayu Surya Prabha, Ni Made Susilawathi, I Wayan Niryana. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

**Funding:** This research did not receive any financial support

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

**Abstract**

**BACKGROUND:** Central nervous system (CNS) tuberculosis (TB) can manifest as meningitis, abscess, tuberculoma or other manifestations. CNS TB is a rare TB complication, and its diagnosis remains a challenge since it has clinical and imaging features that resemble other diseases. The antituberculosis treatment has a significant role in determining a patient's outcome and prevent complications and mortality.

**CASE PRESENTATION:** Here, we report a case of CNS TB manifested as tuberculoma in immunocompetent patient complicated with spinal arachnoiditis. Despite a treatment delay, the patient still showed clinical improvement after proper treatment with a combination of antituberculosis drug and corticosteroid.

**CONCLUSION:** Central Nervous System Tuberculoma Complicated with Spinal Arachnoiditis in Immunocompetent Patient CNS tuberculoma is a rare CNS TB manifestation, and its diagnosis remain a challenge since its clinical symptoms and radiological findings could mimic other cases such as malignancy, pyogenic abscess, toxoplasmosis, sarcoidosis, or neurocysticercosis.

## Introduction

Tuberculosis (TB) is a disease caused by *Mycobacterium tuberculosis* and can occur in any organs of the human body. In the central nervous system, TB can manifest as meningitis, abscess, tuberculoma or other manifestations. CNS TB is a condition with neurological morbidity and mortality and still become a major health problem and threat in developing countries especially with the pandemic of the *human immunodeficiency virus* (HIV) [1], [2], [3].

TB Incidence in Asia & Africa developing countries range from 110-165 cases/100.000 populations per year. About 10% of patient with TB could have CNS involvement. Factors associated with an increased risk of CNS TB are young age, HIV co-infection, alcoholism, malignancy, and immunosuppressive agents. Extrapulmonary TB manifests in about 40% of patients with HIV positive [3].

*Mycobacterium tuberculosis* enters the human body through droplet inhalation, followed by bacteria phagocytosis by macrophage, which induces a cascade of inflammation, protective immunity, and formation of primary complex. In a short term of bacteremia, then the *M. tuberculosis* basil spread hematogenously to any organs including meninges or by the formation of an isolated granuloma on meninges, subpial surface and subependymal of the brain or spinal cord is known as "Rich foci" [4]. Rupture or growth of this tuberculosis lesion then develops into various type of CNS TB [4].

With such various type of TB manifestation, appropriate diagnosis and management are essential to achieve favourable outcomes. In this case report, we discuss a case of a non-HIV woman with 2 types of CNS TB manifestation; tuberculoma and spinal arachnoiditis.

## Case Presentation

A 23-year-old female, Balinese from Buleleng regency, came to Sanglah Hospital Denpasar with headache, nausea and vomiting for One month before hospital admission. The symptoms got worse on the last two weeks, accompanied by a fluctuating fever, dizziness, shaking off both arm when raised, and bilateral leg weakness, but she still could walk without assistance and did mild activities. She also complained a bit of dyspnea with a mild cough. The patient had a history of brain tumour (cerebellar mass) and had undergone VP shunt insertion followed by tumour removal about 1.5 years before, with biopsy result considered as granulomatous lesion suspected as tuberculoma (Figure 1). After the surgery, she felt the symptoms resolved and discontinued the medication. On physical examination, there were subfebrile fever, cerebellar symptoms (spontaneous nystagmus to all directions on both eyes, limb ataxia, tremor, loss of physiological reflexes, muscle atrophy especially on both legs and hypotonia).

On laboratory examination, there was normal white blood count (10.26 x 10 µ/µL) with neutrophil predominance 88.3%, elevated erythrocyte sedimentation rate (66.4 mm/hour), hyponatremia (127 mmol/L) with positive cerebrospinal fluid PCR TB. CSF culture was found negative for *M. tuberculosis* Rapid test for HIV result was non-reactive.

Head MRI with contrast revealed irregular mass with enhancement, and extensive perifocal oedema sized 4.23 x 4.24 x 3.51 cm suggestive as residual/residing mass.

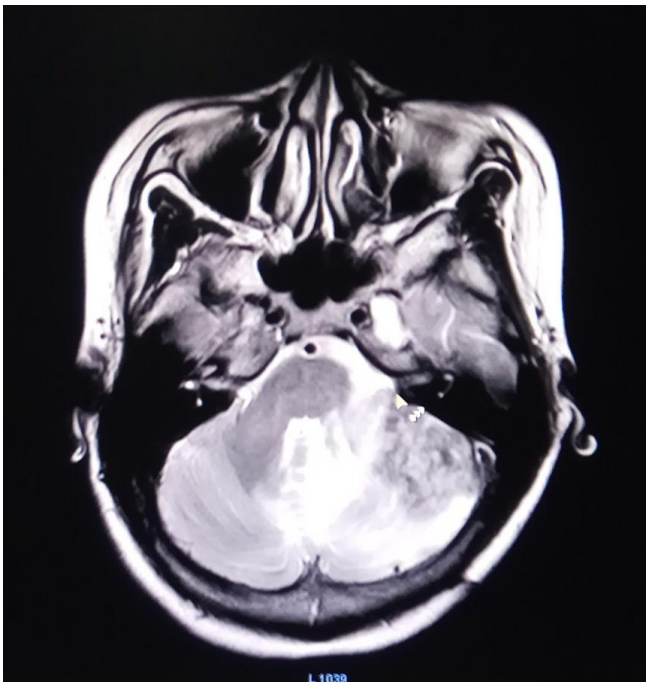


*Figure 1: Chest x-ray showed pneumonia with bilateral hilar lymphadenopathy*

Category I antituberculosis regimen (oral isoniazid 300 mg, rifampicin 600 mg, pyrazinamide 1500 mg and ethambutol 750 mg) also of 1 g intramuscular injection of streptomycin were given immediately. Intravenous dexamethasone injection with a dose of 5 mg was given in interval 6 hours and was tapered off 1 mg each week. The patient was consulted to the Internal Medicine Department and was assessed as pneumonia, she was given cefoperazone I gram/12 hours IV dan levofloxacin, 750 mg/24 hours IV.

On the third day of hospitalisation patient complained a worsening of both leg weakness, she could not lift them. She also felt numbness and tingling sensation from both toes to the navel, accompanied by difficulty in micturition and defecation. On rectal touché examination, the sphincter ani muscle tone was decreased.

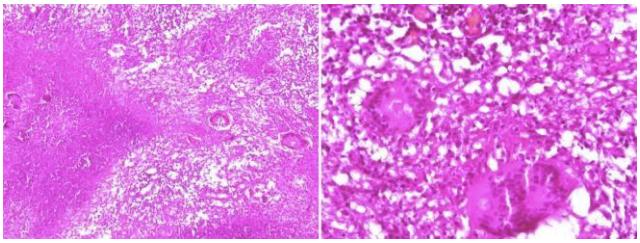


*Figure 2: Histopathological result from a cerebellar mass biopsy; Large necrotic foci, some surrounded by histiocytes epitheloid cell that forms granulomas with compact aggregates and multinucleated giant cell Langhans and specific type*

The patient underwent thoracolumbar MRI with contrast and showed diffuse leptomeningeal enhancement along cervical, thoracal and lumbar spine with enhancement on cauda equina and nerve root on lumbal level suggestive as arachnoiditis (Fig. 3). *Compound Muscle Action Potential* (CMAP) test showed loss of response on bilateral peroneus nerve, lengthening of distal latency and decreased of amplitude on bilateral tibialis nerve especially on the left side and *somatosensory evoked potential* (SSEP) showed lengthening of P37 latency consistent with spinal cord lesion above 12$^{th}$ thoracal.

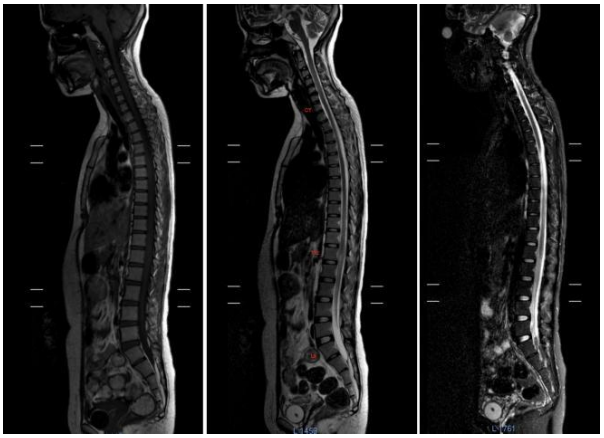


*Figure 3: Whole spine vertebral MRI T1, T2, TSE; diffuse leptomeningeal enhancement along cervical, the thoracal and lumbal segment with the enhancement of cauda equine and spinal nerve roots*

On the 19th day of hospitalisation patient's condition was improved, there was no fever, cerebellar signs were improved (spontaneous nystagmus were diminished, tremor and ataxia were gone), the patient could sit down with help and elevate her right leg. She could defecate without laxantia and micturate without a catheter. The patient was discharged with category I antituberculosis drug and continued streptomycin injection at a health facility near her residence.

After 3 day outpatient care patient came back to the hospital with a distended stomach. Leg weakness and numbness persist as previous time patient was discharged. On plain abdomen and 3 position abdomen x-ray, there was distention of gaster without signs of ileus. The patient was consulted to Digestive Surgery Department, and decompression was done by insertion of a nasogastric tube (NGT), and the antituberculous drug was continued. An evaluation of CSF were performed on day 8 of re-hospitalisation with result yellow colour, pleocytosis (128 cell/ µL) with monocyte predominance (90%), total protein elevation (72.9 mg/dL) and hipoglycoracchia (CSF glucose was 14 mg/dL, with CSF glucose/serum ratio 0.13). Dexamethasone 10 mg was administered intrathecally after CSF sample collection and was repeated once with a 1-week interval.

The patient then was discharged with the improvement of the condition; abdominal distention was relieved, she could elevate her both legs, sat down by herself, numbness and tingling sensation were diminished. She continued antituberculosis drug medication at the health facility in her hometown.

## Discussion

CNS TB occurs in about 1% of TB patients, especially in young age, immunosuppressed patient, malnutrition, alcoholism, and malignancy. Intracranial tuberculoma is a rare condition, found in 1 % CNS TB [5], [6]. Tuberculoma can cause focal neurologic symptoms without systemic condition, and its radiologic finding can mimic malignancy, pyogenic abscess, toxoplasmosis, sarcoidosis or neurocysticercosis [6], [7], [8].

Focal brain lesion involves the process of hematogenous spread from a primary focus on other organs such as the lung. With low cell-mediated immunity and significant inoculation size, a tubercle focus on brain parenchyma can develop into tuberculoma or abscess [4]. Intracranial tuberculoma is a rare complication and usually occur in immunocompromised patients such as HIV patients and its incidence in patients without TB is not clearly described [9], [10].

A serial case report by Guo et al. 2013 [11] reported 11 cases of infratentorial tuberculoma on immunocompetent patients and most of the cases did not show any evidence of systemic tuberculosis; only 1 subject had pulmonary TB. Infratentorial tuberculoma risks patient's life more than supratentorial tuberculoma and rarely reported. Tuberculoma can occur in any body parts. However, adults tend to have supratentorial tuberculoma while infratentorial tuberculoma occurs more in children [11], [12].

The supporting examination for diagnosis, in this case, was a positive result of CSF PCR for *M. tuberculosis*. The microbial CSF culture for *M. tuberculosis* was negative. Lack of sensitivity on current diagnostic tests for TB still became a problem in supporting early diagnosis of TB. Culture is a gold standard. However, its sensitivity is not quite high, ranging from 25 to 70%. TB diagnosis cannot be excluded from a negative microbial test [13]. PCR can detect *M. tuberculosis* weeks before culture in 80-90% of patients with TB whose TB already confirmed by culture [14].

Our patient had a complication of spinal arachnoiditis, which is considered as a late complication of CNS TB and often causes spinal cord and spinal nerve root disorder. Spinal arachnoiditis can develop into various clinical conditions such as radiculomyelitis, spinal tuberculoma, myelitis, syringomyelia, vertebral TB or spinal TB abscess. There is 3 possible pathomechanisms for TB spinal arachnoiditis; by hematogenous spread to meninges and spinal cord parenchyma; gravitation of tuberculous exudates to the lumbosacral area or via direct spread from vertebral TB [15]. With a diffuse leptomeningeal enhancement along cervical, thoracal and lumbal level accompanied by enhancement of cauda equina and spinal nerve roots on whole spine MRI examination, it is possible that our patient had gravitation of infection from infratentorial to meninges along the spinal cord until the spinal nerve roots.

After received category I antituberculosis drug with adjunction of streptomycin injection, the patient getting improved gradually with diminished of cerebellar symptoms and relieved symptoms of radiculomyelitis (increased of muscle strength, numbness and tingling sensation minimised, micturition and defecation returned to normal). Immediate treatment with an antituberculosis drug with the first-line drug (isoniazid, rifampicin, ethambutol, pyrazinamide and streptomycin) is important. At the first 2 months of intensive phase patient should receive at least 4 regimens (isoniazid, rifampicin, ethambutol and streptomycin or pyrazinamide) followed by a maintenance phase with isoniazid and rifampicin for 7-10 months [16].

The patient also received dexamethasone injection with a dose of 5 mg every 6 hours, which tapered off weekly and 2 times intratechal

dexamethasone with a 1-week interval. Corticosteroid for tuberculoma patient with spinal TB could benefit in improvement of neurological outcome despite its controversy, especially in HIV patients. The recommended dose according to latest study is dexamethasone 0.4 mg/kg/day on first week and tapered down 0.1 mg/kg every week until dose 0.1 mg/k/day, continued orally 4mg/day and was tapered down 1 mg/kg each week [2], [15], [17]. Intratechal steroid in TB meningitis (TBM) remains a controversy; even some study does not recommend it. Steroid decreases meningeal inflammation and consequently affect antituberculosis drug penetration [3]. According to a study by Ghosh et al. 1971 [18] in children with TBM, there was no significant difference in the outcome between patients who received oral, intravenous or intratechal steroid.

In conclusion, CNS tuberculoma is a rare CNS TB manifestation, and its diagnosis remains a challenge since its clinical symptoms and radiological findings could mimic other cases such as malignancy, pyogenic abscess, toxoplasmosis, sarcoidosis, or neurocysticercosis. Spinal arachnoiditis as a late complication of TB infection may occur. Appropriate antituberculosis drug medication is important to prevent complications and mortality and should be highlighted since it requires a lot of drug combination and long treatment duration to achieve a favourable outcome of the patients.

## Acknowledgement

The author obtained permission from the patient and her family to report her case.

## References

1. Rock RB, Olin M, Baker CA, Molitor TW, Peterson PK. Central Nervous System Tuberculosis: Pathogenesis and Clinical Aspects. Clinical Microbiology Reviews. 2008:243-261. https://doi.org/10.1128/CMR.00042-07 PMid:18400795 PMCid:PMC2292571

2. Cherian A, Thomas SV. Central nervous system tuberculosis. African Health Sciences. 2011; 11.

3. Changal KH, Raina AH. Central Nervous System Manifestations of Tuberculosis: A Review Article. J Mycobac Dis. 2014; 4:2.

4. Garg RK. Tuberculosis of the central nervous system. Postgrad Med J. 1999; 75:133-140. https://doi.org/10.1136/pgmj.75.881.133 PMid:10448488 PMCid:PMC1741157

5. Pimentel MLV, Alves SMV, Novis SAP, Brandão RZ, Neto EB. Intracranial tuberculomas are developing during treatment of pulmonary tuberculosis: a case report. Arq Neuropsiquiatri. 2000; 58(2-B):572. https://doi.org/10.1590/S0004-282X2000000300028 PMid:10920425

6. Monteiro R. Carneiro JC, Costa C, Duarte R. Cerebral tuberculomas: A clinical challenge. Respiratory Medicine Case Reports. 2013; 9:34. 37. https://doi.org/10.1016/j.rmcr.2013.04.003 PMid:26029627 PMCid:PMC3949551

7. Rock RB, Olin M, Baker CA, Molitor TW, Peterson PK. Central nervous system tuberculosis: pathogenesis and clinical aspects. Clinical Microbiology Reviews. 2008; 21(2):243e61. https://doi.org/10.1128/CMR.00042-07 PMid:18400795 PMCid:PMC2292571

8. Sahaiu SS, Jones B. Brainstem tuberculoma in the immunocompetent: case report and literature review. Clinical Neurology and Neurosurgery. 2008; 110:302e4. https://doi.org/10.1016/j.clineuro.2007.11.002 PMid:18160211

9. Martinez-Vazquez C, Bordon J, Rodriguez-Gonzalez A, Aguado JD, Sopena B, Gallego-Rivera A, Martinez-Cueto P. Cerebral tuberculoma- a comparative study in patients with and without HIV infection. Infection. 1995; 23(3):149-53. https://doi.org/10.1007/BF01793855 PMid:7499003

10. Zavascki AP, Dias AE, Cruz RP, De Oliveira RL, Duquia RP. Intracranial tuberculomas in an immunocompetent patient mimicking brain metastasis of unknown origin. Infection. 2006; 34(3):181-2. https://doi.org/10.1007/s15010-006-5028-5 PMid:16804665

11. Guo N. Huang F. Chen X. Zheng M. Zhong D. Feng Y. Infratentorial Tuberculoma Mimics Tumor in Immunocompetent Adults: An Analysis of 11 Patients. Open Journal of Modern Neurosurgery. 2013; 3:36-40. https://doi.org/10.4236/ojmn.2013.33008

12. Dastur HM. Desai AD. A Comparative Study of Brain Tuberculomas and Gliomas Based on 107 Case Records of Each. Brain. 1965; 88(2):375-96. https://doi.org/10.1093/brain/88.2.375 PMid:5828909

13. Heemsker AD, Donovan J, Thu DDA,Marais S, Chaidir L, Dung VTM, Centner CM, Ha VTN, Annisa J, Dian S, Boyijn L, Mai NTH, Phu NH, Chau NVV, Ganiem AR, Van CT, Geskus RB, Thuong NTT, Ruslami R, Meinties G, van Crevel R, Wilkinson RJ, Twaithes GE. Improving the microbiological diagnosis of tuberculous meningitis: A prospective, international, multicentre comparison of conventional and modified Ziehl-Neelsen stain, GeneXpert, and culture of cerebrospinal fluid. Journal of Infection 77. 2018; 509-515. https://doi.org/10.1016/j.jinf.2018.09.003 PMid:30217659 PMCid:PMC6293313

14. Ryu YJ. Diagnosis of Pulmonary Tuberculosis: Recent Advances and Diagnostic Algorithms. Tuberc Respir Dis. 2018; 78:64-71. https://doi.org/10.4046/trd.2015.78.2.64 PMid:25861338 PMCid:PMC4388902

15. Garcia-Monco JC, Pomar V, Domingo P. CNS Infections. Springer; 2017. https://doi.org/10.1007/978-3-319-70296-4

16. Parekh R, Haftka A, Porter A. A Rare Case of Central Nervous System Tuberculosis. Case reports in infectious diseases. 2014; 2014. https://doi.org/10.1155/2014/186030 PMid:25478256 PMCid:PMC4248556

17. Thwaites GE, Nguyen DB, Nguyen HD, Hoang TQ, Do TT, Nguyen TC. Dexamethasone for the treatment of tuberculous meningitis in adolescents and adults. N Engl J Med. 2004; 351(17):1741-51. https://doi.org/10.1056/NEJMoa040573 PMid:15496623

18. Ghosh S. Seshadri R. Jain KC. Evaluation of Corticosteroids in Treatment of Tuberculous Meningitis. Archives of Disease in Childhood. 1971; 46:51. https://doi.org/10.1136/adc.46.245.51 PMid:21032461 PMCid:PMC1647566

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Jun 30; 7(12):2006-2009.
https://doi.org/10.3889/oamjms.2019.561
eISSN: 1857-9655
***Case Report***

# Unusual Splitting of Medial Cord of the Right Brachial Plexus and Its Relation to the Axillary Artery and Subscapular Artery: A Case Report

Adegbenro Omotuyi John Fakoya[1*], Emilio Aguinaldo[2], Natalia M. Velasco-Nieves[2], Zachary T. Vandeveer[2], Nannette Morales-Marietti[2], Shannon Mathew[2], Abayomi Gbolahan Afolabi[1], Thomas McCracken[1]

*[1]Department of Anatomy, University of Medicine and Health Sciences, Basseterre, St. Kitts and Nevis; [2]University of Medicine and Health Sciences, Basseterre, St. Kitts and Nevis*

**Citation:** Fakoya AOJ, Aguinaldo E, Velasco-Nieves NM, Vandeveer ZT, Morales-Marietti N, Mathew S, Afolabi AG, McCracken T. Unusual Splitting of Medial Cord of The Right Brachial Plexus and Its Relation to The Axillary Artery and Subscapular Artery: A Case Report. Open Access Maced J Med Sci. 2019 Jun 30; 7(12):2006-2009. https://doi.org/10.3889/oamjms.2019.561

**Keywords:** Brachial plexus; Medial cord; Axillary artery; Subscapular artery

***Correspondence:** Adegbenro Omotuyi John Fakoya. Department of Anatomy, University of Medicine and Health Sciences, Basseterre, St. Kitts and Nevis. E-mail: afakoya@umhs-sk.net

**Received:** 30-Apr-2019; **Revised:** 22-Jun-2019; **Accepted:** 23-Jun-2019; **Online first:** 30-Jun-2019

**Copyright:** © 2019 Adegbenro Omotuyi John Fakoya, Emilio Aguinaldo, Natalia M. Velasco-Nieves, Zachary T. Vandeveer, Nannette Morales-Marietti, Shannon Mathew, Abayomi Gbolahan Afolabi, Thomas McCracken. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

**Funding:** This research was supported by the administration of the University of Medicine and Health Sciences, Basseterre, St. Kitts and Nevis

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

**Abstract**

**BACKGROUND:** Variations in human anatomy have been associated with numerous clinical correlations that may affect patient care. In this article, we present a unique variation of the medial cord of the brachial plexus about the axillary artery and subscapular artery. The precise assessment of this unique morphology was performed during a cadaveric dissection.

**CASE PRESENTATION:** Contrary to the general course of the medial cord of the brachial plexus, this report demonstrates a rare splitting of the medial cord around the axillary artery and a second abnormal communication between the posterior and medial cords that show a “nutcracker-like” syndrome involving the subscapular artery.

**CONCLUSION:** Such variations could make surgeries challenging. We also infer that these anatomical variations could make gliding therapy inefficient in any motor dysfunction initiating from the brachial plexus.

## Introduction

The brachial plexus is a continuation of nerves from the spinal cord and are separated into roots, trunks, divisions, cords, and branches. Lesions and malformations in each different section of the brachial plexus can have severe and different consequences [1].

The brachial plexus is a continuation of nerves from the spinal cord that normally exits four cervical vertebrae and one thoracic vertebra. The cervical vertebra involved are C5, C6, C7, C8, and the thoracic input is from T1 [1]. The combination of nerves exits the vertebrae and form the roots that mark the beginning of the brachial plexus. The brachial plexus innervates the muscles of the chest, muscles neighbouring the scapula, and the entire upper limb [1]. They also provide sensory and cutaneous innervation to their respective parts of the body depending on where the nerve separates and its route throughout the upper limb [1]. Medically, it is of utmost importance to know the location of nerves, junctions, separations, and affiliations. Many nerves are accidentally lesioned due to their proximity to major arteries, abnormalities, and surgeries. A

significant landmark of the brachial plexus is the axillary artery; this artery is what gave the cords of the brachial plexus their name due to their respective relations to the artery [1]. The relationship of the cords to the axillary is shown in Figure 1.

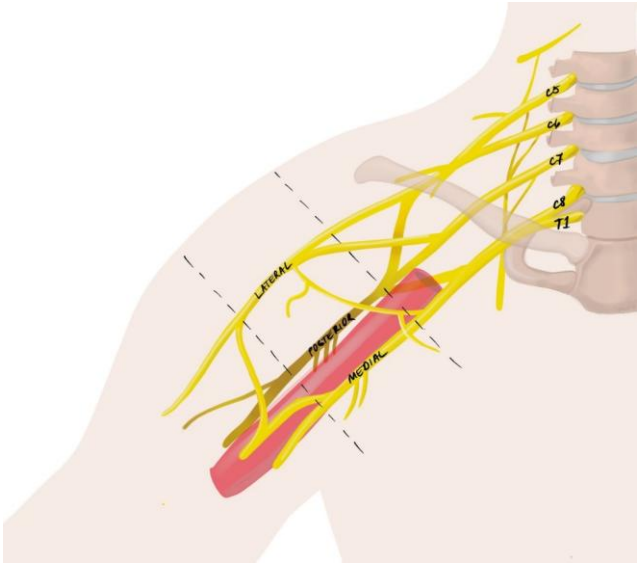



*Figure 1: The cords are labelled in the figure as they relate to the axillary artery*

The axillary artery is a continuation of the subclavian artery that routes inside the intricate plexus of nerves. The axillary artery runs anterior to the posterior cord, laterally to the medial cord, and medial to the lateral cord, as demonstrated in Figure 1. Malformations and variations can obscure the clinical presentations of lesions associated with the brachial plexus, while also making surgeries difficult if nerves and arteries are not in anatomical position.

This case report shows a unique variation of the medial cord about the axillary artery observed during a routine student cadaveric dissection.

Understanding the brachial plexus is of crucial importance when dealing with nerve lesions and surgical procedures of the upper limb. Variations in the brachial plexus [Af1] are multifactorial in presentation and can either hinder or help patients continue to have valued nerve supply for supporting multiple functions. Some variations are more common than others and have been studied significantly; these malformations usually include the addition of C4 or T2 spinal roots to the standard origin of C5-T1 spinal root of the brachial plexus [2]. A contributing branch of C4 to C5 variation in the brachial plexus occurs in about thirty per cent of the population. The median nerve has also been found to be more prone to abnormalities than other branches of the brachial plexus. In about ten per cent of the population, an abnormality has been identified as having an additional branch forming the median nerve [2]. This additional branch comes from the medial cord. Normally the median nerve is formed from the combination of the lateral and medial cord branches joining together [2].

## Case Report

A routine cadaveric dissection of the axilla and upper limbs of a 90-year-old Caucasian female cadaver by medical students at the Anatomy laboratory, University of Medicine and Health Sciences, St. Kitts & Nevis revealed a variant split of the medial cord located distal to its formation from the inferior trunk of the brachial plexus, creating a medial cord with two nerve branches in the right axilla. The branch that was most medial to the axillary artery travelled the normal course of the medial cord running medially to the axillary artery and deep to the pectoralis minor muscle before giving off additional nerves that innervate the forearms and the hand. The other branch travelled in a posterolateral to anteromedial direction over the axillary artery before joining with the normal branch to further create the medial root of the median nerve and the ulnar nerve of the right brachial plexus as shown in figure 2. Both branches that formed the medial cord were joined distally by neural connective tissue splitting around the axillary artery.

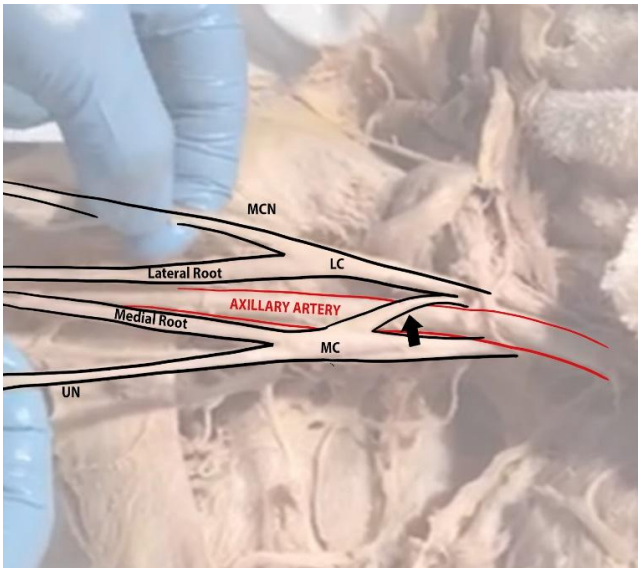



*Figure 2: The medial cord of the brachial plexus split distal to its formation in the right axilla (indicated by the black arrow). LC: Lateral Cord; MC: Medial cord; MCN: Musculocutaneous nerve MN: Median nerve; UN: Ulnar nerve*

As described, the axillary artery coursed through a split in the medial cord, while a communicating branch connected the posterior cord to the medial cord immediately adjacent to the subscapular artery, almost pinching it in between as demonstrated in Figure 3. The nerves ran their normal course to their respective regions in the indicated

limb. The course and origin of the medial cord of the brachial plexus were located normally in the left axilla and upper limb.

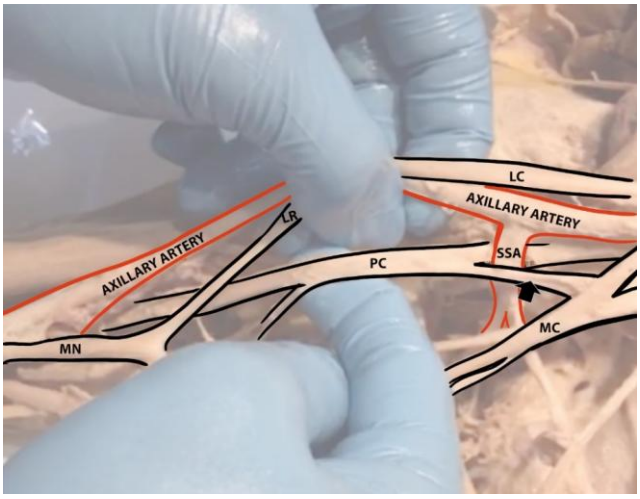



*Figure 3: Brachial plexus cords about the axillary artery. Arrow points to the abnormal presentation of a communicating branch from posterior cord to the medial cord of the brachial plexus. LC: Lateral cord; LR: Lateral root; SSA: Subscapular Artery; PC: Posterior cord; MN: Median Nerve; MC: Medial cord*

## Discussion

The brachial plexus and the upper limb vessels are closely associated with one another. Therefore, the anatomical configuration and form of either structure can affect both nerve conduction and blood supply function. Interestingly, previous studies have shown that variation in the brachial plexus is more often found on the right side of the body, as the case with our female cadaver [2], [3]. The cords of the brachial plexus are of special significance in the axillary region of the body due to their proximity to the third part of the axillary artery. In one case previously reported, the medial cord was observed to be sandwiched in the middle of the axillary and superficial brachial arteries [4]. In another case, it was reported that 4 out of 480 (0.0083%) cadavers had the medial cord divided by the common stem of the lateral thoracic arteries [5]. This paper reveals a pair of medial cord formations, never described in the literature. These anomalies regarding the brachial plexus are thought to be out of an embryologic origin.

Although no significant lesion of both the cords and vessels can be readily appreciated during the dissection, it is difficult to disprove whether the anatomical variation was pathological and rendered the axillary artery susceptible to pinching and thus to symptoms mild of ischemia. This may suggest that in rare cases, upper limb vasoocclusions can result from congenital brachial plexus morphology. Patients that suffer from loss of muscle function due to peripheral nerves anomalies can be treated by gliding therapies as part of a rehabilitation program or perioperative treatment. Gliding therapy consists of a series of active range of movements of the muscles surrounding the targeted nerve, with the purpose of release compression on the nerve [1]. Due to the anomaly found on this cadaveric patient regarding the axillary artery, we believe that if the cadaver had any kind of motor dysfunction originating from the brachial plexus, gliding therapy would not be efficient. Anatomical anomalies are very important to be aware of during surgical procedures. This is especially true when it comes to regions of the brachial plexus and its surrounding areas. An injury to the brachial plexus represents “a severe and difficult-to-handle” traumatic event for the patient. Physicians and surgeons must be aware of the anatomical variations and locations before performing procedures that can drastically affect the lives of their patients. Medical technology can help to play a role in guiding surgeons and anesthesiologists during operative procedures. Diagnostic Sonography is an important tool that can be used to avoid brachial plexus injuries. Also, viewing of such anomalies may aid in orienting patients about possible physiological changes which might occur as a result of such abnormal findings. Sonography is a cost-effective and safe technique which can be used to ensure that nerves and vasculature are normally distributed in the patient, especially if there is an anomaly that is not yet known [2]. To better understand the brachial plexus and possible variations, technology such as sonography and other diagnostic tools must be used. This can lead to the evolution of surgical quality and anaesthesia by leading to facilitated nerve blockage [6]. The location of the axillary artery and its surrounding structures is also important for peripherovascular surgeons and invasive cardiologists due to the use of the axillary artery for coronary bypass. Also, brachial plexus blocks are common in orthopaedic surgeries, but there are novel uses in radical mastectomies for blocking the pectoral nerves [7]. For once we are better able to understand the cause of these variations, we can use them to our advantage in medicine for the benefit of the patient.

In conclusion, knowledge of variations of the brachial plexus and its relation to the axillary artery is important clinically in the planning of surgical and diagnostic procedures. The unusual splitting of the right brachial plexus on the medial cord into two branches which wrap around the axillary artery on the lateral side is an anatomical anomaly that has never been recorded in literature. The embryological origin of this anomaly suggests that congenital brachial plexus morphology can result in changes in the position and shape of the axillary artery. The use of medical technologies, such as sonography, can be used by surgeons to guide and be able to identify any possible variations, to ensure the safety and wellbeing of the patient. Anomalies such as this, are rare and often discovered with little prior information to help

surgeons and other medical professionals in identifying and aiding in treating their patients. Due to this, further research is needed to look deeper into the embryological origin of the cause of this type of anomalies and to look at different surgical and diagnostic procedures that can be used to aid in this outcome.

## References

1. Loh P, Yeoh W, Nakashima H, Muraki S. Deformation of the median nerve at different finger postures and wrist angles. PeerJ. 2018; 6:e5406. https://doi.org/10.7717/peerj.5406 PMid:30123715 PMCid:PMC6087621

2. Yang W, Chui P, Metreweli C. Anatomy of the normal brachial plexus revealed by sonography and the role of sonographic guidance in anaesthesia of the brachial plexus. American Journal of Roentgenology. 1998; 171(6):1631-1636. https://doi.org/10.2214/ajr.171.6.9843302 PMid:9843302

3. Uysal İ, Şeker M, Karabulut A, Büyükmumcu M, Ziylan T. Brachial Plexus Variations in Human Fetuses. Neurosurgery. 2003; 53(3):676-684. https://doi.org/10.1227/01.NEU.0000079485.24016.70 PMid:12943583

4. Nakatani T, Mizukami S, Tanaka S. A rare case: The medial cord of the brachial plexus sandwiched between the axillary and superficial brachial arteries. Kaibogaku Zasshi. 1997; 72:151-154.

5. Miller R. Observations upon the arrangement of the axillary artery and brachial plexus. American Journal of Anatomy. 1939; 64(1):143-163. https://doi.org/10.1002/aja.1000640107

6. Emamhadi M, Chabok S, Samini F, Alijani B, Behzadnia H, Firozabadi F et al. Anatomical Variations of Brachial Plexus in Adult Cadavers; A Descriptive Study. The archives of bone and joint surgery. 2016; 4(3):253-258.

7. Cassai A, Bonvicini D, Ruol M, Correale C, Furnari M. Erector spinae plane block combined with a novel technique for selective brachial plexus block in breast cancer surgery. Korean Journal of Anesthesiology. 2018. https://doi.org/10.4097/kja.d.18.00266 PMid:30481947 PMCid:PMC6547240

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Jun 30; 7(12):2010-2016.
https://doi.org/10.3889/oamjms.2019.071
eISSN: 1857-9655
***Public Health***

# Variations in Family Attitudes towards Coping with People Living with Mental Illness

Tomike I. Olawande[1*], Mofoluwake P. Ajayi[1], Emmanuel O. Amoo[2], Charles T. Iruonagbe[1]

*[1]Department of Sociology, College of Business and Social Science, Covenant University, Ota, Ogun State, Nigeria; [2]Demography and Social Statistics, College of Business and Social Science, Covenant University, Ota, Ogun State, Nigeria*

**Citation:** Olawande TI, Ajayi MP, Amoo EO, Iruonagbe CT. Variations in Family Attitudes towards Coping with People Living with Mental Illness. Open Access Maced J Med Sci. 2019 Jun 30; 7(12):2010-2016. https://doi.org/10.3889/oamjms.2019.071

**Keywords:** Family; Attitudes; Caregivers; People Living with Mental Illness (PLWMI); Sustainable Development Goals

***Correspondence:** Tomike I. Olawande. Department of Sociology, College of Business and Social Science, Covenant University, Ota, Ogun State, Nigeria. E-mail: tomike.olawande@covenantuniversity.edu.ng

**Received:** 25-Feb-2019; **Revised:** 16-May-2019; **Accepted:** 17-May-2019; **Online first:** 30-Jun-2019

**Copyright:** © 2019 Tomike I. Olawande, Mofoluwake P. Ajayi, Emmanuel O. Amoo, Charles T. Iruonagbe. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

**Funding:** This research was financially supported by the management of Covenant University, Ota, Ogun State, Nigeria

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

**Abstract**

**BACKGROUND:** The major challenge faced in Nigeria regarding the care of people living with mental illness are a stigma, caregiver burden and financial aid. This has led to high premature deaths and has also hindered the attainment of Sustainable Development Goal 3 target 4. In an attempt to find lasting solutions to increased mortality caused by mental illness, it is important to evaluate family attitudes towards people living with mental illness.

**AIM:** To examine variations in family attitudes towards coping with people living with mental illness in Nigeria.

**SUBJECTS AND METHODS:** Thirty In-Depth Interview (IDI) were conducted among caregivers of people living with mental illness (those presently receiving treatment and those recovering). During all the interview sessions, field notes were taken. Responses from interviewees were transcribed and analysed with the aid of 'systematic-content analysis.

**RESULTS:** It found out that family members of people living with mental illness or caregivers usually suffer from stress and stigma, which affects adherence to treatment.

**CONCLUSION:** Caregivers must have sufficient knowledge and support to assume the responsibilities of caring for the mentally ill, leading to recurrence. The caregiver remains in contact with professional healthcare workers and helps the mentally ill with decision-making and matters affecting their daily life.

## Introduction

Mental health, as non-communicable disease, is an indispensable part of a person's capability to carry on with an actual existence of self-actualisation, capacity to maintain social interactions and to resolve everyday decisions [1]. Mental illness clarifies the syndrome that is usually portrayed by mood deregulation and behaviour of both men and women [2], and it is inextricably linked to development. However, the Sustainable Development Goals (SDGs) showed that by 2030, prevention and management of mental health and well-being would lead to a 33% decline in premature death due to non-communicable diseases (NCD). To achieve the Sustainable Development Goals (SDGs), effective management of people living with mental illness is very crucial. To achieve this, it is not possible to over-emphasise the role and attitudes of family members or caregivers in managing people living with mental illness.

Caregiver refers to someone who provides unpaid care and support to any member of the family or relative, acquaintance or neighbour who has been diagnosed with mental illness. The function of a caregiver has been recognised as vital, both functionally and economically. A fundamental part of caregiving is the ability to be an excellent communicator with a person living with mental illness (who has recovered or who is recovering).

The majority of household caregiving is normally provided by parents (mothers and fathers), spouse or husband, or relatives. Members of mentally ill households or relatives usually feel stigma pain [3], [4], [5]. Not only for people with mental illness, but

also their families, mental illness is traumatic. Many studies have found that caregivers of mentally ill people suffer from stress, face a significant burden and receive poor assistance from mental health professionals in general. [6]. Disability and the severity of symptoms are the most important indicator of burden. Effective management, therefore, represents the first step towards reducing the burden. High emotional pressure can reflect the family's hard work in helping the patient and is interfered with by controlling attitudes, stigma, burden and the perception of the caregiver of mentally ill people [7].

As medical care expands to find better approaches to help manage people living with mental illness, caregivers play an important and ever-growing role. Caring for mentally ill people requires untiring work, vigour, understanding, and an undeniable impact on the daily life of caregivers. Caregivers are at high risk for various challenges to physical and mental health. They sometimes suffer from an alarming level of constant concern, anxiety, frustration, depression, and risky behaviour. They neglect their care and are more likely to die than non - caregivers of equivalent age. [8]. Social support in Nigeria is considered to be of great value about the problem of caring for people with mental illness [9].

Mental health care behaviour in African indigenous society is often influenced by the household decision-making process in mental illness management [10]. In general, mentally ill people's relatives decide where to seek their help. The belief system of the roots of mental illness influences the opinion of a family about the probable cause of mental disorders experienced in society by a family member. [11]. First of all, mental retardation is diagnosed much earlier than serious mental illness in family life. Mentally disabled people are usually diagnosed at birth or early childhood. As a result, most mentally ill siblings have always known that they have significant disabilities with their brother or sister. Their formative years included satisfaction and difficulties in living with a visible "family difference" [12], and many siblings were involved in parental surrogacy, especially sisters [13], [14].

Access to better treatment for people with mental illness, including medication prescriptions, psychosocial interference and services for reintegration, is a key factor in relieving the burden of caregivers. Emergency management readiness, the provision of legally delegated community treatment to prevent hospitalisation and the support of an informed balance [15] are also necessary. People with mental illness are suffering from episodic crises that can undermine family and household stability. Therefore, in society, the course of mental retardation is better accepted than mentally ill people. Women are often caregivers of the family. Women seek health care more frequently for their children and themselves. The challenge of family responsibility in advanced and non-industrialized countries to help people with mental illness is a collective problem. Different health and social systems in different countries may affect a family's duty to care.

Families may be responsible for taking care of persons with mental illness in a convincing manner of the facilities, resources and care available to persons with mental illness and their caregivers. Knowing the detailed lifestyle that could affect the burden of family caregivers and the techniques that transmit these issues in the care of a mentally ill member of the family can be an important part of developing an all-inclusive family-centred care ideal. According to [16], households are the main sustainable caregiver and a valuable supply for mentally ill people despite differences in needs and concerns between individuals and ethnic groups. Studies in the UK have shown that caregivers have some collective needs. Expressive support, assistance with seclusion, recognition of reliable and suitable services, evidence and recognition of their role and impact in helping caregivers [17]. It is the responsibility of extended Arab families to arrange their members to prevent or retard the effects of parental loss and psychological disorder [18]. Instead of unfamiliar persons, the violent behaviour of mentally ill people is most often directed towards families, caregivers and acquaintances. Regrettably, Nigeria does not have statistics on the percentage of population attributable risk (PAR per cent) that would indicate the proportion of mental illness-related violence.

In addition to the above, it is worthy to note that in reducing premature death caused by mental illness, the attitudes of caregivers are often neglected in the study of the management of people living with mental illness. This is because it is necessary to examine the attitudes of caregivers to manage mental illness; as has already been established, it is prone to the stigma that can lead to a relapse and ultimately affect the rehabilitation process of mental illness. This study, therefore, examines variations in family attitudes/caregivers in the management of mentally ill people in Nigeria. The study also investigates whether the treatment of mentally ill people has been a positive exploration in pursuing and achieving the Sustainable Development Goals (SDGs) target 3.4.

## Subjects and Methods

This study utilised In-Depth Interview (IDI) to examine the variations in family attitudes towards coping with people living with mental illness in Nigeria. Interviews are the most common source of qualitative data for health scientists. Interviews allowed for in-depth analysis to explain attitudes of caregivers, who are family members and coping strategies towards people living with mental illness in a view to reduce premature death among men as listed in the

Sustainable Development Goals (target 3.4). The choice of these methods was necessary to enhance the realisation of the objectives of this study.

Framework analysis was used to analyse the data. In the work of [19], the framework analysis focuses on real-life findings using a method of content analysis that summarises and classifies responses into themes [20]. Also, framework analysis is suitable for applied research that deals with practical methodologies to solve problems, in particular, health issues.

Hospitals and Local Government Areas (LGAs) in Ogun State have been selected intentionally. Four Federal and State-owned hospitals were purposively selected. The selection criteria for psychiatric hospitals are 1. The presence of psychiatric patient facilities and services and 2. Availability of psychiatric patients.

The multistage sampling procedure was adopted to purposively select Ogun State due to the presence of a first-generation Neuropsychiatric Hospital. The state was clustered into six dialectical groups and purposively selected three LGAs with psychiatric healthcare facilities. People living with mental illness could not be interviewed because they were not mentally stable. Family members or people in selected psychiatric hospitals who care for mentally unstable people (now suffering plus those who recovered) who are Yoruba descendants in Ogun State were randomly selected, screened, recruited and interviewed in their clinic days after obtaining informed consent. Caregivers under 18 years of age were excluded. Caregivers who had been or is currently in a marital relationship were the target group of interest. At the commencement of the interview, the basic characteristics such as gender, marital status, level of education and religion were assessed.

The clinic hall at the various psychiatric hospitals, which is void of distractions was chosen for the interviews. These locations allowed interviewees to be easily accessible, comfortable and to freely discuss health issues. Thirty Interviews were conducted across the three Local Government Areas in the state. The few respondents were due to a limited number of mentally ill persons (invited persons). Volunteers were interviewed. However, the number of interviewees was manageable, and there was enough data. Each of the interviews lasted 50 to 90 minutes. Discussions continued until new information was made available and the theoretical saturation was achieved. The interviews were moderated by a social worker who was selected to avoid partiality and misconception of health conditions. Discussions were held in Yoruba (local southwestern language, Nigeria).

Nonetheless, some individuals used Pidgin English or a combination of Yoruba and English. The in-depth interview guide was adopted from the Caregiver Burden Inventory, Orientation towards mental illness scale (OMI) [21], [22] cited by [23]. Interviewees were asked about their experiences and knowledge of care given to people living with mental illness as well as coping strategies in reducing premature deaths among men.

The socio-demographic characteristics of the interviewees (marital status, mentally ill relationship and level of education) have made the findings more representative and contributed to the creation of contrasting beliefs. The interviewees reviewed the notes to ensure the validity and integrity of the data were correct.

Other researchers who did not participate in the study but had qualitative expertise also examined the transcriptions. Transcripts were read without numbers to discover themes, confirm that the transcripts follow the same approach to transcription and eliminate bias in the identification of themes. It should also be noted that the analytical results were submitted to non-participating colleagues and women who considered the findings of the research to be appropriate.

### *Statistical Analysis*

During all the interview sessions, field notes were taken. Responses from interviewees were transcribed and analysed with the aid of ‘systematic-content analysis’ Qualitative data were thematically, and content analysed [19]. The field notes and transcripts were read severally to have a proper understanding of the data. In classifying, repeated answers and mutual themes, ‘scissors and paste’ approach was used [24], [20], [19]. Transcript data that have been revised repeatedly and field notes have enhanced the identification of the concepts. Concepts have been coded and systematised into categories for each transcription and then combined. The topics have been further refined by more concepts, divisions or combinations [20]. Answers from interviewees that could not be grouped directly into themes discussed and subsequently re-grouped between reviewers. The importance of the answers and the links to other topics were discussed.

A Microsoft Excel sheet document that details the answers to each question was generated. Each of the question was inserted on detached sheets within an Excel sheet. In the used excel sheet as created, five columns were labelled, the first column was interviewees’ identification number (ID); the second column was for coding; the third column or responses of the interviewees; the fourth column was for the themes of the coding categories while the fifth (last) column was for analysis of the questions. Afterwards, the responses were cross-tabulated by socio-demographic characteristics for easy appraisals and evaluations. The results were buttressed with existing literature. Analysis of data adapted the qualitative

research review guidelines (RATS). RATS stress the importance of research questions. It also criticizes the suitability of methods while ensuring transparency and consistency of the interpretive approach [25]. Data presentation and research findings were used with consolidated criteria for reporting qualitative research (COREQ) [6].

### *Ethical Consideration*

At the commencement of the interviews, the aim of the study was made known to the interviewees, and their consents sought and obtained to be part of the study. They were also assured of the confidentiality of data, and the place of the interview was made free of interference as much as possible. Participants' permissions were also sought before recording the interviews on tape. Approvals from Aro Neuropsychiatric Hospital, Abeokuta with approval Number PR003/16 and Federal Medical Centre, Abeokuta with approval number FMCA/470/HERC/05/2016 were obtained from institutional ethical boards.

**Table 1: Socio-Demographic Characteristics of In-Depth Interview Participants**

| Selected Characteristics of Caregivers | Frequency | Percentage |
|---|---|---|
| Gender | | |
| Male | 8 | 26.7 |
| Female | 22 | 73.3 |
| Total | 30 | 100.0 |
| Age in years | | |
| 18-29 | 7 | 23.3 |
| 30-39 | 10 | 33.3 |
| 40 and above | 13 | 43.3 |
| Total | 30 | 100.0 |
| Marital Status | | |
| Single | - | - |
| Married | 25 | 83.3 |
| Separated | 5 | 16.7 |
| Total | 30 | 100.0 |
| Educational Level | | |
| No Education | - | - |
| Primary Education | - | - |
| Secondary Education | 17 | 56.7 |
| Tertiary Education | 13 | 43.3 |
| Total | 30 | 100.0 |
| Religion | | |
| Christianity | 20 | 66.7 |
| Islam | 10 | 33.3 |
| Traditional | - | - |
| Total | 30 | 100.0 |
| Relation to Patients | | |
| Parent | 15 | 50 |
| Spouse | 10 | 33.3 |
| Sibling | 5 | 16.7 |
| Total | 30 | 100.0 |
| Local Government Areas | | |
| Abeokuta North | 10 | 33.3 |
| Abeokuta South | 12 | 40 |
| Sagamu | 8 | 26.7 |
| Total | 30 | 100.0 |

Source: Author's Computation.

## Results

A total of 30 caregivers of mentally ill people were interviewed with a response rate of 100%. Of these, 73% were females, and 27% were males. Findings showed that 23% were under 29 years of age, those in age categories 30-39 were 33%, the highest proportion was 40 years and above with 43%. Most (83%) of the participants were married, while 17% were separated. The distribution of religion showed that the respondents in Nigeria practised the two main religions. The study found that a greater proportion of respondents practised Christianity (67%) while 33% practised Islam. It is also worthy to note that 57% of the participants had secondary education, while 43% of the participants had tertiary education. Surprisingly, 50% had relations with parents, while 17% had a relation with siblings.

### *Perception of mental illness*

Caregivers indicated the causes of mental illness. Few caregivers used the right terminology for the disease. However, experienced medical and social workers' facilitators helped to classify the disease described by participants. The most common causes of mental illness described by participants were: hereditary *('were Iran'), a* mental illness that one is born with *('were amutorunwa')*. A small number also mentioned mental illness due to affliction *('were afise')*.

A caregiver aged 30-39 expressed that '*breaking a family taboo is the primary cause of mental illness. In Yorubaland, it is called 'two idle'***.** Another married caregiver, aged 40 years and above pointed out that mental illness is due to insufficient rehabilitation. Other causes of mental illness as experienced are described in the following excerpts:

*"Omo on mejo ko'gbodo ya were bi koni taye ninun" (Translated thus; "*No mental illness shall affect a child of eight years or less without supernatural causes*")* (Caregiver, Married, Islam, Spouse).

*'The cause of mental illness in the life of my son is as a result of substance/drug abuse* (Caregiver, Separated, Christian, Parent).

### *Attitudes towards people living with mental illness*

Answers from a married caregiver whose husband had a mental illness (30-39 years of age) said that the stigma attached to the disease humiliates him. A caregiver said that "a kind of label is a psychiatric hospital because it is isolated." While one participant stated that stigma is a major obstacle to recovery, it can limit the social functioning of family members and mentally ill people.

A man aged 40 years and above indicated that caregivers' self-stigma is a psychological challenge. An extract of another caregiver in the same age group indicates:

'*I have avoided making friends because my younger sister is mentally ill' (Caregiver, Single, Islam,*

*Sibling).*

Many caregivers avoided being identified with the mentally ill people they care for due to public stigma.

*'I worried that people would find out about the illness' (Caregiver, Female, Married, Spouse).*

*'I worried that neighbours would treat me differently' (Caregiver, Male, Islam, Parent).*

Most caregivers believed that self-stigma could hurt the mentally ill seeking treatment, adherence and rehabilitation.

'*I find it very difficult to give my husband his drugs; this has affected him from recovering fast' (Caregiver, Female, Christian, Spouse).*

*My daughter was involved in the murder; I took her to a mental home. She was found to have a mental illness. Her health condition is improving, but I'm afraid to take her home so as not to commit murder (Caregiver, male, Christian, Parent).*

*'My family has a mental illness. No one is willing to marry my daughter because they all believe that it is hereditary (Caregiver, female, Christian, Parent).*

### *Limitations to the utilisation of mental healthcare services*

In addition to the main topics identified, the results showed that caregivers faced challenges in the use of mental health services. Most participants indicated that access to and use of mental health services was problematic in the mental illness recovery and rehabilitation process. The general impression was that *"financing was a major impediment to the use of mental health services."*

*"The drugs prescribed by the professional healthcare workers are too expensive." (Caregiver, Single, Sibling).*

*"To make sure my daughter was treated, I had to lend money to relatives and friends (Caregiver, Separated, Parent).*

There was consensus, especially among older caregivers, that the distance is also a major barrier to the utilisation of mental healthcare services. Few of their responses are as follows:

*"The biggest obstacle to hospital use is distance. I had to stay in a hotel so my son could receive adequate treatment at the time". (Caregiver, Female, Married, Parent).*

*"This child is my third child to go to this psychiatric hospital. The hospital is far from where I live." (Caregiver, male, Married, Parent).*

*Some caregivers pointed out that while the mental hospital is far away, the services offered are too costly. (Caregiver, Female, Married, Parent).*

### *Coping Strategies towards People Living with Mental illness*

Several preventative coping strategies in minimising premature deaths among men as listed in the Sustainable Development Goals (SDGs) were described in the interviews. These approaches were used to manage caregivers' attitudes towards the mentally ill. A popular response from the interviewees was that instituting family support groups and counselling caregivers by professional healthcare providers will be of great importance in dealing with self-stigma among caregivers of mentally ill people.

Primary themes of coping strategies included counselling caregivers by professional healthcare workers, seeking government support in the treatment of people living with mental illness and establishment of family support groups to eradicate or minimise self-stigma among caregivers of mentally ill.

### *Seeking Counselling from Professional healthcare workers*

Across all discussion, seeking counselling from professional healthcare worker was one of the essential steps in coping with the mentally ill. Majority of the participants complained of the need to be counselled. The general perception was that professional healthcare workers were 'divine helpers' in the treatment of mental illness, especially as it relates to the recovery process and rehabilitation. Interviewees thought that health workers were trained discreetly. The following are extracts from some of the respondents in all age groups: '*Hiding anything from medical personnel will be of no help to me. They are here to help me. Their counsel me go a long way' (Caregiver, aged 18-29).*

*'Professional healthcare workers are highly respected in this country and are held in high esteem, for me I need their advice so that my wife will get well as quick as possible' (Caregiver, aged* 30-39*).*

*'Counselling caregivers will go a long way. It will help to guide me whenever there is a relapse' (Caregiver, aged 40 and above).*

### *Seeking government support in the management of the mentally ill*

The analytical results revealed a popular approach to coping among caregivers seeking government support in treating people with mental illness. Virtually all the caregivers described this coping strategy as very important. Other comments in support of this response indicated that' the best option is government intervention in the treatment of mental illness. A caregiver (aged 30-39) indicated that 'the

drugs are too expensive to purchase, hence the need for subsidy. However, another participant (in age 40 and above) believed that *'If the government can create more psychiatric hospitals in every state in the country, it will be easier to have access to the psychiatric hospitals.* Almost a quarter of all participants indicated that they expected support from the government in the management of these health problems. In this connection, another caregiver (18-29 years old) considered government assistance in reducing premature mental illness deaths. *'for me, I want the government to reinforce the National Health Insurance Scheme (NHIS).*

### *Establishment of family support groups*

The next strategy for coping with the mentally ill was the establishment of family support groups. Most caregivers reported that family social support could help them feel worthy. Discussions highlighted that family support groups are vital means for families who have a loved one with mental illness, knowing that other caregivers had the same knowledge is a relief to families who often speak about mental illness to relatives, friends and neighbours. Having been told that other caregivers have been able to work out the same health issues can inspire hope. A caregiver aged 37 reported that *'having social groups is the best way to ease stress'.* Another caregiver aged 29 indicated that '*learning from the experience of other caregivers will assist in speedy recovery of the mentally ill'.* This was supported by three caregivers who stated that sharing of knowledge by other caregivers is very important.

## Discussion

This study provides evidence on the levels of support and unpaid caregivers attitudes towards PLWMI with the view of reducing premature deaths among men. These findings go beyond existing studies on the attitudes of caregivers and the management of mental health and wellness. In addition to adding to the knowledge base on family/caregiver attitudes, the results also helped achieve goal 3.4 of the Sustainable Development Goals (SDGs), which states that by 2030, premature mortality from non-communicable diseases should be reduced by one-third through prevention and treatment, and mental health and well-being promoted. This study dealt exclusively with different attitudes of family/caregivers in managing people living with mental illness. Amongst the important contributions of this research is the discovery that caregivers experience financial and social burden. Caregivers, who undergo undesirable levels of severe burden, are responsible for caring for people living with mental illness [26]. To take care of PLWMI, caregivers need support and understanding. Also, PLWMI dominates caregivers, which can lead to an increase in distress and its inability to deal with the crisis [23]. In [13]'s work, unwanted quality of life for caregivers can lead to poor quality of care and a deterioration in their quality of life. Failure to deal with the situation could lead to the possibility of PLWMI being abused, leading to a further deterioration of the disease.

It was also discovered that caregivers were not willing to disclose the patients' mental illness and being ashamed or embarrassed by it. As a result of stigma experienced by the caregivers, they avoided social gatherings or been seen with the mentally ill. This is likely to happen because been seen with the mentally ill; the caregiver is at risk of stigma and discrimination by friends, colleagues or the populace in the society. In the works of [27], stigma in caregivers is on the increase and needs to be eradicated.

It was also discovered that caregivers had to travel miles to get access to mental healthcare services in Nigeria. In the work of [28], women have more frequent access to mental health facilities, receive more treatment, and have a higher rate of psychiatric hospitalisation than men. These variations may affect women's diagnosis and therapy. These variations can have an impact on diagnosis and therapy for women. The results of the study confirm the previous work carried out by [29], which showed that access to mental health services is increasing closely with the health centres. This proximity is necessary to reduce the transport rate and the thoroughness of access to modern health services from a distance.

### *Limitation of the Study*

The study's limitation includes the use of a purposive sample of medical facilities that restrict the generalizability of results. The limited number of interviews may also be a study limitation. The study appraised the attitudes of caregivers towards PLWMI in Nigeria. Responses of attitudes of professional healthcare workers towards PLWMI were neglected. Another significant limitation of the study is the language barrier. The service of a knowledgeable Yoruba interpreter was employed by the principal investigator. This is to allow the Yoruba interpreter to interpret the information provided by the caregivers in the Yoruba Language so as to have rich and in-depth information. On the contrary, some complications with the translator could have existed with the fact that the translator spoke both Yoruba and English Language as some words could have been lost in transition. In view of the above, the reliability of the gathered empirical material may have been negatively affected by the issues mentioned; the research was strengthened by the fact that an appreciable number

of literatures were compared with the interviews conducted. Finally, this study only established responses by caregivers and the key socio-demographic characteristics, the type of mental illness been experienced were not collected.

In conclusion, this study has added to the knowledge about caregivers ' attitudes toward PLWMI. This study concludes that the rehabilitation process and management of PLWMI is crucial to the achievement of target 3.4 of the Sustainable Development Goals (SDGs). The non-availability of mental healthcare services and the poor utilisation of psychiatric hospitals in Nigeria which include but not limited to stigma, finance and distance should be urgently addressed by the government. The study recommends that the government find lasting solutions to the challenges of PLWMI by providing support and adequate healthcare facilities to reduce thirty - three per cent of premature death from non-communicable diseases through prevention, management and promotion of mental health. The existing policy document on mental health, formulated by the Government of Nigeria in 1991, which includes a promotion, advocacy, prevention, rehabilitation and management.

## Acknowledgement

The Authors appreciate the management of Covenant University for funding the Publication.

## References

1. World Health Organization. Department of Mental Health, Substance Abuse, World Health Organization, World Health Organization. Mental Health Evidence, Research Team. Mental health atlas 2015. World Health Organization, 2015.

2. World Health Organisation. Mental health: Maternal mental health, 2017. www.who.int/mental health/maternal child/maternal mental health. Accessed 15th February, 2017.

3. Larson JE, Corrigan P. The stigma of families with mental illness. Academic psychiatry. 2008; 32(2):87-91. https://doi.org/10.1176/appi.ap.32.2.87 PMid:18349326

4. Corrigan PW, Miller FE. Shame, blame, and contamination: A review of the impact of mental illness stigma on family members. Journal of Mental Health. 2004; 13(6):537-48. https://doi.org/10.1080/09638230400017004

5. Olawande TI. Gender Differentials in the Perception and Treatment of Mental Illnesss among the Yoruba of Ogun State, Nigeria. Unpublished PhD Thesis, 2017. https://doi.org/10.4172/1522-4821.1000384

6. Saunders JC. Families living with severe mental illness: a literature review. Issues in Mental Health Nursing. 209; 24 (2): 175-198. https://doi.org/10.1080/01612840305301 PMid:12554427

7. Ohaeri JU. The burden of caregiving in families with a mental illness: a review of 2002. Current Opinion in Psychiatry. 2003; 16(4):457-65. https://doi.org/10.1097/01.yco.0000079212.36371.c0

8. Edition F. Diagnostic and statistical manual of mental disorders. Am Psychiatric Assoc. 2013.

9. Esan OB, Kola L, Gureje O. Mental disorders and earnings: results from the Nigerian National Survey of Mental Health and Well-Being (NSMHW). The journal of mental health policy and economics. 2012; 15(2):77-82.

10. Kaur N. Caregiving burden and social support among caregivers of schizophrenic patients. Delhi Psychiatry Journal. 2014; 17:337-42.

11. World Health Organisation. Gender disparities in mental health. Department of mental health and substance dependence. Geneva: World Health Organisation, 2015.

12. Mehrotra K, Nautiyal S, Raguram A. Mental health literacy in family caregivers: A comparative analysis. Asian journal of psychiatry. 2018; 31:58-62. https://doi.org/10.1016/j.ajp.2018.01.021 PMid:29414389

13. Stoneman Z, Brody GH, Davis CH, Crapps JM. Role relations between children who are mentally retarded and their older siblings: Observations in three in-home contexts. Research in Developmental Disabilities. 1989; 10(1):61-76. https://doi.org/10.1016/0891-4222(89)90029-2

14. Wilson J, Blacher J, Baker, BL. Siblings of children with severe handicaps. Mental Retardation. 2001; 27(3):167-173.

15. Awad AG, Voruganti LN. The burden of schizophrenia on caregivers. Pharmacoeconomics. 2008; 26(2):149-62. https://doi.org/10.2165/00019053-200826020-00005 PMid:18198934

16. Chan SW. Global perspective of burden of family caregivers for persons with schizophrenia. Archives of psychiatric nursing. 2011; 25(5):339-49. https://doi.org/10.1016/j.apnu.2011.03.008 PMid:21978802

17. World Health Organisation, 2017. Accessed Online www.who.int/mentalhealth/prevention

18. El-Islam, MF. Arab culture and mental healthcare. Transcultural Psychiatry. 2008; 45(4):1-8. https://doi.org/10.1177/1363461508100788 PMid:19091731

19. Green J, Thorogood N. Qualitative methods for health research. London: Sage Publications Ltd., 2004:279.

20. Amoo EO, Omideyi AK, Fadayomi TO, Ajayi MP, Oni GA, Idowu AE. Male reproductive health challenges: appraisal of wives coping strategies. Reproductive health. 2017; 14(1):90. https://doi.org/10.1186/s12978-017-0341-2 PMid:28754166 PMCid:PMC5534028

21. Novak M, Guest C. Application of a multidimensional caregiver burden inventory. The gerontologist. 1989; 29(6):798-803. https://doi.org/10.1093/geront/29.6.798 PMid:2516000

22. Prabhu GG. Mental illness, public attitudes and public education. Indian J Clin Psychol. 1983; 10:13-26.

23. Mokgothu MC, Du Plessis E, Koen MP. The strengths of families in supporting mentally-ill family members. Curationis. 2015; 38(1):1-9. https://doi.org/10.4102/curationis.v38i1.1258 PMid:26018195 PMCid:PMC6091649

24. Olanrewaju FO, Omotoso F, Alabi JO. Boko Haram insurgency and the management of internally displaced women in Nigeria: A situational analysis. African Population Studies. 2018; 32(1): 4022-4033.

25. Clark JP. Qualitative research review guidelines-RATS. Peer review in health sciences. 2003:219-35.

26. Gupta MD, Gauri V, Khemani S. Decentralized Delivery of Primary Health Services in Nigeria. Africa Region Human Development Working Paper Series Washington, DC, The World Bank. 2003.

27. Shibre T, Negash A, Kullgren G, Kebede D, Alem A, Fekadu A, Fekadu D, Medhin G, Jacobsson L. Perception of stigma among family members of individuals with schizophrenia and major affective disorders in rural Ethiopia. Social Psychiatry and Psychiatric Epidemiology. 2001; 36(6):299-303. https://doi.org/10.1007/s001270170048 PMid:11583460

28. Rhodes AE, Goering PN, To T, Williams JI. Gender and outpatient mental health service use. Social science & medicine. 2002; 54(1):1-0. https://doi.org/10.1016/S0277-9536(01)00002-8

29. Omonona BT, Obisesan AA, Aromolaran OA. Healthcare access and ultilization among rural households in Nigeria. Journal of Development and Agricultural Economics. 2015; 7(5):195-203. https://doi.org/10.5897/JDAE2014.0620

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Jun 30; 7(12):2017-2023.
https://doi.org/10.3889/oamjms.2019.475
eISSN: 1857-9655
***Public Health***

# Exploring the Human Factors Affecting Health Service Managers: A Qualitative Study

Mansour Ranjbar[1], Masoumeh Neishabouri[2], Mohammad Ali Heidari Gorji[2*]

*[1]Educational Development Center, Mazandaran University of Medical Sciences, Sari, Iran; [2]Department of Medical-Surgical Nursing, Nasibeh School of Nursing and Midwifery, Mazandaran University of Medical Sciences, Sari, Iran*

**Citation:** Ranjbar M, Neishabouri M, Heidari Gorji, MA. Exploring the Human Factors Affecting Health Service Managers: A Qualitative Study. Open Access Maced J Med Sci. 2019 Jun 30; 7(12):2017-2023. https://doi.org/10.3889/oamjms.2019.475

**Keywords:** Health Facility Administrators; Health Services; Qualitative Research

***Correspondence:** Mohammad Ali Heidari Gorji. Department of Medical-Surgical Nursing, Nasibeh School of Nursing and Midwifery, Mazandaran University of Medical Sciences, Sari, Iran. E-mail: drheidarigorji@yahoo.com

**Received:** 19-Apr-2019; **Revised:** 27-May-2019; **Accepted:** 28-May-2019; **Online first:** 30-Jun-2019

**Copyright:** © 2019 Mansour Ranjbar, Masoumeh Neishabouri, Mohammad Ali Heidari Gorji. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

**Funding:** This research has been financially supported by the deputy of research and technology, Mazandaran University of Medical Sciences, Sari, Iran

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

**Abstract**

**BACKGROUND:** In recent decades, managing health-service systems has faced multiple challenges. Identifying and resolving these challenges promote the efficiency and effectiveness of hospital activities.

**AIM:** The present study aimed to explore the human factors affecting health service managers.

**MATERIAL AND METHODS:** In this qualitative study, in-depth unstructured interviews were conducted with 29 employees who were in close contact with the health service managers. All the interviews were transcribed verbatim. Data were collected using purposeful sampling and were analysed using conventional content analysis via MAXQDA software.

**RESULTS:** A group of 29 participants were interviewed (male 65.5%, female 34.4%). "Managing managers" has been identified as the primary theme with four supporting secondary themes including the inappropriate appointment of managers, the impact of human and social needs of managers, influential employees, and disrupting organisational communications. These are the challenges faced by managers in managing human resources in health-service organisations.

**CONCLUSION:** Results showed that employees manage their managers within the organisation so that they can prevent managers from concentrating on their management affairs and tasks resulting in the distortion of management practices. The results of this study can help the key policy makers and planners in health-service organisations to guide the organisation to pursue its goals through appointing appropriate managers and identifying influential employees.

## Introduction

Managing health-service systems have faced multiple challenges in recent decades. Identifying and resolving these challenges can promote the efficiency and effectiveness of organisation activities [1]. It is currently believed that the most challenging health-service systems issue today is the issue of management [2] so that the efficiency and effectiveness of health-service systems depend to a great extent on the management and effective use of resources in that organisation [3]. The management and role of health-care system managers in improving the quality of health care are of paramount importance [4]. Arguably, management is one of the most important, complex and difficult knowledge nowadays [5], [6]. It is a process by which different resources are organised for achieving a goal [7]. The main objective of management in health-service systems is to provide health services of high quality [3], [7]. Health-service systems' managers are often responsible for planning, budgeting, managing human resource, and monitoring service quality [8]. Improvement and promotion of health-service systems require strengthening the capacity of managers [9] because health-service systems' managers can promote communication among various components of the health system and can provide human relationships within the organisation and among different system factors [10]. By developing and exploiting their inner abilities, knowledge and work experience, the competent and knowledgeable managers can realise the goals of the organisation through recruiting the least amount of possible resources in the best way and improving the effectiveness and efficiency of the

organisation [11]. Human resource is arguably the most important component of the organisation at the managerial and non- managerial levels and has been recognised as the main factor in success or failure of the organisation in achieving maximum productivity [7]. It has been reported that human resource communication plays a significant role in the systematic analysis of factors, affecting the organisation's management system and makes a major contribution towards optimising the performance of human resources in achieving organisational productivity [12]. The role of managers in the coordination of human resource functions is highlighted in reaching the overall goals of the system [13]. Managers of health-service systems face multiple challenges and obstacles for managing these important and complex organisations. These problems include lack of proper policy making [14], structural problems of universities [1] and managerial instability [12]. Although extensive research has been carried out on the classification of the duties and skills that managers needed, far too little attention has been paid to human factors influencing management [8], [15], [16], [17]. Human factors are effective in the management of the topic managers, which is less considered. Therefore, there is a strong need for managers' analysis as an important and influential factor in the human resources of organisations.

Regarding the culture of management, which deals with the administration of human affairs [18], this phenomenon requires its comprehensive examination in the cultural context to identify the factors that influence it. Considering that quantitative research does not have the flexibility and depth to understand and explain the depth and accuracy of the views and experiences of individuals [19]; therefore, it is necessary to deeply analyse this issue using appropriate tools such as qualitative research. Thus, this study aimed to explore the human factors affecting health service managers.

## Material and Methods

This qualitative study was performed using conventional content analysis — this study conducted at Mazandaran University of Medical Sciences, Sari, Iran, in 2017. The participants (n = 29) were employees working at Mazandaran University of Medical Sciences, who were in contact with the health service managers. The participants were selected using purposeful sampling. The inclusion criteria were at least one year of working experience in close contact with the health service managers. To obtain a wide range of experiences and views, sampling was performed using the maximum variance approach [20], from both sexes with different roles and responsibilities within the organisation and management experience. Data were collected using unstructured interviews with open-ended questions such as “What are the perceived human factors that affect health service managers?" Then, to clarify the details and improve the depth of the interview content, probing questions such as “Please explain more”, why and how? " were asked. Data collection and analysis were done simultaneously. When data saturation was achieved, the interview process was discontinued due to the duplicated data. The interviews were conducted individually in a quiet room at a hospital or university. The time and location of the interview were determined by employees’ agreement. The duration of the interview varied between 20 to 90 minutes.

Data analysis was carried out according to the content analysis approach proposed by Graneheim and Lundman [21]. Immediately after each interview, the interviews were transcribed word by word. Then, to gain a general sense of the data, the text of the interview was repeatedly read out and analysed before the next interview. The interview transcripts were read line by line, and their semantic units were extracted and coded. Codes with similar meanings were subsumed under the more general categories of secondary themes based on similarity and relevance. Finally, the analysis process was completed by identifying the main elements of the process. MAXQDA software was used to manage the data.

To verify the accuracy of the results, four criteria of credibility, dependability, confirmability and transferability were taken into consideration [19]. Credibility and dependability are activities that improve the ability to believe and accept, as well as the stability of the results. The researcher has been involved in the subject for a long time and allocated enough time to collect data. That is, the researcher was present as an employee at the University of Medical Sciences; this helped him to gain the trust and acceptance of the participants and understand their experiences. Also, member check was done through 3 employees to increase the credibility of data. To improve the credibility and dependability of the collected data, we used maximum variance sampling among the employees with work experience, organisational posts, as well as different organisations supervised under the university.

Meanwhile, the researcher provided a transparent description of the research steps taken from the start in order to ensure the reliability of the data. Conformability is an agreement about the accuracy, relevance and meaning of the data. To ensure this criterion, accurate review and external checking as member of the research group analyzed the data. Transferability describes the process of applying and generalizing the results of research in one situation to other similar situations; therefore, the results were detailed in order to allow its use in similar environments. It should be noted that this study was approved by the University Ethics Committee. The researcher provided some explanations for the

participants regarding the purpose of the study, voluntary participation, as well as study exclusion. Written informed consent was obtained from all participants for participation in the research and recording the interviews.

## Results

In total, 29 interviews were conducted. Nineteen participants were male, and eight were female, and their age range was between 28 to 63 years (work experience ranges between 4 to 22 years). Using the constant comparative analysis, "managing manager" was identified as the primary theme with four supporting secondary themes including "inappropriate appointment of managers", "impact of human and social needs of managers", "influential employees" and "disrupting organisational communications". These themes demonstrate the barriers of the proper management faced with health-service managers (Table 1).

**Table 1: Main and secondary factors affecting the managing of managers**

| Main Theme | Secondary Theme | Category |
|---|---|---|
| Managing managers | Inappropriate appointment of managers | Inadequate managerial and organizational components |
| | | lack of focus on the organisation's affairs and tasks |
| | Impact of human and social needs of managers | The need for belonging and love |
| | | The need for respect and value from the staff |
| | | The need for security and trustworthiness |
| | Influential employees | Experienced employees |
| | | Employees engaged in close relationships with management |
| | Disrupting organizational communications | Application of employee communication tips |
| | | Appointment of consultants based on the relationships |

### *Inappropriate appointment of managers*

Inadequate management components refer to the individual and organisational characteristics of managers that are necessary for the management of health-service systems. This concept involves personality traits and a lack of focus on the organisation's affairs.

### *Inadequate managerial components*

Components such as impact, uncertainty and inadequate managerial authority are considered factors are influencing the managers' ability to manage. Also, the appointment of inexperienced managers with lack of necessary technical knowledge and organisational affairs skill are among the most important factors for managing the managers within the organisations. It is axiomatic that having a high degree of power, decisiveness and authority is considered one of the main requirements of management among the managers in case of necessity. However, the lack of these personal characteristics will certainly cause great inefficiencies for the manager, and consequently, the manager is rarely able to use the best of his management authority or exercise effective control over his or her subordinates. In this regard, one interviewer (with 8 years of experience) commented: "*When the manager doesn't* have *the* inherent talent, skill *or insight for management …. this implies that he is unable to show the ability to make decisions quickly and effective …. or does not have strong personality and easily lose against his employees*" (P.19).

### *Lack of focus and concentration on organisational affairs*

Along with the personality traits of managers, managers need to focus on organisational issues and devote a lot of time and energy for better and more efficient management of organisational affairs. On the contrary, if a manager possesses adequate power and personal authority, but does not devote a lot of time and energy for managing the organisational affairs, he may lack necessary competence and core skills needed for managing the organisational affairs. Therefore, he has no choice but to obey his subordinates. One of the participants (with 12 years of experience) commented: "*It is very important for the manager to possess sufficient knowledge and* required expertise *as well as the level of* mastery *in his career…Nevertheless, if the manager has sufficient knowledge and necessary skills but can manage multiple tasks simultaneously; surely, he is unable to* devote sufficient time *to the* organisation's affairs*, for example, a cardiothoracic surgeon is appointed as the chief of hospital. It is certain that this person does not have a strong sense of responsibility to* handle *varied* tasks simultaneously" (P.4).

### *Human and social needs of the manager*

Human and social needs of manager display the managers' willingness to get more acceptance from the employee. This notion encompasses the need for belonging, being loved, respect, value and the sense of security as well as reassurance in the manager by the employee.

### *The need for belonging and love by the employee*

The desire for belonging and love is a human need that affects the manager. Some managers converge their emotions and interests with employees by focusing on their interests and try to be accepted by their employees rather than perform their respective and assigned duties. In these situations, while paying special attention to the

individual manager's characteristic traits, the employees try to recognise the strengths and weaknesses of the manager accurately and constantly remind them to the manager. As a result, they struggle to build up a strong sense of belonging, being together and love so that they can gradually bring managers under their control to ultimately achieve their intended goals. One of the interviewees (with 6 years of experience) alluded to the notion: "*The manager is also a human and has, of course, his own special social needs… He, like any other person, likes to be accepted in public... But a manager can oversee or manage his employee successfully if he was able to sustain a limitation of intimacy and privacy with his subordinates*" (P. 15).

### *The need for respect and value from the employee*

One of the factors influencing managers is disguising the respect and feeling valued for managers through the inflated and exaggerated respect, praise and flattery, showing gratitude and appreciation even for the least important things, instilling the feeling of being the best manager with an emphasis on having high academic talent and capability on behalf of the employees. By exploiting this human need, they can influence his management practices, and as a result, they can accomplish their goals more easily. One interviewee (with 11 years of experience) expressed the belief that: "*Some managers can be easily influenced by flattering employees. I have worked with a manager who easily ignored the poor performance of his subordinates just by expressing some compliment or admiration sentences from the employees (say: "*whatever you say sir") (P 9).

### *The need for security and assurance*

If managers failed to fulfil their security requirements by using desirable ways, this could set the grounds for other individuals and in-power groups to exercise their control. The employees struggle to provide a sense of security for the vulnerable managers through displaying loyalty and support for the manager, lack of receiving criticism from the employees, lack of presenting organisation's problems and failing to express dissatisfaction or complaint. Adopting the solutions mentioned above helps the employees to draw the manager's attention and gradually exercise their control over their manager's actions and consequently, realise their defined objectives.

In this regard, one of the participants with 20 years of experience commented: "*When the employees report to their manager that everything is going well inside the organisation and it works out in your favour; it seems that there is no problem. Surely, something is wrong. My experience has shown me that these managers take a strong stance against the criticism and lack of job security and therefore, deceive their employees by concealing managerial challenges and problems"* (P 28).

### *Influential employees*

Influential employees refer to those employees who can influence managers within the organisation because of their expertise, work experience and holding position within the organisations. This concept applies to employees with work experience and a history of direct and close contact with management.

### *Experience employees*

This category refers to a group of employees with a long work history and usually had a previous management experience within the organisation. They tarnish or harm the scientific knowledge and practical capability of the manager by relying on their knowledge and expertise and hurt their performance. An interviewee with 4 years of experience pointed out: "*A manager with insufficient knowledge or experience cannot make decisions quickly and effectively against employees with a long work history **…** Due to the lack of expertise, he is forced to surrender to higher-level professionals for decision making and other issues to move the system forward*" (P.19).

### *Employees holding key positions to the management*

This category refers to people within the organisation who hold key positions to the management providing access to information and decision-making processes, and have had work experience with managers who were able to manage them.

In this respect, one of the participants with 22 years of experience said: "*Employees holding key positions to the manager can easily affect the decisions of the manager by giving biased and sometimes false information. In these organisations, the manager does not play an integral role in moving the organisation forward and takes no responsibility or liability except signing the letters and announcements*" (P.29).

### *Disrupting organisational communications*

Disrupting organisational communications are described as factors affecting the managing of managers and involves using the communication tricks (tips) by the employee and the appointment of advisers based on the relationships within the organisation.

### *Application of communication tips*

Communication tricks are used by individuals and groups that attempt to influence the managers. They include taking the lead in greetings and respect for the manager, establishing eye contact, approving the manager's speech using non-verbal sign, pretending, showing, and doing things differently.

One of the interviewees with 10 years of experiences expressed the belief that: "*In the 10 years I have worked closely with the doctor (the manager's name), I understood that he (manager) has no fault...we have employees who are able to impose their wills upon the manager using communication techniques such as (whatever you say is true; but with regards, I think that). They stand on ceremony with the manager and finally, prove their own words*".

### *Appointment of consultants based on relationships*

Furthermore, inaccurate and faulty selection of consultant managers and ambiguity in their limits of authority may hurt the managerial decision-making ability, and the manager can be inadvertently dominated by the surrounded employees. As a result, these situations may cause a lot of difficulties for the manager; it may negatively impact the manager's concentration ability and decisions making the process can easily become bogged down, and this may set the conditions for the interference by other people. One of the participants with 18 years of experience said: "*During the management of the doctor (doctor's name), his counsellor managed all the affairs, not the doctor. Many people imposed their wills and decisions upon the manager and even sometimes issued a notification instead of the manager*" (P 2).

## Discussion

According to the results of this study, the inadequate appointment of managers, the impact of the managerial and human needs of the managers, human and social needs of managers, influential employees and disrupting organisational communications have been identified as the human factors affecting the health-service systems managers. These factors conceptualised as "Managing Managers" by employees. A possible explanation for this might be related to the fact that some employees try to influence their managers by using mechanisms and techniques. These actions are incompatible with the common targeted organisational frameworks and procedures and can violate the appropriate approaches used for employee communication with managers. Also, they can gradually become tools for dominating and manipulating the manager by the subordinate staff. In other words, the employees "manage" their manager instead of being managed by their manager. The employees manage the manager and adjust his manager's behaviours, decisions and policies for their benefit. The appointment of inexperienced managers without adequate technical knowledge and out-of-organization is one of the most important factors in managing managers within the organisations. The majority of scholars in management science believe that the managers' beliefs and attitudes have a direct impact on their behaviour, and the managers' behaviour has a significant effect on their internal organisational performance as well [8], [22], [23]. In other words, managers need skills to protect them from being "managed" [24]. Also, the environment of health-service systems makes it necessary for us to have these skills in the twenty-first century [9]. The managers' belief in the effective role of managerial capabilities is considered one of the main prerequisites for its successful implementation and is of paramount importance as a first step in achieving the goals of the organisation [17]. Therefore, also, to possess adequate knowledge and skills in their career path, managers must be able to develop dynamic capabilities and try to constantly strengthen their managerial capacities [9], [25]. The human and social needs of managers are another factor for affecting the managing of managers. The desire for belonging and to be loved by employees is another important factor involved in the satisfaction and motivation of managers that is used by the subordinators embedded in the phenomenon of "human and social needs". Indeed, the need for belonging and loving components have been classified as the main components of basic needs and motivational factors both in the hierarchy of Maslow's needs and McClelland's motivational theory [7]. Further, the results of our study demonstrated that the need for respect and appreciation is another important source of satisfaction and motivation, which has been introduced in the Maslow Needs Hierarchy as respect esteem and received attention in Skinner as well as Deci and Ryan theories [26].

It was also found that the need for security and reassurance is a human need that is used to manage managers and to disguise the respect and feeling valued for managers through the inflated and exaggerated respect and praise is considered as an important factor in drawing the individual's attention based on the Maslow Needs Hierarchy and other motivational/psychological theories. Meanwhile, the presence of influential employees is another factor in managing managers. An implication of this possibility is that the influence of inter-organizational individuals or groups (including the old staff deployed and organized in the form of inter-agency groups), the existence of a conflict between the individual and organizational interests all shift the attention towards the provision of personal interests through non-formal

procedure [27] and might lead the employees to exercise their control over the manager. This interfering influence on manager's decisions and policies, on the one hand, and “managing " the manager for personal and collective interests, on the other hand, is one of the most decisive ways to achieve those benefits. In this regard, researchers demonstrated the use of experienced workforce and structural problems as the main challenges faced by the managers. Since the responsible and efficient workforce is considered the most important assets for an organisation [28], [29], identifying the influential employees can improve their efficiency and realise their goals in line with organisational objectives. The next important and effective factor in managing the managers is disrupting organisational correlations. The results of our study revealed that the human nature of inter-organisational forces, their behaviours, reactions, relationships, which have been regarded as "human communication", can make a significant contribution to the quality of the employee’s performance and managers in general, and organisational goals in particular. In this regard, recognising the "communication system" and its pathological analysis to optimise its performance in the organisation seem to be necessary [30].

Additionally, "managing" managers by employees will lead to changes in the procedures, performance and macro and micro operating levels of planning inside the organisation and finally makes the organisation deviate from the established and normal course of the program's mainstream organisation. This disruption is dangerous for the organisation because the managers, who play the primary role within the organisation, monitor employee’s performance and guide the organisation in the direction of the assigned mission, are the main source giving rise to this disruption within the organisation. Therefore, organisational productivity is negatively affected, and the efficiency and productivity growth will be lost within the organisation [31].

To the best of our knowledge, this is the first qualitative study to consider specifically human factors influencing management in health-service systems in Iran. The use of content analysis methods used here is ideally placed to understand the complexity of the interaction between managers, organisation activities, health-service systems environment and human factors. The findings are specific to the people involved in this study; however, the use of content analysis allows themes to transcend beyond basic description and to resonate with other similar situations and locations.

In conclusion, the results of this study provide a broad range of context-specific human factors that can affect the management of health service managers and can lead to their inefficient management. Since managing the managers is influenced by personality factors of both manager and employee; therefore, it is recommended that in addition to enhancing concentration and accuracy towards the managerial personality traits, management work experience should be carefully analyzed for selecting health service managers in terms of exercising appropriate managerial authority to prevent the creation of a managing the manager phenomenon.

## Acknowledgements

This study is a part of a research project funded by Mazandaran University of Medical Sciences, Sari, Iran. Thereby, the researchers wish to express their gratitude for the Deputy of Research and Technology University as well as all the participants who helped to conduct the study.

## References

1. Martins JM, Isouard G, Freshman B. Human dimension of health service management. Aust Health Rev. 2017.

2. Young TK, Chatwood S. Delivering more equitable primary health care in Northern Canada. CMAJ. 2017; 189(45):E1377-E1378. https://doi.org/10.1503/cmaj.170498 PMid:29133538 PMCid:PMC5687925

3. Alonso JM, Clifton J, Díaz-Fuentes D. The impact of New Public Management on efficiency: an analysis of Madrid's hospitals. Health Policy. 2015; 119(3):333-40. https://doi.org/10.1016/j.healthpol.2014.12.001 PMid:25533550

4. Tabibi S, Heidari S, Nasiri-pour A, Hosseini Shokouh M, Ameryoun A, Mashayekhi F. Assessment of Professional and Non-professional Managers' Performance among Selected Hospitals in Tehran. Journal of Hospital. 2014; 13(2):45-53.

5. Handel MJ. Theories of lean management: an empirical evaluation. Soc Sci Res. 2014; 44:86-102. https://doi.org/10.1016/j.ssresearch.2013.10.009 PMid:24468436

6. Lunden A, Teräs M, Kvist T, Häggman-Laitila A. A systematic review of factors influencing knowledge management and the nurse leaders' role. J Nurs Manag. 2017; 25(6):407-420. https://doi.org/10.1111/jonm.12478 PMid:28580645

7. Tetui M, Coe AB, Hurtig AK, Bennett S, Kiwanuka SN, George A, Kiracho EE. A participatory action research approach to strengthening health managers' capacity at district level in Eastern Uganda. Health Res Policy Syst. 2017; 15(Suppl 2):110. https://doi.org/10.1186/s12961-017-0273-x PMid:29297346 PMCid:PMC5751402

8. Belrhiti Z, Booth A, Marchal B, Verstraeten R. To what extent do site-based training, mentoring, and operational research improve district health system management and leadership in low- and middle-income countries: a systematic review protocol. Syst Rev. 2016; 5:70. https://doi.org/10.1186/s13643-016-0239-z PMid:27116915 PMCid:PMC4847191

9. Prashanth NS, Marchal B, Devadasan N, Kegels G, Criel B. Advancing the application of systems thinking in health: a realist evaluation of a capacity building programme for district managers in Tumkur, India. Health Res Policy Syst. 2014; 12:42. https://doi.org/10.1186/1478-4505-12-42 PMid:25159487 PMCid:PMC4245764

10. Labrague LJ, McEnroe-Petitte DM, Leocadio MC, Van Bogaert P, Cummings GG. Stress and ways of coping among nurse managers: An integrative review. J Clin Nurs. 2018; 27(7-8):1346-1359. https://doi.org/10.1111/jocn.14165 PMid:29148110

11. Rockers PC, Bärnighausen T. Interventions for hiring, retaining and training district health systems managers in low- and middle-income countries. Cochrane Database Syst Rev. 2013; (4):CD009035. https://doi.org/10.1002/14651858.CD009035.pub2 PMid:23633365

12. Ziari A, Abachizade K, Rassouli M, Mohseny M. Assessment of barriers of implementation of clinical governance in educational hospitals of Shahid Beheshti University of Medical sciences: A qualitative study. Journal of Hospital. 2015; 13 (4): 93-103.

13. Ahanchian MR, Emami Zeydi A, Armat MR. Conflict management styles among Iranian critical care nursing staff: a cross-sectional study. Dimens Crit Care Nurs. 2015; 34(3):140-5. https://doi.org/10.1097/DCC.0000000000000106 PMid:25840129

14. Tetui M, Zulu JM, Hurtig A-K, Ekirapa-Kiracho E, Kiwanuka SN, Coe A-B. Elements for harnessing participatory action research to strengthen health managers' capacity: a critical interpretative synthesis. Health Res Policy Syst. 2018; 16(1):33. https://doi.org/10.1186/s12961-018-0306-0 PMid:29673346 PMCid:PMC5907405

15. Jones A. Organisational commitment in nurses: is it dependent on age or education? Nurs Manag (Harrow). Nurs Manag (Harrow). 2015; 21(9):29-36. https://doi.org/10.7748/nm.21.9.29.e1298 PMid:25629349

16. Karimi Moonaghi H, Emami Zeydi A, Mirhaghi A. Patient education among nurses: bringing evidence into clinical applicability in Iran. Invest Educ Enferm. 2016; 34(1):137-151. https://doi.org/10.17533/udea.iee.v34n1a16 PMid:28569983

17. Purfarzad Z, Vanaki Z, Ghamari Zare Z, Farmahini Farahani M, Ghorbani M. Assessment charge nurses' managerial self-efficacy. Quarterly Journal of Nursing Management. 2014; 2 (4):8-16.

18. Carney, M.Understanding organizational culture: the key to successful middle manager strategic involvement in health care delivery? J Nurs Manag. 2006; 14(1):23-33. https://doi.org/10.1111/j.1365-2934.2005.00592.x PMid:16359443

19. Graneheim UH, Lindgren BM, Lundman B. Methodological challenges in qualitative content analysis: A discussion paper. Nurse Educ Today. 2017; 56:29-34. https://doi.org/10.1016/j.nedt.2017.06.002 PMid:28651100

20. Arieli D, Tamir B, Man M. Teaching qualitative research as a means of socialization to nursing. Nurse Educ Today. 2015; 35(6):795-9. https://doi.org/10.1016/j.nedt.2015.02.021 PMid:25799426

21. Graneheim UH, Lundman B. Qualitative content analysis in nursing research: concepts, procedures and measures to achieve trustworthiness. Nurse Educ Today. 2004; 24(2):105-12. https://doi.org/10.1016/j.nedt.2003.10.001 PMid:14769454

22. Suhonen R, Stolt M, Virtanen H, Leino-Kilpi H. Organizational ethics: a literature review. Nurs Ethics. 2011; 18(3):285-303. https://doi.org/10.1177/0969733011401123 PMid:21558106

23. Jahrami H, Marnoch G, Gray AM. Leadership competencies in the context of health services. Health Serv Manage Res. 2008; 21(2):117-30. https://doi.org/10.1258/hsmr.2008.007027 PMid:18482935

24. Pettigrew LM, De Maeseneer J, Anderson M-IP, Essuman A, Kidd MR, Haines A. Primary health care and the Sustainable Development Goals. Lancet. 2015; 386(10009):2119-21. https://doi.org/10.1016/S0140-6736(15)00949-6

25. Naghneh MHK, Tafreshi MZ, Naderi M, Shakeri N, Bolourchifard F, Goyaghaj NS. The relationship between organizational commitment and nursing care behavior. Electron Physician. 2017; 9(7): 4835-4840. https://doi.org/10.19082/4835 PMid:28894543 PMCid:PMC5587001

26. Muse L, Harris SG, Giles WF, Feild HS. Work-life benefits and positive organizational behavior: is there a connection? J Organiz Behav. 2008; 29: 171-92. https://doi.org/10.1002/job.506

27. Karami A, Farokhzadian J, Foroughameri G. Nurses' professional competency and organizational commitment: Is it important for human resource management? PLoS One. 2017; 12(11): e0187863. https://doi.org/10.1371/journal.pone.0187863 PMid:29117271 PMCid:PMC5678726

28. Custer N. Test Anxiety and Academic Procrastination Among Prelicensure Nursing Students. Nurs Educ Perspect. 2018; 39(3): 162-163. https://doi.org/10.1097/01.NEP.0000000000000291 PMid:29505500

29. Lentz S, Luther B. Nursing Care Management: Influence on Bundled Payments. Orthop Nurs. 2017; 36(1): 28-33. https://doi.org/10.1097/NOR.0000000000000309 PMid:28107297

30. Coiera E. Communication systems in healthcare. Clin Biochem Rev. 2006; 27(2):89-98.

31. Nabilou B, Yusefzadeh H, Rezapour A, Ebadi Fard Azar F, Salem Safi P, Sarabi Asiabar A, et al. The productivity and its barriers in public hospitals: case study of Iran. Med J Islam Repub Iran. 2016; 30:316.

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Jun 30; 7(12):2024-2030.
https://doi.org/10.3889/oamjms.2019.566
eISSN: 1857-9655
***Public Health***

# Potential Risk Factors of Developmental Cognitive Delay in the First Two Years of Life

Ebtissam M. Salah El Din[1], Thanaa M. Rabah[2], Ammal M. Metwally[2], Maysa S. Nassar[1], Mona A Elabd[1*], Ashraf Shalaan[3], Wafaa Kandeel[3, 4], Lobna A. El Etreby[2], Sanaa Y. Shaaban[5]

*[1]Child Health Department, Medical Division, National Research Centre, Giza, Egypt; [2]Department of Community Medicine Research, Medical Division, National Research Centre, Giza, Egypt; [3]Biological Anthropology Department, Medical Division, National Research Centre, Giza, Egypt; Medical Research Centre of Excellence (MRCE), Giza, Egypt; [4]Theodor Bilharz Research Institute, Imbaba, Giza, Cairo, Egypt; [5]Pediatric Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt*

**Citation:** El Din EMS, Rabah TM, Metwally AM, Nassar MS, Elabd MA, Shalaan A, Kandeel W, El Etreby LA, Shaaban SY. Potential Risk Factors of Developmental Cognitive Delay in the First Two Years of Life. Open Access Maced J Med Sci. 2019 Jun 30; 7(12):2024-2030. https://doi.org/10.3889/oamjms.2019.566

**Keywords:** Cognitive development; Infants; Maternal education; Micronutrient deficiency; (Bayley-III) scales

***Correspondence:** Mona A. Elabd. Child Health Department, Medical Division, National Research Centre, Giza, Egypt. E-mail: Mona_elabd@yahoo.com

**Received:** 20-Apr-2019; **Revised:** 22-Jun-2019; **Accepted:** 23-Jun-2019; **Online first:** 30-Jun-2019

**Copyright:** © 2019 Ebtissam M. Salah El Din, Thanaa M. Rabah, Ammal M. Metwally, Maysa S. Nassar, Mona A Elabd, Ashraf Shalaan, Wafaa Kandeel, Lobna A. El Etreby, Sanaa Y. Shaaban. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

**Funding:** This research did not receive any financial support

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

**Abstract**

**BACKGROUND:** The first two years of life constitute a critical period of rapid change. The events during this phase prepare the child for subsequent developmental competency.

**AIM:** To determine the potential risk factors that affect an infant's cognitive development in the first two years of life in a sample of Egyptian infants

**SUBJECTS AND METHODS:** A cross-sectional comparative study included 655 male and female infants. Their age ranged from 3 – 24 months. Bayley Scales of Infant and Toddler Development (Bayley III) were used for cognitive assessment. Perinatal and nutritional data were recorded. Levels of serum Zinc, Copper, Iron, vitamin B12 and complete blood count (CBC) were assessed in a subsample of 193 infants.

**RESULTS:** Infants having below the average cognitive composite score (CCS) represented 38.47% of the whole sample. The risk of having a low average (CCS) was determined by multiple factors. Poor maternal education and low family income were the most significant social risk factors (OR = 2.19, p = 0.0003; OR = 1.64, p = 0.002 respectively). Prematurity and complicated labor represented significant perinatal risks (OR = 1.22, p = 0.005; OR = 2.39, p =0.001respectively). Bottle feeding versus breastfeeding in the first six months of life was the most significant nutritional predictor of low average (CCS) (OR = 1.79, p = 0.001). Infants with low average (CCS) had significantly lower levels of serum zinc and vitamin B12 than those with average scores.

**CONCLUSION:** Multiple factors appear to interact affecting the early cognitive development of Egyptian infants. Prematurity, complicated labour, poor maternal education, low family income and micronutrient deficiency are the main risk factors. Studying these factors is of great value in directing governmental intervention efforts.

## Introduction

The first 2 years of life is a critical period of rapid growth and brain development. During this period, nutrition and environmental factors play important roles in growth and cognitive development of children [1]. Early cognitive development is related to the development of memory and social skills, language acquisition, logical reasoning, planning, and problem-solving [2]. Child cognitive development is influenced by genetic and environmental factors which interact in complex ways to determine how the brain develops and functions [3]. The child has a genetically determined potential for cognitive development. However environmental factors, such as prenatal and postnatal maternal and infant wellbeing [4], [5], nutritional factors as adequate breastfeeding and complementary feeding [6], socioeconomic conditions and the parents' ability to create a good and stimulating home environment may also have a positive influence on the child's cognitive development [7]. Factors such as malnutrition, micronutrient deficiency, poverty-related health problems, home environment, parenting practices, and living in poor neighbourhoods with high levels of crime and unemployment are all factors that may impact brain development in children and therefore influence the possibility of education [8]. The effects of under-nutrition may begin before the child is born [9].

Undernourished pregnant women are more likely to give birth to underweight babies who are generally more at risk. Disruption of normal development can result in dysregulation of neural systems during vulnerable periods of brain development, leading to pronounced neurocognitive deficits, delays in the development of IQ, language, social-emotional functioning, poor academic achievement and poor productivity in adulthood [10]. Neurocognitive assessment is crucial for early detection of developmental disorders, especially in the first years of life, subsequently enabling optimising the design of intervention strategies that respond to individual needs [11]. In developing countries, diminished data represents a big challenge for choosing the most appropriate cost-effective intervention procedure. Research in the area of early child development is highly needed to detect modifiable risk factors and direct intervention efforts.

The objective of this study is to inspect potential risk factors that affect an infant's cognitive development in the first two years of life in a sample of Egyptian infants.

## Material and Methods

*Study design:* A cross-sectional comparative study of Egyptian infants in the first two years of life. They were classified according to their performance on the cognitive domain of Bayley Scales of Infant and Toddler Development (Bayley-III) into two categories: infants having below the average composite score and infants having average and above average score. Potential risk factors that might impair the cognitive performance of these infants were studied.

### *Inclusion criteria*

Infants were enrolled if they were over 1 month and not more than 24 months of age, belonging to the middle socioeconomic class and their caregivers consented to participate in the study.

### *Exclusion criteria*

Infants were excluded if they demonstrated any obvious congenital anomalies, features of genetic diseases, or had a history of any metabolic or physical problems which may affect their cognitive development.

### *Study setting*

Infants were recruited from Developmental and Behavioral paediatrics Clinic at the National Research Centre, and Pediatric Nutrition Clinic of Ain Shams University in the period from September 2016 to September 2018.

### *Sample Size Calculations*

We are planning a study of independent cases and controls with 2 control(s) per case. Prior data indicate that the probability of exposure among controls is 0.26. If the true odds ratio for disease in exposed subjects relative to unexposed subjects is 1.8, we will need to study 190 patients and 380 controls to be able to reject the null hypothesis that this odds ratio equals 1 with probability (power) 0.85. The Type I error probability associated with this test of this null hypothesis is 0.05. We will use a continuity-corrected chi-squared statistic or Fisher's exact test to evaluate this null hypothesis.

Two hundred fifty-two infants with below average cognitive composite score were recruited as cases and 403 infants with average and above average cognitive composite score as controls [12].

### *Methods*

Socio-demographic assessment: Special questionnaire was designed for this study, which included questions about maternal age, maternal and paternal education and occupation, marital status, family income and order of childbirth [13].

Assessment of maternal and prenatal history: including parity, history of chronic diseases as hypertension, diabetes or hypothyroidism, diseases acquired during pregnancy as gestational diabetes or preeclampsia, gestational age of the infant, mode of delivery, postnatal problems as cyanosis, jaundice or convulsions and whether the infant was admitted to NICU or not.

Thorough physical examination and anthropometric measurements of weight and height: All measurements were made according to techniques described in the Anthropometric Standardization Reference Manual [14]. Weight-for-age z scores (WAZ), height-for-age z-scores (HAZ) and Body mass index- for age z-score for all children were calculated based on the WHO growth standards [15] with the help of Anthro-Program of PC.

Infant Feeding Practices in the first six months of life: was assessed to identify infants who were predominately breastfed, artificially-fed (who were consuming other milk including infant formula, fresh, tinned, and powdered milk from cows or other animals or mixed fed (artificial plus breast milk). The time of introduction of complementary feeding was recorded whether before or after the sixth month of age.

Cognitive ability assessment: Using the Bayley Scales of Infant and Toddler Development (Bayley-III). These scales were developed by Nancy

Bayley (16) to assess the development of infants and toddlers between the age range of 1 month to 42 months. Bayley-III consists of 5 subscales, i.e. Cognitive Scale, Language Scale (Receptive Communication and Expressive Communication), Motor Scale (Fine Motor and Gross Motor), Social-Emotional Scale and Adaptive Behavior Scale. In this study, only the cognitive domain is being measured. The Cognitive Scale includes items that assess sensorimotor development, exploration and manipulation, object relatedness, concept formation, memory, and other aspects of cognitive processing. The test is administered according to the infant's age-specific start point. Each correct response is given a score of 1, and the total raw score is then converted into its composite score.

*Biochemical assessment:* Serum Fe, Zn and Cu concentrations were measured using an atomic absorption flame emission spectrophotometer [17], [18]. Serum vitamin B12 was assessed using the Bayer Centaur chemiluminescence method. Complete blood count was performed by an automated cell counter. Haemoglobin concentration < 11 g/dl, was used as a cutoff point for the diagnosis of anaemia [19]. It was considered that iron deficiency existed when serum Iron < 45 ug/dL [20]. Other nutritional deficiencies, were assessed according to the following cutoff values; vitamin B12 < 203 pg/mL, (21) Zinc < 65 ug/dL [22] and Copper < 63.7 ug/dL [23].

### *Ethical Considerations*

The study complies with the International Ethical Guidelines for Biomedical Research Involving Human Subjects [24]. The Research and Ethical Committee of NRC cleared the study protocol. The number of ethical approvals was 11020.

### *Informed Consent*

It was obtained from the parents enrolled in the study Confidentiality: Mothers and children were identified by a serial number, and the information at the individual level was kept strictly confidential.

## Results

In this study, 655 male and female infants were recruited. Forty-four per cent were males. Their age ranged from 3 to 24 months, with a mean of 14.7 ± 6 months. Anthropometric measurements revealed that the majority of infants were of normal weight for their age (91%), normal height (88%) and normal weight for height (91%)(Table 1). The mean cognitive composite score was 80.32 ± 12.48. The infants were classified according to their cognitive composite scores into 2 categories: Below average group whose score was less than 85, and average and above average group whose score was 85 or above (Table 1).

**Table 1: Characteristics of the studied sample**

| Variable | Number (%) N = 655 |
|---|---|
| Sex | |
| Male | 290 (44.3%) |
| Female | 365 (55.7%) |
| Mean age in months | 14.7 ± 6.0 |
| Infants < 6 months | 231 (35.3%) |
| Infants 6 - < 12 months | 191 (29.1%) |
| Infants 12- 24 months | 233 (35.6%) |
| WAZ | |
| (mean ± SD) | -0.2048 ± 1.19 |
| Normal | 596 (91.0%) |
| Underweight | 46 (7.0%) |
| Overweight | 13 (2.1%) |
| HAZ | |
| (mean ± SD) | -0.4985 ± 1.49 |
| Normal | 578(88.2%) |
| Stunted | 77 (11.7%) |
| WHZ | |
| (mean ± SD) | 0.09 ± 1.17 |
| Normal | 597 (91.1%) |
| Wasted | 24 (3.7%) |
| Overweight& obese | 34 (5.2%) |
| Cognitive composite score | |
| (mean ± SD) | 80.32 ± 12.482 |
| Average& above average | 403 (61.52%) |
| Below average | 252 (38.47%) |

WAZ: weight for age z-score; HAZ: Height for age z-score; WHZ: Weight for height Z-score.

It was found that the risk of having below average cognitive composite score was significantly associated with the father's income. In infants belonging to lower-middle-income families, the risk was 1.64 times higher than infants belonging to upper-middle-income families (OR = 1.64), and the P value was < 0.01). Being a low educated mother carried a highly significant risk (P < 0.001) of having a below average infant 2.01 times more than a highly educated mother (OR = 2.01). Other social variables as maternal age, maternal occupation and child order of birth looked to not influence cognitive development of this sample (Table2).

**Table 2: Social risk Factors for below average cognitive composite score**

| | Infants having below average score (n = 252) | Infants having average &above average score (n = 403) | OR (95%CI) | P-value |
|---|---|---|---|---|
| Child Order | | | | |
| (≥ 3) n = 443 | 172 (68.3%) | 271 (67.2%) | 1.05 | 0.78 |
| (< 3) n = 212 | 80 (31.7%) | 132 (32.8%) | (0.75-1.49) | |
| Mother's age | | | | |
| (≤ 25 years) n = 241 | 96 (38.1%) | 145 (36.0%) | 1.09 | 0.58 |
| (> 25 years) n = 414 | 156 (61.9%) | 258 (64.0%) | (0.78-1.54) | |
| Father'sincome | | | | |
| (lowermiddle) *n = 312 | 139 (55.2%) | 173 (42.9%) | 1.64 | 0.002 |
| (Upper Middle) **n = 343 | 113 (44.8%) | 230 (57.1%) | (1.18-2.27) | |
| Mother's education | | | | |
| (illiterate or read and write) n = 161 | 84 (33.3%) | 77 (19.1%) | 2.19 | 0.0003 |
| (High education) ***n = 494 | 168 (66.7%) | 326 (80.9%) | (1.38-3.48) | |
| Mother's occupation | | | | |
| (Housewife) n = 503 | 196 (77.7%) | 307 (76.2%) | 1.09 | 0.63 |
| (Working mother) = 152 | 56 (23.9%) | 96 (18.8%) | (0.74-1.62) | |

*Low- Middle Income: Father is Unemployed, Day by day worker, Farmer & Laborer; **Upper-Middle-Income: Father is Employee, Professional & Employer or dealer; ***High Education= High School and University.

As regards perinatal medical circumstances presented in Table 3, preterm infants were more at risk of having below average cognitive composite score OR = 1.22, p-value = 0.005. Infants born by cesarean section were 1.26 times at risk of having below average cognitive composite score than vaginally born infant although this data didn't reach statistically significant ratio p = 0.15. Complicated labour had a highly significant effect on cognitive composite score outcome as infants who experienced delivery problems were twice at risk of having below

average cognitive composite score OR = 2.39, p-value 0.0001. As regards chronic maternal illness before and during pregnancy didn't appear to have a significant effect on the infant cognitive composite score outcome in the current study.

**Table 3: Association of perinatal medical factors with below average cognitive composite score**

| | Infants having below average score (n = 252) | Infants having average &above average score (n = 403) | OR (95%CI) | P-value |
|---|---|---|---|---|
| History of maternal chronic disease before pregnancy | | | | |
| Yes (n = 124) | 45 (17.9%) | 79 (19.6%) | 0.89 | 0.57 |
| No (n = 531) | 207 (82.1%) | 324 (80.4%) | (0.58-1.36) | |
| Maternal diseases acquired during pregnancy | | | | |
| Yes (n = 89) | 35 (13.9%) | 54 (13.4%) | 1.04 | 0.858 |
| No (n = 566) | 217 (86.1%) | 349 (86.6%) | (0.64-1.69) | |
| Gestational age | | | | |
| Preterm < 37 weeks (n = 49) | 28 (11.1%) | 21 (5.2%) | 1.22 | 0.005 |
| Full-term ≥ 37 weeks (n = 606) | 224 (88.9%) | 382 (94.8%) | (0.65-2.28) | |
| Type of labor | | | | |
| CS (n = 372) | 152 (60.3%) | 220 (54.6%) | 1.26 | 0.15 |
| Normal (n = 283) | 100 (39.7%) | 183 (45.4%) | (0.91-1.76) | |
| Complicated labor | | | | |
| Yes (n = 86) | 49 (19.4%) | 37 (9.2%) | 2.39 | 0.001 |
| No (n = 569) | 203 (80.6%) | 366 (90.8%) | (1.47-3.88) | |

As shown in Table 4, The risk of having below average cognitive composite score in bottle-fed infants was near twice times higher than breastfed infants ($p < 0.001$, OR = 1.79). At the same occasion, bottle-fed infants had the probability of getting below average cognitive composite score 1.70 times greater than mixed fed infants with a significant P-value < 0.05.

Time of introduction of complementary food could not be considered as a predictor of the cognitive composite score. Starting CF before six months seemed to carry a risk of getting below the average cognitive composite score (OR = 1.12). However, the P value was not significant (P > 0.05).

**Table 4: Infant feeding practices as risk factors for below average cognitive composite score**

| Feeding practices | Infants having below average score (n = 252) | Infants having average &above average score (n = 403) | Or (95% ci) | P |
|---|---|---|---|---|
| Type of feeding | | | | |
| Bottle feeding vs Breastfeeding | | | | |
| Bottle fed (240) | 113 (44.8%) | 127 (31.5%) | 1.79 | 0.001** |
| Breast fed (322) | 107 (42.5%) | 215 (53.3%) | (1.25-2.56) | |
| Bottle feeding vs Mixed feeding | | | | |
| Bottle fed (240) | 113 (44.8%) | 127 (31.5%) | 1.7 | 0.03* |
| Mixed fed (93) | 32 (12.7%) | 61 (15.1%) | (1.00-2.00) | |
| Mixed feeding vs Breastfeeding | | | | |
| Mixed fed (93) | 32 (12.7%) | 61 (15.1%) | 1.05 | 0.833 |
| Breast fed (322) | 107 (42.5%) | 215 (53.3%) | (0.63-1.76) | |
| Time of introduction of complementary food | | | | |
| Before the age of six months (332) | 132 (52.1%) | 200 (49.6%) | 1.12 (0.78-1.62) | 0.53 |
| After the age of six months (323) | 120 (47.6%) | 203 (50.3%) | | |

A subsample of 193 infants was investigated for some micronutrient's serum levels (Iron, copper, zinc and vitamin $B_{12}$) and anaemia is owing to their association with cognitive development. It was found that none of the examined infants has subnormal levels of serum iron or serum copper, while 17 infants had subnormal zinc level, 9 had subnormal vitamin B12 level, and 89 infants were anaemic.

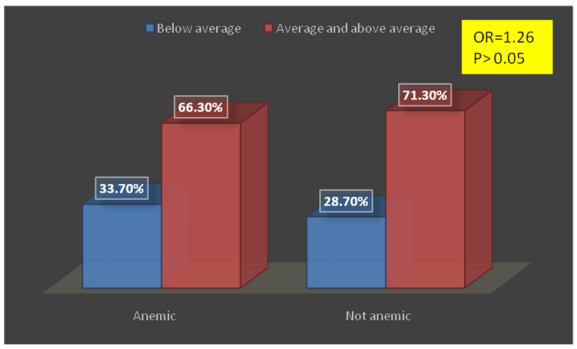
**Table 5: Studied biochemical parameters as risk factors for below average cognitive composite score**

| Biochemical parameter | N[1] | Mean ± SD in infants having below average score | Mean ± SD in infants having average & above average score | Cutoff values indicating a deficiency | T | P |
|---|---|---|---|---|---|---|
| Cu (µg/dl) | 193 | 116.7 ± 41.2 | 130.0 ± 44.6 | < 63.7 µg/dl | 1.969 | 0.056 |
| Zn (µg/dl) | 193 | 83.5 ± 31.8 | 101.7 ± 47.9 | < 65 µg/dl | 4.396 | 0.015* |
| Vitamin $B_{12}$ (pg/ml) | 193 | 981.6 ± 422.0 | 1109.4 ± 433.3 | < 203 pg/ml | 3.392 | 0.028* |
| Fe (µg/dl) | 193 | 163.0 ± 47.4 | 157.4 ± 54.2 | < 45 µg/dl | 0.701 | 0.484 |
| Hemoglobin (gm/dl) | 193 | 10.6 ± 1.2 | 10.8 ± 1.4 | < 11 gm/dl | 1.123 | 0.766 |

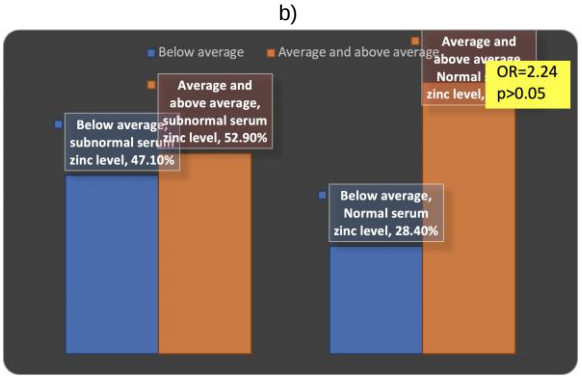
[1]Biochemical parameters were done for a subsample of 193 children; *significant at p < 0.05.

As shown in Table 5, there are significantly lower levels of serum zinc and serum vitamin B12 in infants with below average cognitive composite score if compared with their peers with average and above average scores.

a)




b)




c)

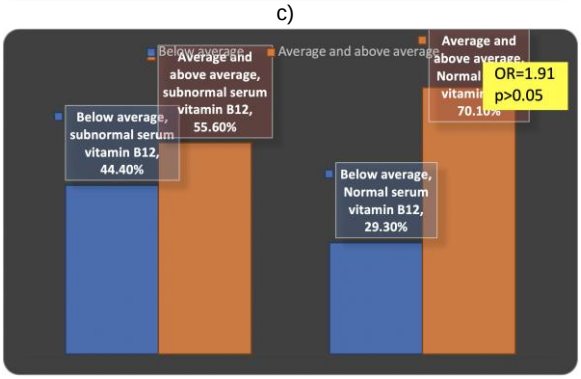



*Figure 1: a) Anemia as a risk factor for below average cognitive composite score; b) Subnormal serum zinc as a risk factor for below average cognitive composite score; c) Subnormal serum vitamin $B_{12}$ as a risk factor for below the average cognitive composite score*

Figures 1a, 1b, and 1c show that as being anaemic; having subnormal serum level of serum zinc, or subnormal serum level of vitamin B12 appeared to carry a non-significant risk for cognitive development in the studied sample (P was > 0.05 for each factor). It has been found that 33.7% of infants who were anaemic had a below average cognitive composite score on Bayley III scales (OR = 1.262), P > 0.05); 47.1% of infants having subnormal serum level of zinc had a below average cognitive composite score (OR = 2.24 (0.74-6.79), P > 0.05). Also, about 44.4% of infants having subnormal serum level of vitamin B12 had a below average cognitive composite score (OR = 1.91), P > 0.05).

In this study, logistic regression analysis (Table 6) revealed the multifactorial interaction between infant feeding variables, socio-economic variables, maternal and perinatal health variables in predicting the cognitive developmental outcome. Complicated labour, prematurity and low educated mothers were highly significant predictors of below average cognitive development. Mode of feeding in the 1st six months was an important predictor for infant cognitive development. Father's occupation, as the main source of household income, appeared as an influential variable for cognitive development.

**Table 6: Logistic Regression of Factors Affecting cognitive composite score**

| | B | S.E. | Wald | df | Sig. | AOR | 95%CI for AOR |
|---|---|---|---|---|---|---|---|
| Feeding in the first six months of life | | | | | | | |
| Breastfeeding | | | | | | ® | |
| Mixed | 1.293 | 0.553 | 5.464 | 2 | 0.019 | 0.275 | 0.098-0.873 |
| Bottle | 0.628 | 0.237 | 6.991 | | 0.008 | 1.88 | 1.27-2.91 |
| Gestational age | | | | | | | |
| Full term | | | | | | ® | |
| Preterm | 0.636 | 0.237 | 7.230 | 1 | 0.007 | 0.529 | 0.33-0.84 |
| Mother education | | | | | | | |
| High | | | | | | ® | |
| Illiterate, R&W | 0.782 | 0.270 | 8.415 | 1 | 0.004 | 0.458 | 0.27-0.78 |
| Complicated labour | | | | | | | |
| No | | | | | | ® | |
| Yes | 0.721 | 0.256 | 7.623 | 1 | 0.003 | 0.489 | 0.75-2.31 |
| Father income | | | | | | | |
| Upper middle | | | | | | ® | |
| Lower middle | 0.943 | 0.643 | 3.531 | 1 | 0.03 | 0.834 | 1.23-3.71 |
| Constant | 5.268 | 1.168 | 20.332 | 1 | 0.000 | 94.065 | |

## Discussion

Breastfeeding initiation and duration are the points of concern for many researchers due to its relation and influence on early growth and development, especially cognitive and brain development. Breastfeeding could benefit development through nutrients in breast milk, especially essential fatty acids, reduced infant morbidity, or closer mother-child relations as it was found that there are improvements in motor development with longer duration of exclusive breastfeeding and that early introduction of supplementary bottle feeding was associated with poorer motor and cognitive function [25].

The first years of life constitute a critical period of rapid personal change, and the events of this phase prepare the child for subsequent developmental competency [26] In the current study, infants were classified according to the cognitive composite score cutoff point [85] into 2 groups (Below average group—and average and above average group). The risk of having below average cognitive composite score was significantly associated with low father's income and low maternal education. Other social variables as maternal age, maternal occupation and child order of birth had no association with a cognitive composite score of these infants; this is in agreement with Andrade & Shaffer et al., who found that family income, maternal education and socioeconomic factors indirectly affect children's early cognitive development. The lower the maternal schooling and family income, the poorer the psychosocial stimulation, as children are deprived of play materials and school stimulation, negatively affecting their cognitive development. The study findings corroborate those described in the literature, indicating that maternal schooling affects children cognitive development using environmental organisation, parental expectations and practices, provision of materials for child's cognitive stimulation, and variety in daily stimulation [27], [28]. As regards perinatal medical circumstances, preterm infants were more at risk of having below average cognitive composite score, this finding may be contributed to a lack of breastfeeding in preterm infants due to admission in incubator and some preterm infants are not physically or developmentally able to suckle, swallow and breathe in a coordinated manner this is in agreement with Kandeel et al., who found that mothers with a preterm newborn had a higher tendency toward artificial feeding than exclusive breastfeeding [29]. Infants born by cesarean section were at risk of having below average cognitive composite score than vaginally born infant although, this result is in agreement with Cain Polidano et al., who found through a longitudinal study that Cesarean birth may be directly and indirectly associated with negative child cognitive outcomes [30], cesarean procedures also poses postnatal maternal health risks [31] also there is potential knock-on effects for the child's development through altered mother-child interactions [32] and lower rates of breastfeeding and its beneficial effects on early stage of brain development [33].

Complicated labour had a highly significant effect on cognitive composite score outcome as infants who experienced delivery problems were twice at risk of having below average cognitive composite score this result is in agreement with Pappas et al., who found that moderate to severe neonatal encephalopathy resulting from complicated labour contributes to a wide range of neurodevelopmental and cognitive impairments among survivors [34]. As

regards chronic maternal illness before and during pregnancy didn't appear to have a significant effect on the infant cognitive composite score outcome in the current study which disagrees with Thach et al., who found that child cognitive development is affected by antenatal iron deficiency anaemia and common mental problems [35]. The risk of having below average cognitive composite score in bottle-fed infants was near twice times higher than breastfed infants. At the same occasion, bottle-fed infants had the probability to get below average cognitive composite score 1.70 time greater than mixed fed infants , this result support and explain the importance of breastfeeding and also exclusive breastfeeding in the first 6 months of life the period of early brain development and agreed with James W Anderson et al., who found through a meta-analysis study that breastfeeding was associated with significantly higher scores for cognitive development than was formula feeding [36].

Time of introduction of complementary food could not be considered as a predictor of the cognitive composite score in this study. Starting CF before six months seemed to carry a non-significant risk of getting below the average cognitive composite score. This result is with an agreement with Sargoor et al., who found that there is no relation between age of introduction of complementary foods, and cognitive function [37]. We found that there were significantly lower levels of serum zinc and vitamin B12 in infants with below average cognitive composite score; it is believed that zinc is a vital nutrient for the brain. It has an important role in neurogenesis, maturation, migration of neurons and synapse formation [38]. Deficits in vitamin B12 (cobalamin) have negative consequences on the developing brain during infancy. Maureen et al. examined two mechanisms linking folate and vitamin B12 deficiency to abnormal behaviour and development in infants: disruptions to myelination and inflammatory processes [39]. In the current study, logistic regression analysis revealed the multifactorial interaction between infant feeding variables, socio-economic variables, maternal and perinatal health variables in predicting the cognitive developmental outcome. Complicated labour, prematurity and low educated mothers were highly significant predictors of below average cognitive development. Mode of feeding in the 1st six months was an important predictor for infant cognitive development as a nutritive value of breastfeeding in this period is essential for brain development. These results are in agreement with that of Metwally et al., who found that incorporative variables affected the infant social-emotional development. Breastfeeding, the level of maternal education and micronutrient sufficiency were the most important predictors of the infant mental health [40].

There may be some possible limitations in this study: Cross-sectional design is commonly preferred owing to its reasonable cost and feasibility. However, it cannot support a causal relationship. All participants in the current study were confined to the middle social class, as the majority of attendants of our clinic. Infants of low and high social classes were not included, which may constrain the generalisation of results. Time was a constraint, preventing the detailed recording of complementary feeding quality and parenting behaviour, which may reveal important influences on cognitive development.

In conclusion, multiple factors appear to interact, affecting the early cognitive development of Egyptian infants. Prematurity, complicated labour, poor maternal education, low family income and micronutrient deficiency are the main risk factors. Studying these factors is of great value in directing governmental intervention efforts. Providing good antenatal and natal care for all pregnant mothers especially poor ones, attention towards woman education, raising awareness about the importance of breastfeeding and providing adequate health care services for children will promote cognitive development in Egyptian children.

## References

1. Nurliyana AR, Shariff ZM, Taib MN, Gan WY, Tan KA. Early nutrition, growth and cognitive development of infants from birth to 2 years in Malaysia: a study protocol. BMC pediatrics. 2016; 16(1):160. https://doi.org/10.1186/s12887-016-0700-0 PMid:27687906 PMCid:PMC5043613

2. Santos LM, Santos DN, Bastos AC, Assis AM, Prado MS, Barreto ML. Determinants of early cognitive development: hierarchical analysis of a longitudinal study. Cadernos de Saúde Pública. 2008; 24:427-37. https://doi.org/10.1590/S0102-311X2008000200022

3. Tucker-Drob EM, Briley DA. Continuity of genetic and environmental influences on cognition across the life span: a meta-analysis of longitudinal twin and adoption studies. Psychol Bull. 2014; 140(4):949-79. https://doi.org/10.1037/a0035893 PMid:24611582 PMCid:PMC4069230

4. Peng Zhu, Meng-Sha Sun, Jia-Hu Hao, Yu-Jiang Chen, Xiao-Min Jiang, Rui-Xue Tao, Kun Huang, Fang-Biao Tao. Does prenatal maternal stress impair cognitive development and alter temperament characteristics in toddlers with healthy birth outcomes? Developmental Medicine & Child Neurology. 2014; 56(3): 283-289. https://doi.org/10.1111/dmcn.12378 PMid:24512346

5. Walker SP, Wachs TD, Grantham-McGregor S, Black MM, Nelson CA, Huffman SL, Baker-Henningham H, Chang SM, Hamadani JD, Lozoff B, Gardner JM. Inequality in early childhood: risk and protective factors for early child development. The lancet. 2011; 378(9799):1325-38. https://doi.org/10.1016/S0140-6736(11)60555-2

6. Prado EL, Dewey KG. Nutrition and brain development in early life. Nutr Rev. 2014; 72(4):267-84. https://doi.org/10.1111/nure.12102 PMid:24684384

7. de La Rochebrochard E, Joshi H. Children born after unplanned pregnancies and cognitive development at 3 years: social differentials in the United Kingdom Millennium Cohort. American journal of epidemiology. 2013; 178(6):910-920. https://doi.org/10.1093/aje/kwt063 PMid:23887043 PMCid:PMC3775543

8. Ali SS. A brief review of risk-factors for growth and developmental delay among preschool children in developing countries. Advanced Biomedical Research. 2013; 2:91. https://doi.org/10.4103/2277-9175.122523 PMid:24520553 PMCid:PMC3908499

9. Lu LH, Dapretto M, O'hare ED, Kan E, McCourt ST, Thompson PM, Toga AW, Bookheimer SY, Sowell ER. Relationships between brain

activation and brain structure in normally developing children. Cerebral cortex. 2009; 19(11):2595-604. https://doi.org/10.1093/cercor/bhp011 PMid:19240138 PMCid:PMC2758677

10. Spratt EG, Friedenberg SL, Swenson CC, Larosa A, De Bellis MD, Macias MM, Summer AP, Hulsey TC, Runyan DK, Brady KT. The Effects of Early Neglect on Cognitive, Language, and Behavioral Functioning in Childhood. Psychology (Irvine). 2012; 3(2):175-182. https://doi.org/10.4236/psych.2012.32026 PMid:23678396 PMCid:PMC3652241

11. Silverstein SM, Berten S, Olson P, Paul R, Williams LM, Cooper N, Gordon E. Development and validation of a World-Wide-Web-based neurocognitive assessment battery: WebNeuro. Behavior Research Methods. 2007; 39(4):940-9. https://doi.org/10.3758/BF03192989 PMid:18183911

12. On line calculation http://biostat.mc.vanderbilt.edu/PowerSampleSize Version 3.0, January 2009. William D. Schlesselman: Case-control Studies: Design, Conduct, Analysis. New York: Oxford U. Press, 1982:144-152.

13. El-Shakhs A. Social level and the economic scale of the family: the scale manual.

14. Lohman TG, Roche AF, Martorell R. Anthropometric standardization reference manual. Champaign, IL: Human Kinetics Books; 1988.

15. 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/

16. Bayley N. Bayley scales of infant and toddler development-third edition. San Antonio, TX: Harcourt Assessment Journal of psychoeducational assessment. 2006; 25(2):180-90. https://doi.org/10.1177/0734282906297199

17. Chou PP. Zinc. In: Pesce AJ, Kaplan LA, editors. Methods in clinical chemistry. St. Louis, Missouri: The C.V. Mosby Company, 1987:596-602.

18. Taylor A, Bryant TN. Comparison of procedures for determination of copper and zinc serum by atomic absorption spectroscopy. Clin Chim Acta. 1981; 110:83-90. https://doi.org/10.1016/0009-8981(81)90304-1

19. World Health Organization. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. World Health Organization; 2011.

20. WHO C. Assessing the iron status of populations: including literature reviews: report of a Joint World Health Organization. Centers for Disease Control and Prevention Technical Consultation on the Assessment of Iron Status at the Population Level, Geneva, Switzerland. 2004:6-8.

21. de Benoist B. Conclusions of a WHO Technical Consultation on folate and vitamin B12 deficiencies. Food and nutrition bulletin. 2008; 29(2_suppl1):S238-44. https://doi.org/10.1177/15648265080292S129 PMid:18709899

22. Hess SY, Peerson JM, King JC, Brown KH. Use of serum zinc concentration as an indicator of population zinc status. Food and nutrition bulletin. 2007; 28(Suppl 3):S403-29. https://doi.org/10.1177/15648265070283S303 PMid:17988005

23. Murray RK, Jacob M, Varghese J. Plasma Proteins & Immunoglobulins. Bender DA, Botham KM, Weil PA, Kennelly PJ, Murray RK, Rodwell VW. Harper's Illustrated Biochemistry. 29th. New York, McGraw-Hill, 2011.

24. CIOMS/WHO. International Ethical Guidelines for Biomedical Research Involving Human Subjects. Geneva: CIOMS, 1993.

25. Shereen Jegtvig. Longer breastfeeding tied to better development. Health News. December 25, 2013

26. Berkman DS, Lescano AG, Gilman RH, Lopez SL, Black MM. Effects of stunting, diarrhoeal disease, and parasitic infection during infancy on cognition in late childhood: a follow-up study. Lancet. 2002; 359:564-71. https://doi.org/10.1016/S0140-6736(02)07744-9

27. Andrade AS, Santos DN, Bastos AC, Pedromônico MR, Almeida-Filho N, Barreto ML. Ambiente familiar e desenvolvimento cognitivo infantil: uma abordagem epidemiológica. Rev Saúde Pública. 2005; 39:606-11. https://doi.org/10.1590/S0034-89102005000400014 PMid:16113911

28. Shaffer D. Psicologia do desenvolvimento: infância e adolescência. São Paulo: Pioneira Thomson Learning; 2005.

29. Kandeel WA, Rabah TM, Zeid DA, El-Din EM, Metwally AM, Shaalan A, El Etreby LA, Shaaban SY. Determinants of Exclusive Breastfeeding in a Sample of Egyptian Infants. Open access Macedonian journal of medical sciences. 2018 Oct 25;6(10):1818-1823. https://doi.org/10.3889/oamjms.2018.359 PMid:30455755 PMCid:PMC6236050

30. Polidano C, Zhu A, Bornstein JC. The relation between cesarean birth and child cognitive development. Scientific reports. 2017; 7(1):11483. https://doi.org/10.1038/s41598-017-10831-y PMid:28904336 PMCid:PMC5597642

31. Liu S, Heaman M, Joseph KS, Liston RM, Huang L, Sauve R, Kramer MS. Risk of maternal postpartum readmission associated with mode of delivery. Obstetrics & Gynecology. 2005; 105(4):836-42. https://doi.org/10.1097/01.AOG.0000154153.31193.2c PMid:15802414

32. Kelly Y, Sacker A, Del Bono E, Francesconi M, Marmot M. What role for the home learning environment and parenting in reducing the socioeconomic gradient in child development? Findings from the Millennium Cohort Study. Archives of Disease in Childhood. 2011; 96(9):832-7. https://doi.org/10.1136/adc.2010.195917 PMid:21666278

33. Zanardo V, Svegliado G, Cavallin F, Giustardi A, Cosmi E, Litta P, Trevisanuto D. Elective cesarean delivery: does it have a negative effect on breastfeeding?. Birth. 2010; 37(4):275-9. https://doi.org/10.1111/j.1523-536X.2010.00421.x PMid:21083718

34. Pappas A, Korzeniewski SJ. Long-Term Cognitive Outcomes of Birth Asphyxia and the Contribution of Identified Perinatal Asphyxia to Cerebral Palsy. Clin Perinatol. 2016; 43(3):559-72. https://doi.org/10.1016/j.clp.2016.04.012 PMid:27524454

35. Tran TD, Biggs BA, Tran T, Simpson JA, Hanieh S, Dwyer T, Fisher J. Impact on infants' cognitive development of antenatal exposure to iron deficiency disorder and common mental disorders. PLoS One. 2013; 8(9):e74876. https://doi.org/10.1371/journal.pone.0074876 PMid:24086390 PMCid:PMC3781140

36. Anderson JW, Johnstone BM, Remley DT. Breast-feeding and cognitive development: a meta-analysis. The American journal of clinical nutrition. 1999; 70(4):525-35. https://doi.org/10.1093/ajcn/70.4.525 PMid:10500022

37. Veena SR, Krishnaveni GV, Srinivasan K, Wills AK, Hill JC, Kurpad AV, Muthayya S, Karat SC, Nalinakshi M, Fall CH. Infant feeding practice and childhood cognitive performance in South India. Archives of disease in childhood. 2010; 95(5):347-54. https://doi.org/10.1136/adc.2009.165159 PMid:19946010 PMCid:PMC3428883

38. Prado EL, Dewey KG Nutrition and brain development in early life. Nutrition Reviews. 2014; 72(4):267-284. https://doi.org/10.1111/nure.12102 PMid:24684384

39. Black MM. Effects of vitamin B12 and folate deficiency on brain development in children. Food and nutrition bulletin. 2008; 29(2_suppl1):S126-31. https://doi.org/10.1177/15648265080292S117 PMid:18709887 PMCid:PMC3137939

40. Metwally AM, El-Din EM, Shehata MA, Shaalan A, El Etreby LA, Kandeel WA, Shaaban SY, Rabah TM. Early Life Predictors of Socio-Emotional Development in a Sample of Egyptian Infants. PloS one. 2016; 11(7):e0158086. https://doi.org/10.1371/journal.pone.0158086 PMid:27379907 PMCid:PMC4933375

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Jun 30; 7(12):2031-2035.
https://doi.org/10.3889/oamjms.2019.346
eISSN: 1857-9655
***Public Health***

# From Job Satisfaction to Organizational Commitment: The Mediating Influence of Perceived Treatment of Diversity among Nigeria's Public Healthcare Employees

Anthonia Adenike Adeniji, Adewale Omotayo Osibanjo, Tunde Charles Iruonagbe, Tomike Olawande, Ayodotun Stephen Ibidunni*, Maxwell Ayodele Olokundun

*Covenant University, Ota, Nigeria*

**Citation:** Adeniji AA, Osibanjo AO, Iruonagbe TC, Olawande T, Ibidunni AS, Olokundun MA. From Job Satisfaction to Organizational Commitment: The Mediating Influence of Perceived Treatment of Diversity among Nigeria's Public Healthcare Employees. Open Access Maced J Med Sci. 2019 Jun 30; 7(12):2031-2035. https://doi.org/10.3889/oamjms.2019.346

**Keywords:** Job satisfaction; Organizational commitment; Workforce diversity; Public healthcare; Nigeria

***Correspondence:** Ayodotun Stephen Ibidunni. Covenant University, Ota, Nigeria. E-mail: ayodotun.ibidunni@covenantuniversity.edu.ng

**Received:** 31-Jan-2019; **Revised:** 27-May-2019; **Accepted:** 28-May-2019; **Online first:** 30-Jun-2019

**Copyright:** © 2019 Anthonia AdenikeAdeniji, Adewale Omotayo Osibanjo, Tunde Charles Iruonagbe, Tomike Olawande, Ayodotun Stephen Ibidunni, Maxwell Ayodele Olokundun. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

**Funding:** This research was financially supported by the Management of Covenant University, Nigeria

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

**Abstract**

**BACKGROUND:** In the Nigerian, like most developing economies', health sector, employees' perceptions about treatments of diversity are crucial not only to their satisfaction with the job but equally to their commitment to the organisation. The importance of this view of the public health sector, is that it could induce political behaviours, result in conflict situations and hence promote tensed work environments, if not properly managed. Despite these facts, there is again, a dearth of existing literature that shows an element of empirical analysis to demonstrate the moderating influence of workforce diversity on job satisfaction and organisational commitment of public healthcare employees in Nigeria.

**AIM:** This study is focused on investigating the mediating effect of employees' perceived treatments of diversity in the workplace on the relationship between job satisfaction and their commitment to the organisation**.**

**METHODS:** The survey method was used to collect data. One hundred thirty-three public health workers from the Lagos State Health Ministry in Nigeria were involved in this research. The research used questionnaires as the main tools to perform this research. The statistics on the reliability of the tools used in this study were 0.747. The statistical analysis was carried out using SPSS (version 22) and AMOS (version 23) software for this study.

**RESULTS:** The results from the statistical analysis indicate that job satisfaction significantly relates with all dimensions of workforce diversity in the following ways: education (r = 0.19), gender (r = 0.48), religion (r = -0.20), ethnicity (r = 0.42) and position (r = 0.15). The mediating effects of workforce diversity on the relationship between job satisfaction and employee commitment is also evident from the statistical analysis, especially with respect to education (affective commitment = 0.16, normative commitment = 0.18, continuance commitment = 0.18); gender (affective commitment = 0.32, normative commitment = 0.42); and religion (continuance commitment = 0.14).

**CONCLUSION:** This study concluded that not only is job satisfaction significant to ensuring the commitment of healthcare workers to their organisation, but more critical is the role of workforce diversity as viable leverage for transiting the interest of employees from the level of job satisfaction to organisational commitment.

## Introduction

Nigeria's health sector is a part of its economy, which require critical discourse, especially concerning issues surrounding infrastructure, government funding, health workers motivation and satisfaction, among others. Considering the strategic role that health workers play within this sector, it is imperative that researchers and practitioners give the required attention to exploring and interrogating issues that can enhance their performance, to ensure that they function optimally [1], [2]. It is not untrue that where the workers in the Nigerian health sector are unfairly treated, the ailing patients will be at the receiving end of such injustice. Consequently, existing studies have attempted to show the relationships between healthcare workers satisfaction and the motivation towards work [3]. There have also been studies to demonstrate the factors that influence health workers satisfaction with their work [4], [5]. Not withstanding, the case with Nigeria requires more than simply understanding the basic elements that explain job satisfaction of health workers. As [6] opined, there are insufficient empirical details in the literature to explain health workers' motives for linking their level of job satisfaction with their motives to remain committed, or otherwise, to their organisation.

Literature has asserted that employees'

perception about treatments of diversity at the workplace is critical to enhancing, or otherwise, their behaviours and commitment to the job [7]. According to [8], [9] it is not sufficient to measure employees' levels of satisfaction, but workplace diversity can influence the type of commitment they show to the organisation. In the Nigerian health sector, employees' perceptions about treatments of diversity are crucial not only to their satisfaction with the job but equally to their commitment to the organisation [10], [11]. The importance of this view of the public health sector, is that it could induce political behaviours, result in conflict situations and hence promote tensed work environments, if not properly managed. Despite these facts, there is again, a dearth of existing literature that shows an element of empirical analysis to demonstrate the moderating influence of workforce diversity on job satisfaction and organisational commitment of public healthcare employees in Nigeria.

Therefore, this study is focused on investigating the mediating effect of employees' perceived treatments of diversity in the workplace on the relationship between job satisfaction and their commitment to the organisation.

## Material and Methods

The method of survey was used to collect data. This research involved 133 public health workers from the Ministry of State Health of Lagos in Nigeria. Public health workers, especially in the Ministry of Health of Nigeria, are important for this research because of the increased awareness of the diverse workforce of the Ministry and the need to manage this diversity in a way that does not hurt employee satisfaction and commitment to the organisation [14]. Furthermore, due to the importance of the Ministry of Health for the population and the overall well-being of each country, this research is considered essential to maintaining employee interest and motivation in the provision of quality services [15].

### *Sample Size & Sampling Procedure*

The sample size was determined using a sample size determination formula with a 5 per cent sampling error. Apply the Yamane sample size procedure [16].

The formula

$N = N / (1 + N(e)^2)$

where

n = sampling size

e = sampling error (0.05)

N= the population (that is 200 employees in the healthcare location)

$n = 200 / (1+200 (0.05)^2)$

$n = 200 / 1+ 200 (0.0025)$

$n = 200 / 1+ 0.5$

$n = 200 / 1.5$

n = 133.33 (approximately 133)

n = 133

Therefore, the sample size is 133

For this research, the technique adopted is the simple random sampling technique. Simple random sampling which is a subdivision of the probability sampling is used and is applicable as a result of the small number and similarity of the population and all subdivision of the population have an equal chance of being selected. This technique allows every member of the population to be a respondent by selecting the respondents without any form of partiality.

### *Execution of Field Research*

The research used questionnaires as the main tools to perform this research. The questionnaire was used in this study for the following reasons; it allowed the respondents to express themselves more freely and clearly and to gather the answers in a standardized manner; It saved time by allowing information to be collected as soon as possible and also by facilitating the collection of possible information from a large sample of respondents. The research questionnaire consisted of a 5-point Likert scale in which the respondent was expected to agree strongly, agree, disagree and strongly disagree with carefully constructed questions, ranging from very positive to very negative, to measure the influence of diversity in the workforce on the commitment and satisfaction of employees. The questionnaire has been tested by the pilot using the Cronbach reliability test. The investigation questionnaire was divided into two parts. Section A was designed to provide information on the background of respondents, while section B was designed to provide information on the nature of workforce diversity, employee satisfaction and level of commitment (affective, regulatory and continuing) in the Ajeromi General Hospital.

### *Measures*

This research took advantage of the ideas from existing research studies. Workforce diversity issues were developed based on [17], [18], work satisfaction items were developed based on [19], while organisational commitment items were adapted from [20], [21]. The data collected was encoded and entered into version 22 of SPSS. The analysis of data

was carried out using descriptive statistics and structural equation modelling (SEM).

### *Ethical Consideration*

The management of the questionnaire was based on the readiness of respondents to respond to the research instrument. The participants in the study were also assured of confidentiality and anonymity, as their names were not reflected in the questionnaire.

### *Validity and Reliability of Research Instrument*

The alpha coefficient (Cronbach's alpha) has been used in this research to test the reliability of the measurement scale. The Cronbach alpha is a method to determine the internal consistency of a measuring instrument by identifying all possible ways to split the items in the instrument and then investigate the correlation degree. Values of the coefficient from 0 to 1. A highly reliable instrument has a coefficient value near 1, where a score very close to 0 indicates a very low or no reliability of the instrument. It is widely accepted that the score above 0.7 shows the instrument's reliability. The SPSS (Social Science Statistical Package) was used to test the reliability of the research instrument. The statistics on the reliability of the tools used in this study were 0.747. The validity of the instrument was determined by the validity of the content.

### *Statistical Analysis*

The statistical analysis was carried out using SPSS (version 22) and AMOS (version 23) software for this study. Descriptive analysis showed the total number of respondents according to the analysed categories as well as the percentages of each category. The inferential statistics which established the multivariate relationships between public healthcare workers' diversity issues and commitment to their organisation was analysed using structural equation modelling (SEM) at a significant level of $p < 0.000$.

## Results

Sixty-four (48.1%) were male, and the remaining 69 (51.9%) were female. This research involves more female participants than male respondents.

The age distribution of the respondents revealed that 68 respondents were aged between 18 and 34 years; 45 respondents were aged between 35 and 44 years. The range of 13 respondents is 45 to 54 and 7 falls within the range of 55 years. This means that the age group was between 18 and 34 years old, with the majority of respondents and had 68 participants in this research.

**Table 1: Socio-demographic Distribution of Respondent**

| Demographic Variable | | Frequency | Percentage |
|---|---|---|---|
| Gender | Male | 64 | 48.1 |
| | Female | 69 | 51.9 |
| Age | 18-34 | 68 | 51.1 |
| | 35-44 | 45 | 33.8 |
| | 45-54 | 13 | 9.8 |
| | 55 and above | 7 | 5.3 |
| Years of Work experience | less than 5 years | 54 | 40.6 |
| | 5-10 years | 49 | 36.8 |
| | 11-15 years | 16 | 12.0 |
| | 16 years above | 14 | 10.5 |
| Educational Qualification | O.N.D | 24 | 18.0 |
| | N.C.E | 9 | 6.8 |
| | H.N.D | 43 | 32.3 |
| | Bachelor's degree | 35 | 26.3 |
| | Master's degree | 22 | 16.5 |

Based on years of working experience (40.6%) of respondents worked for less than 5 years, the table shows that (36.8%) of respondents worked for 5-10 years and (12.0%) for 11-15 years. The table shows that there were some respondents 16 years ago (10.5%). Table 4 above shows the background of the students surveyed 24 (18.0%) were O.N.D., 9 (6.8%) were N.C.E., 43 (32.3%) were H.N.D., 35 (26.3%) were Bachelor, and 22 (16.5%) were Masters. This means that the majority of the education background was (32.3%), meaning H.N.D. There were 43 respondents.

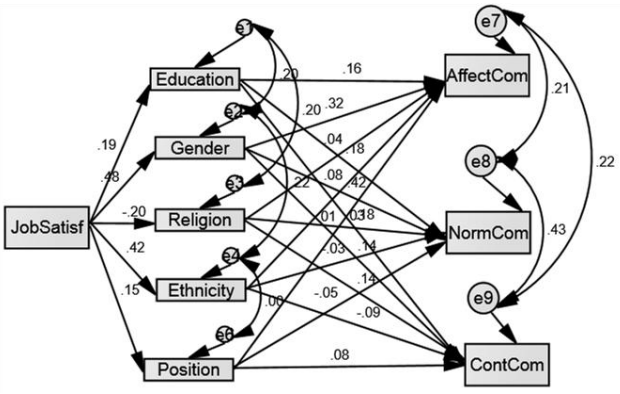



*Figure 1: Regression Path of Job Satisfaction, Workforce Diversity & Employee Commitment*

Figure 1 shows the model for analysing the multifaceted relationships between workforce diversity, job satisfaction and commitment of employees. Table 2 also shows structural regression weights of multi-variable analysis. Chi-square / Degree of Freedom (Cmin / df) = 3.876, Goodness of Fit Index (GFI) = 0.949, Normal Fit Index (NFI) = 0.854, Comparative Fit Index (CFI) = 0.872, Root Mean Square Error Approximation (RMSEA) = 0.148 are used to ensure the model fit of the analysis. The values are important based on the arguments presented in the study [28], [29], [30].

**Table 2: Structural Regression Weights for Job Satisfaction, Workforce Diversity and Employee Commitment**

| | | | Estimate | S.E. | C.R. | P | Label |
|---|---|---|---|---|---|---|---|
| Education | <--- | JobSatisf | 0.215 | 0.098 | 2.184 | *** | Relationship Exist |
| Gender | <--- | JobSatisf | 0.418 | 0.067 | 6.223 | *** | Relationship Exist |
| Religion | <--- | JobSatisf | -0.179 | 0.078 | -2.290 | *** | Relationship Exist |
| Ethnicity | <--- | JobSatisf | 0.301 | 0.057 | 5.312 | *** | Relationship Exist |
| Position | <--- | JobSatisf | 0.118 | 0.069 | 1.723 | *** | Relationship Exist |
| AffectCom | <--- | Education | 0.092 | 0.048 | 1.901 | *** | Relationship Exist |
| NormCom | <--- | Education | 0.110 | 0.049 | 2.239 | *** | Relationship Exist |
| ContCom | <--- | Education | 0.121 | 0.059 | 2.040 | *** | Relationship Exist |
| AffectCom | <--- | Gender | 0.245 | 0.067 | 3.642 | *** | Relationship Exist |
| NormCom | <--- | Gender | 0.341 | 0.069 | 4.954 | *** | Relationship Exist |
| ContCom | <--- | Gender | 0.129 | 0.083 | 1.553 | 0.120 | No Relationship |
| AffectCom | <--- | Religion | 0.029 | 0.059 | 0.486 | 0.627 | No Relationship |
| NormCom | <--- | Religion | 0.025 | 0.060 | 0.417 | 0.677 | No Relationship |
| ContCom | <--- | Religion | 0.121 | 0.072 | 1.670 | *** | Relationship Exist |
| AffectCom | <--- | Ethnicity | 0.078 | 0.079 | 0.983 | 0.326 | No Relationship |
| NormCom | <--- | Ethnicity | -0.025 | 0.081 | -0.312 | 0.755 | No Relationship |
| ContCom | <--- | Ethnicity | -0.099 | 0.098 | -1.011 | 0.312 | No Relationship |
| AffectCom | <--- | Position | 0.005 | 0.066 | 0.074 | 0.941 | No Relationship |
| NormCom | <--- | Position | -0.041 | 0.068 | -0.612 | 0.540 | No Relationship |
| ContCom | <--- | Position | 0.074 | 0.081 | 0.909 | 0.363 | No Relationship |

The data supports a relationship between diversity of workforce and job satisfaction, diversity of workforce and organizational commitment and influence on the organizational commitment to work. The results of these data support existing research results [21], [22], [23], [24]. Specifically, the results from the statistical analysis indicate that job satisfaction significantly relates with all dimensions of workforce diversity in the following ways: education (r = 0.19), gender (r = 0.48), religion (r = -0.20), ethnicity (r = 0.42) and position (r = 0.15). The mediating effects of workforce diversity on the relationship between job satisfaction and employee commitment is also evident from the statistical analysis, especially with respect to education (affective commitment = 0.16, normative commitment = 0.18, continuance commitment = 0.18); gender (affective commitment = 0.32, normative commitment = 0.42); and religion (continuance commitment = 0.14).

## Discussion

The study focused on investigating the mediating effects of diversity in the workforce on job satisfaction and the organisational commitment of public health workers in Nigeria. For the analysis of data collected from respondents, the structural equation model was used. This study fills a research gap in the existing literature by empirically demonstrating the moderating influence of diversity in the workforce on the satisfaction of public health workers in Nigeria and their organisational activities. Through the understanding of such relationships, managers and governments in the healthcare sector are ably posed to curbing incidences that could arise as a result of adverse politicking and conflicts. Moreover, the findings of this study has been corroborated by existing literature that argued that diversity at the workplace can be harnessed not only to ensure sustainable satisfaction of employees, but to motivate them into creativity and commitment in the organization [13], [14], [15], [16], [17], [18], [19], [20], [21], [22], [23], [24], [25]. This study constitutes a significant departure from conventional studies on workforce diversity by adopting a mediating perspective of the concept. In other words, the strength of workplace diversity is projected as a means of achieving higher levels of satisfaction from healthcare workers. The perspective adopted in this study is significant to enhancing passion and emotional affinity of healthcare workers toward caring for patients, hence sustaining a healthy community [10], [11], [12]. Based on the findings from the study, educational diversity at the workplace is a very strategic linkage between job satisfaction and employees' commitment (including affective, normative and continuance commitment). Indeed, the need to appreciate the roles of educational diversity reflects in the capacity of each to function across different cadres, yet achieving the common goal of the organisation [26], [27]. In the same way, diversities based on gender and religion has been evidence from the statistical point of view of this study to be credible leverages for stimulating employees' interest and commitment to the organisation.

This study concludes that not only is job satisfaction significant to ensuring the commitment of healthcare workers to their organisation, but more critical is the role of workforce diversity as viable leverage for transiting the interest of employees from the level of job satisfaction to organisational commitment. Specifically, three levels of workforce diversity, namely; education, gender and religion, were found to be strategic to enhancing healthcare workers perception about diversity treatments in the healthcare sector. Consequently, this study recommends that policymakers and managers in the Nigerian health care sector must pay adequate attention to creating a balance in the educational, gender and religious diversity of healthcare workers in Nigeria to sustain their satisfaction and stimulate their commitment to the organisation.

## Acknowledgements

Authors of this research work express sincere appreciation to the Management of Covenant University for giving full sponsorship to the publication of the research work in this journal.

## References

1. W Welcome MO. The Nigerian health care system: Need for integrating adequate medical intelligence and surveillance systems. Journal of pharmacy & bioallied sciences. 2011; 3(4):470-8. https://doi.org/10.4103/0975-7406.90100 PMid:22219580 PMCid:PMC3249694

2. Eboh A, Akpata GO, Akintoye AE. Health Care Financing in

Nigeria: An Assessment of the National Health Insurance Scheme (NHIS). European Journal of Business and Management. 2016; 8(27):24-34.

3. Butawa NN, Sule AG, Omole VN, Yere JK, Dogo M, Gyuro J. Assessment of job satisfaction among health workers in a tertiary hospital in Zaria, Northern Nigeria. Savannah Journal of Medical Research and Practice. 2013; 2(2):54-62.

4. Khamlub S, Harun-Or-Rashid MD, Sarker MA, Hirosawa T, Outavong P, Sakamoto J. Job satisfaction of health-care workers at health centers in Vientiane Capital and Bolikhamsai Province, Lao PDR. Nagoya journal of medical science. 2013; 75(3-4):233-241.

5. Kolo ES. Job satisfaction among healthcare workers in a tertiary center in kano, Northwestern Nigeria. Nigerian Journal of Basic and Clinical Sciences. 2018; 15(1):87-91. https://doi.org/10.4103/njbcs.njbcs_31_17

6. Chirdan OO, Akosu JT, Ejembi CL, Bassi AP, Zoakah AI. Perceptions of working conditions amongst health workers in state-owned facilities in northeastern Nigeria. Annals of African Medicine. 2009; 8(4):243-249. https://doi.org/10.4103/1596-3519.59579 PMid:20139547

7. McKay PF, Avery DR, Morris MA. Mean racial-ethnic differences in employee sales performance: The moderating role of diversity climate. Personnel psychology. 2008; 61(2):349-74. https://doi.org/10.1111/j.1744-6570.2008.00116.x

8. Gupta R. Workforce Diversity and Organizational Performance. International Journal of Business Management Invention. 2013; 2(6):36-44.

9. Black & Enterprise. Managing a multicultural workforce. Black Enterprise Magazine (July). Canada. Equal Opportunities International. 2001; 15(5):1-27.

10. Ibidunni AS, Falola HO, Ibidunni OM, Salau OP, Olokundun MA, Borishade TT, Amaihian AB, Peter F. Workforce diversity among public healthcare workers in Nigeria: Implications on job satisfaction and organisational commitment. Data in brief. 2018; 18:1047-53. https://doi.org/10.1016/j.dib.2018.03.127 PMid:29900272 PMCid:PMC5996598

11. Watson WE, Johnson L, Zgourides GD. The influence of ethnic diversity on leadership, group process, and performance: An examination of learning teams. International Journal of Intercultural Relations. 2002; 26(1):1-6. https://doi.org/10.1016/S0147-1767(01)00032-3

12. Osibanjo AO, Abiodun AJ, Adeniji AA. Impact of job environment on job satisfaction & commitment among Nigerian nurses. Proceedings of the 22nd International Business Information Management Association Conference. 2013; 3:1743-1752.

13. Falola HO, Olokundun MA, Salau OP, Oludayo OA, Ibidunni AS. Data article on the effect of work engagement strategies on faculty staff behavioural outcomes in private universities. Data in brief. 2018; 18:1383-7. https://doi.org/10.1016/j.dib.2018.04.035 PMid:29900319 PMCid:PMC5997958

14. Abiodun AJ, Ibidunni AS, Kehinde OJ. Demographic determinants of communication and information technology appreciation and usage among health care professionals. The International Journal of Health Economics. 2015; (5):5-19.

15. Ayeni AW, Iyiola OO, Ogunnaike OO, Ibidunni AS. Negativity of Globalization: The Ebola Virus Disease and its Health Impact on Nigerians. African Journal of Business Management. 2017; 11(3):47-56. https://doi.org/10.5897/AJBM2016.8133

16. Yamane T. Statistics, an Introductory Analysis. 2nd Ed., New York: Harper and Row, 1967.

17. Podsiadlowski A, Gröschke D, Kogler M, Springer C, Van Der Zee K. Managing a culturally diverse workforce: Diversity perspectives in organizations. International Journal of Intercultural Relations. 2013; 37(2):159-75. https://doi.org/10.1016/j.ijintrel.2012.09.001

18. Glazer G, Tobias B, Mentzel T. Increasing healthcare workforce diversity: urban universities as catalysts for change. Journal of Professional Nursing. 2018; 34(4):239-44. https://doi.org/10.1016/j.profnurs.2017.11.009 PMid:30055674 PMCid:PMC6069531

19. Jensen KW, Liu Y, Schøtt T. Entrepreneurs innovation bringing job satisfaction, work-family balance, and life satisfaction: In China and around the world. International Journal of Innovation Studies. 2017; 1(4):193-206. https://doi.org/10.1016/j.ijis.2017.11.002

20. Hanaysha J. Examining the effects of employee empowerment, teamwork, and employee training on organizational commitment. Procedia-Social and Behavioral Sciences. 2016; 229:298-306. https://doi.org/10.1016/j.sbspro.2016.07.140

21. Chang E, Chin H. Signaling or experiencing: Commitment HRM effects on recruitment and employees' online ratings. Journal of Business Research. 2018; 84:175-85. https://doi.org/10.1016/j.jbusres.2017.11.002

22. Sourouklis C, Tsagdis D. Workforce diversity and hotel performance: A systematic review and synthesis of the international empirical evidence. International Journal of Hospitality Management. 2013; 34:394-403. https://doi.org/10.1016/j.ijhm.2012.11.013

23. Brzeziński S, Bąk A. Management of employees' commitment in the process of organization transformation. Procedia Economics and Finance. 2015; 27:109-15. https://doi.org/10.1016/S2212-5671(15)00979-X

24. Ibidunni AS, Ogunnaike OO, Abiodun AJ. Extending the knowledge strategy concept: Linking organizational knowledge with strategic orientations. Academy of strategic management journal. 2017; 16(3):1-11.

25. Foster C, Punjaisri K, Cheng R. Exploring the relationship between corporate, internal and employer branding. Journal of Product & Brand Management. 2010; 19(6):401-9. https://doi.org/10.1108/10610421011085712

26. Charbonneau D, Wood VM. Antecedents and outcomes of unit cohesion and affective commitment to the Army. Military Psychology. 2018; 30(1):43-53. https://doi.org/10.1080/08995605.2017.1420974

27. Choi S. Demographic diversity of managers and employee job satisfaction: Empirical analysis of the federal case. Review of Public Personnel Administration. 2013; 33(3):275-98. https://doi.org/10.1177/0734371X12453054

28. Byrne BM. Testing for the factorial validity, replication, and invariance of a measuring instrument: A paradigmatic application based on the Maslach Burnout Inventory. Multivariate Behavioral Research. 1994; 29(3):289-311. https://doi.org/10.1207/s15327906mbr2903_5 PMid:26765139

29. Hipp JR, Bollen KA. Model fit in structural equation models with censored, ordinal, and dichotomous variables: Testing vanishing tetrads. Sociological methodology. 2003; 33(1):267-305. https://doi.org/10.1111/j.0081-1750.2003.00133.x

30. Lomax RG, Schumacker RE. A beginner's guide to structural equation modeling. psychology press; 2004 Jun 24. https://doi.org/10.4324/9781410610904

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Jun 30; 7(12):2036-2043.
https://doi.org/10.3889/oamjms.2019.483
eISSN: 1857-9655
***Public Health***

# Epidemiology of Vehicle Fire Fatalities of Road Traffic Injuries in Kerman Province, Iran: A Cross-Sectional Study

Ali Khodadadizadeh[1], Katayoun Jahangiri[1*], Davoud Khorasani-Zavareh[1], Reza Vazirinejad[2]

*[1]Department of Health in Disasters and Emergencies, School of Public Health and Safety, Shahid Beheshti University of Medical Sciences, Tehran, Iran; [2]Social Determinants of Health Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*

**Citation:** Khodadadizadeh A, Jahangiri K, Khorasani-Zavareh D, Vazirinejad R. Epidemiology of Vehicle Fire Fatalities of Road Traffic Injuries in Kerman Province, Iran: A Cross-Sectional Study. Open Access Maced J Med Sci. 2019 Jun 30; 7(12):2036-2043. https://doi.org/10.3889/oamjms.2019.483

**Keywords:** Vehicle fire fatalities (VFFs); Road traffic injuries (RTIs); Fatality; Iran

***Correspondence:** Katayoun Jahangiri. Department of Health in Disaster and Emergencies, School of Public Health and Safety, Shahid Beheshti University of Medical Sciences, Tehran, Iran. E-mail: k.jahangiri@sbmu.ac.ir

**Received:** 16-Apr-2019; **Revised:** 22-May-2019; **Accepted:** 23-May-2019; **Online first:** 30-Jun-2019

**Copyright:** © 2019 Ali Khodadadizadeh, Katayoun Jahangiri, Davoud Khorasani-Zavareh, Reza Vazirinejad. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

**Funding:** This research was financially supported by the Shahid Beheshti University of Medical Sciences, Iran

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

**Abstract**

**BACKGROUND:** Vehicle fires are one of the most important causes of fatalities in road traffic injuries (RTIs), but there are no accurate statistics about vehicle fire fatalities (VFFs) due to RTIs in Iran.

**AIM:** This study aimed to investigate the Epidemiology of vehicle fire fatalities (VFFs) due to road traffic injuries (RTIs) in Iran.

**METHODS:** In this cross-sectional study, a researcher-made checklist was used to collect the required data from the files of RTI fatalities in the Kerman Legal Medicine Organization (KLMO), or coroner's office. All reported victims of vehicle fires in the ten years from 2007 to 2017 were included in the study. The data were analysed using SPSS ver. 18, with p = 0.05 considered as the level of significance.

**RESULTS:** The authors found 124 cases of vehicle fire fatalities in Kerman, with a mean age of 30.45 ± 12.41, of which 50% were in the 25-49 years age group. Most frequently, the victims were Iranian (91.9%), married (66.1%), self-employed (51.6%), and urban dwellers (79.8%), and had died because of burns (91.9%). In 46.8% of cases, the victims were the driver, and in the remaining 53.2%, they were the passenger of the crashed vehicle. Most frequently, vehicle fires occurred on extra-urban roads (90.3%), during spring (35.5 %) or summer (32.3%), due to a vehicle-to-vehicle collision (66.9%), between sedans (69.9%), and at night (63.7%). Most victims died at the scene of the incident (87.9%) and had been transferred to hospital by an ambulance (71%).

**CONCLUSION:** This study indicated that car fires caused the death of young and middle-aged people. The authors suggest the implementation of preventative measures promoting car safety; establishing speed management; establishing laws governing driving, manufacturing and importation of vehicles; construction of safe roads; identifying accident-prone points; installing road warning signs; establishing more roadside stations; ensuring stricter police monitoring; and improving vehicle safety standards and public awareness about the risks of speeding.

## Introduction

Road traffic injuries (RTIs) are the main causes of early death, disability and a major health issue in low and middle-income countries[1]. RTIs are the cause of deaths, financial losses, and preventable health threats, even in developed countries [2]. It has been reported that most victims of RTIs are people aged <50 years old[3]. RTIs are the second leading cause of death from fatal injuries in Iran [2]. According to the World Health Organization (WHO), Iran had an RTI mortality rate of 34.1 deaths per 100,000 in 2013 [4] and had the third-highest rate of fatal RTIs in 2015 [5].

Vehicle fire, as a notable cause of RTI-related fatality, has received increasing attention since the 1960s [6]. According to the National Fire Protection Association's (NFPA) 2010 report, in the United States, 31 vehicle fires happen every hour, and one person dies because of vehicle fire every day. Between 2003 and 2007, about 287,000 vehicle fires caused 1,525 injuries and 480 fatalities in the US. During this period, on average, 8,200 highway vehicle

fires per year occurred due to accidents and overturning [7]. Generally, highway vehicle fires in the United States were the cause for 17% of reported fires, 12% of deaths, 8% of civilian injuries and 9% of the direct property damage from reported fires [8].

Based on US Fire Administration's National Fire Incident Reporting System from 2014 to 2016, there were 171,500 highway vehicle fires in the United States with an annual death rate of 345 people, 1300 injured and $ 1.1 billion in financial losses [9]. In 2015, vehicle fires constituted about 6.4% of all fires in Australia, 6.2% in Belarus, 4.6% in Poland, 7.8% in Romania, 13.5% in Russia, 10.3% in Slovenia, 23.5% in Sweden, 4.4% in Ukraine, 15.5% in the USA, 7.5% in Bulgaria, 5.9% in Croatia, 10.4% in the Czech Republic, 6.7% in Estonia, 9.6% in Finland, 3.4% in Hungary, 5.1% in Latvia, 4.8% in Liechtenstein, 9.1% in Lithuania, 13.3% in Moldova, 31.4% in New Zealand, and 13.1% of all fires around the world [10].

The causes of RTIs fatalities, the severity of injuries among drivers and passengers, and the factors associated with the severity of crashes and injuries in Iran have been extensively researched [11], but studies conducted in Iran have somewhat neglected the role of vehicle fires in road fatalities, and, unfortunately, there are as yet no accurate statistics on the number of Iranian fatalities due to vehicle fires. In published articles concerning RTIs in Iran, the rate of VFFs ranges from 0.9%-5% [4], [12], [13]. To our best knowledge, since the research on VFFs is scare nothing particularly in Iran, this study aims to provide the epidemiology of VFFs in Iran. It can give a base of the problem in the country, and provide a suggestion for prevention measures .

## Material and Methods

### *Study area and setting*

In this cross-sectional study, researchers studied the RTIs files archived by the Kerman Legal Medicine Organization (KLMO) over ten years, from 2007 to 2017. KLMO is a branch of a national organisation which is responsible for identifying death causes and issuing death certificates in the province [14]. According to Iranian national laws, all road deaths after a collision must be recorded and investigated to find accurate causes of death through an autopsy process in forensic clinics [15]. Kerman is the largest Iranian Province with an area of 183,285 $km^2$, and consists of 23 counties, with a population of 3,164,718 habitats. Geographically, this province is located in the south central region of the Iranian Plateau between 26°29′-31°58′ northern latitudes and 54°21′-59°34′ eastern longitudes. About 700,000 vehicles are registered, and nearly 1750 km of highway (the third largest in Iran) exist in Kerman. Kerman roads accommodate about 10,000,000 trips per year, but also have the highest rate of RTIs in Iran. It is reported that in 2016 alone, the RTIs in this province led to 13,624 injuries and 920 deaths [14], [16], [17], [18], [19]. Therefore, this province can be a suitable field for investigating the problem of vehicle fires and related causes, from both regional and international perspectives (Figure 1).

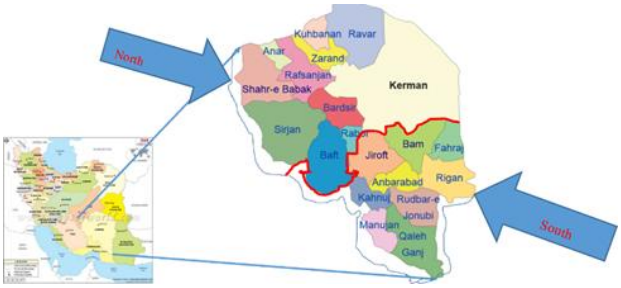



*Figure 1: Kerman province map*

### *Research population and subjects*

From 11,347 RTIs that were recorded by KLMO for 10 years, 124 deaths were caused by VFFs. All of the registered deaths were included in our study.

### *Data collection instrument*

Data collection was conducted initially by trained experts with using a researcher-made checklist and then entered to SPSS 18. In this research, to determine the validity of the checklist, content validity and the expert panel was used. Also, Cronbach's alpha was used to determine the reliability of the checklist. The reliability was 0.89. The principal investigator then extracted data from the records of KLMO. Information about the atmospheric conditions on the dates of crashes was also collected from the records available on the Iran Meteorological Organization website.

The first part of the checklist was related to socioeconomic variables including gender, age, marital status, and occupation, place of residence, education level, nationality, crash location, crash time, lighting situation, crash season, road type, and environmental conditions. The next part of the checklist consisted of pre-hospital parameter variables, including the method of transport to hospital (three categories), ultimate cause of death, place of death, and victim's status in the vehicle (driver, passenger), and vehicle-related variables, namely the type of victim's vehicle and the type of other vehicles involved in the crash.

### *Data analysis*

The data was imported into the SPSS ver. 18. Initially, frequency, percentage, range, mean ±

Standard Deviation on the socioeconomic status of the VFF were used. It included sex, age, marital status, educational level, occupation, residence, nationality, crash time, crash season, lighting situation, road type, crash location, crash type and place of death. Chi-squared and Fisher tests were used for assessing the associations between socioeconomic status, pre-hospital status and type of victim's vehicle (two categories: sedan and other vehicles). A P-value of less than 0.05 was considered significant.

### *Ethical considerations and authority permission*

This study was approved by Shahid Beheshti University of Medical Sciences, Tehran, Iran, with the ethics code IR.SBMU.RETECH.REC.1396.203. All forensic records were collected with the permission of this university and KLMO.

## Results

### *Socioeconomic status*

The results showed that in the 10 years between 2007 and 2017, 124 VFFs caused by RTIs occurred in Kerman. In terms of sex, 106 victims (85.5%) were male. In terms of sex and victim's vehicle, most of the deaths among men occurred in sedans and other vehicles, and most of the deaths among women occurred in sedans. This difference was statistically significant ($P < 0.05$). In terms of victims' ages, the highest frequency, 16 (12.9%), belonged to 22-year-olds. The mean age of victims was 30.45 ± 12.41, with a range of 2-75 years. In terms of victim's age group, the highest frequency was related to 25-49-years, and in terms of victim's age group and victim's vehicle, the difference was statistically significant ($P < 0.05$). In terms of victim's marital status, 66.1% were married. Nearly 70 % of those who had sedans were married, and over 41% of those who had other vehicles were single ($P < 0.05$). Regarding the level of education, the highest frequency of VFF was observed among people with primary school education. The difference between of victim's education level and victim's vehicle type was statically significant ($p < 0.05$). In terms of victim occupation, those who were self-employed, in sedans and other vehicles had the highest frequency ($P < 0.05$). In terms of the victim's place of residence, 99 victims (79.8%) were urban citizens. In terms of victim's residency and victim's vehicle, the numbers of urban victims were more concerning all vehicles ($P > 0.05$). In terms of nationality, 114 victims (91.9%) were Iranian, and 10 victims (8.1%) were Afghan (Table 1).

### *Crash time*

Fifty per cent of crashes occurred between 1:00 and 6:00 a.m. and 25% occurred between 18:01 and 24:00 p.m. Most crashes occurred on Saturdays, with 26 cases (21%), and on Fridays, with 25 cases (20.2%). The number of deaths was higher in sedans on Fridays, (21 or 25.3%) and Saturdays (18 or 21.7%). The difference was statistically significant (Chi-square = 12.5; $P < 0.05$). Most VFFs occurred in March, April, May and June (Figure 2). Most VFFs occurred during spring and summer (Table 1). The year with the highest frequency of VFFs was 2012, with 27 cases (about 25%) and the years with the lowest frequency were 2011 and 2016, each with 4 cases (3.2%). According to study results, there was no significant relationship between VFFs and type of victim's vehicles ($P > 0.05$), (Figure 3).

### *Road conditions*

In terms of lighting conditions, more than two-thirds of VFFs occurred during the night and involved sedans, and more than half involved other vehicles ($P < 0.05$). The road with the highest frequency of VFFs was the extra-urban road (99.2%). In term of the type of extra-urban road, the highest frequency was related to freeways, highways and trunk roads, and the lowest frequency was related to other roads. More than 96% of VFFs in sedans and 78% of VFFs in other vehicles occurred in extra-urban roads ($P < 0.05$), (Table 1).

### *Crash location*

The highest number of VFFs occurred in southern Kerman province (9 counties) with 65 cases (52.4%). In terms of crash location and victim's vehicle, nearly 60% of victims in the cities of southern Kerman province were in sedans, and nearly 61% of victims in the cities of northern Kerman province were in other vehicles ($P < 0.05$), (Table 1).

### *Crash type*

Most crashes (66.9%) involved vehicle-to-vehicle collisions. One incident (accounting for 0.8% of all crashes) involved a vehicle falling off the road. In terms of crash type and victim's vehicle, sedans accounted for the most vehicle-to-vehicle collisions and overturned (Table 1).

### *Pre-hospital parameters*

The most frequent place of death was the scene of the crash (about 90%) and the least frequent place was in the hospital with fifteen cases (12. 1%). In terms of the place of death and the victim's vehicle, the difference was not statistically significant ($P > 0.05$).

**Table 1: Socioeconomic status of the vehicle fire fatalities (VFF) in Kerman Province, Iran (2007-2016)**

| Variable | | Victim's vehicle | | | | |
|---|---|---|---|---|---|---|
| | | Sedan N (%) | Others* N (%) | | Total N (%) | |
| | | 83(66) | 41 (33.1) | | 124 (100) | |
| | | Frequency (%) | Frequency (%) | | Frequency (%) | P value* |
| Sex | Male | 67 (80.7) | 39 (95.1) | | 106 (85.5) | 0.032 |
| | Female | 16 (19.3) | 2 (4.9) | | 18 (14.5) | |
| | | | | M ± SD | | |
| Age | < 25 | 33 (39.7) | 21 (51.2) | 30.45 ± 12.41 | 54 (43.5) | 0.045 |
| | 25-49 | 47 (56.6) | 15 (36.5) | | 62 (50) | |
| | > 50 | 3 (3.6) | 5 (12.2) | | 8 (6.5) | |
| Marital status | Single | 25 (30.1) | 17 (41.4) | | 42 (33.8) | 0.002 |
| | Married | 58 (69.9) | 24 (58.5) | | 82 (66.1) | |
| Educational level | Primary school (< 6) | 22 (26.5) | 17 (41.5) | | 39 (31.5) | 0.046 |
| | Lower secondary school (6-8) | 24 (28.9) | 12 (29.3) | | 36 (29) | |
| | Upper secondary school (9-12) | 31 (37.3) | 5 (12.9) | | 36 (29) | |
| | University | 9 (10.8) | 4 (9.7) | | 13 (10.5) | |
| Occupation | Primary school and university student | 9 (10.8) | 2 (4.9) | | 11 (8.9) | 0.001 |
| | Housewife | 9 (10.8) | 2 (4.9) | | 11 (8.9) | |
| | Employee and retired | 6 (7.2) | 2 (4.9) | | 8 (6.4) | |
| | Worker and farmer | 5 (6) | 6 (14.6) | | 11 (8.9) | |
| | Self-employed | 49 (59.0) | 15 (36.6) | | 64 (51.6) | |
| | Driver | 5 (6.0) | 13 (31.7) | | 18 (14.5) | |
| | Military | 0 (0.0) | 1 (2.4) | | 1 (0.8) | |
| Residency | Urban | 68 (81.9) | 31 (75.6) | | 99 (79.8) | 0.409 |
| | Rural | 15 (18.1) | 10 (24.4) | | 25 (20.2) | |
| Nationality | Iranian | 75 (90.4) | 39 (95.1) | | 114 (91.9) | 0 .360 |
| | Afghan | 8 (9.6) | 2 (4.9) | | 10 (8.1) | |
| Crash time | 1:00-6:00 | 42 (50) | 20 (48.8) | | 62 (50) | 0.888 |
| | 6:0l-12:00 | 8 (9.6) | 4 (9.8) | | 12 (9.7) | |
| | 12:01-18:00 | 10 (12.0) | 7 (17.1) | | 17 (13.7) | |
| | 18:01-24:00 | 23 (27.7) | 10 (24.4) | | 33 (26.6) | |
| Crash Season | Spring | 27 (32.5) | 17 (41.5) | | 44 (35.5) | 0.602 |
| | Summer | 29 (34.9) | 11 (26.8) | | 40 (32.3) | |
| | Autumn | 15 (18.1) | 9 (22) | | 24 (19.4) | |
| | winter | 12 (14.5) | 4 (9.8) | | 16 (12.9) | |
| Lighting situation | Day | 25 (30.1) | 20 (48.8) | | 45 (36.3) | 0.042 |
| | Night | 58 (69.8) | 21 (51.2) | | 79 (63.7) | |
| Road type | Freeways, highways, and trunk roads | 80 (96.4) | 32 (78.0) | | 112 (90.3) | 0.001 |
| | Others | 3 (3.6) | 9 (22.0) | | 12 (9.7) | |
| Crash location | North of Kerman Province (10 counties) | 34 (41) | 25 (60.9) | | 59 (47.5) | 0.035 |
| | South of Kerman Province (9 counties) | 49 (59) | 16 (39.02) | | 65 (52.4) | |
| Crash type | Collision with each other | 58 (69.9) | 25 (61) | | 83 (66.9) | 0.755 |
| | Collision with a fixed object | 6 (7.2) | 4 (9.8) | | 10 (8.1) | |
| | Overturn | 16 (19.3) | 10 (24.4) | | 26 (21) | |
| | Fall | 1 (1.2) | 0 (0.0) | | 1 (0.8) | |
| | Vehicle fire | 2 (2.4) | 2 (4.9) | | 4 (3.2) | |

*(Bus, Trailer, Tanker, Ambulance and Truck (Pickup truck, Small truck)); **Chi-square.

The highest number of deaths in sedans and other vehicles occurred at the crash scene. Regarding the method of transport to the hospital, the majority of victims were transported by ambulance. In terms of transfer type and the victim's vehicle, the difference was statistically significant (P < 0.05). In terms of injury type, the most frequent class was multiple trauma, and the least frequent class was chest and abdomen injury. The most frequent type of injury based on Sedan and other vehicles were related to head, face and multiple trauma (P < 0.05). The most frequent final cause of death burned. More than 80 victims were related to sedans (Table 2). More than half a per cent (50%) of the victims were passengers and more than two-thirds were drivers. Most of the passengers (60.2%) were in sedans, and most of the drivers (61%) were in other vehicles (buses, trailers, tankers, or ambulances) (P < 0.05), (Table 2).

### *Vehicle involved conditions*

Regarding the type of vehicle collision with the victim's vehicle, the highest frequency was a sedan with sedan and other vehicles. Nearly 5% of car fires did not involve any collision. Trucks were responsible for nearly 31% of collisions with sedans and other cars (P > 0.05), (Table 2).

**Table 2: Frequency and percentage of vehicle fire fatalities (VFFs) in terms of pre-hospital conditions in Kerman Province, Iran (2007-2016).**

| Variable | | Victim's vehicle N (%) | | | |
|---|---|---|---|---|---|
| | | Sedan | Others* | Total | |
| | | 83 (66) | 41(33.1) | 124(100) | |
| | | Frequency (%) | Frequency (%) | Frequency (%) | P value** |
| Place of death | At the scene of the incident | 75 (90.4) | 34 (82.9) | 109 (87.9) | 0.232 |
| | Hospital | 8 (9.6) | 7 (14.6) | 15 (12.1) | |
| Transmission method | Ambulance | 56 (67.5) | 32 (78.0) | 88 (71) | 0.019 |
| | Police car | 14 (16.9) | 0 (0.0) | 14 (11.3) | |
| | Passing cars | 13 (15.7) | 9 (22.0) | 21 (17.7) | |
| The location of the body damage | Head and face | 27 (32.5) | 7 (17.1) | 34 (27.4) | 0.001 |
| | Chest and abdomen | 0 (0.0) | 2 (4.9) | 2 (1.6) | |
| | Hands and arms | 1 (1.2) | 8 (19.5) | 9 (7.3) | |
| | Multiple trauma | 55 (66.3) | 24 (58.5) | 79 (63.7) | |
| | Bleeding | 0 (0.0) | 1 (2.4) | 1 (0. 8) | |
| The final cause of death | Multiple trauma | 1 (1.2) | 0 (0.0) | 1 (0.8) | 0.555 |
| | Burn | 82 (98.8) | 40 (97.6) | 122 (98.4) | |
| The type of vehicle involved with the victim's vehicle | Sedan | 39 (47.0) | 14 (34.1) | 53 (42.7) | 0.447 |
| | Truck | 25 (30.1) | 13 (31.7) | 38 (30.6) | |
| | Others | 15 (18.1) | 12 (29.3) | 27 (21.8) | |
| | No collision | 4 (4.80) | 2 (4.9) | 6 (4.8) | |
| Victim's status in the vehicle | Passenger | 50 (60.2) | 16 (39.0) | 66 (53.2) | 0.026 |
| | Driver | 33 (39.8) | 25 (61.0) | 58 (46.8) | |

*(Bus, Trailer, Tanker, Ambulance and Truck (Pickup truck, Small truck)); ** Chi-square.

It was also found that VFFs accounted for 10.92 rate/1000 of all deaths caused by RTIs, with an annual mean of 10 deaths in Kerman Province (Table 3). The data showed that 24.55% of all deaths due to burn injuries in this area were related to vehicle fires.

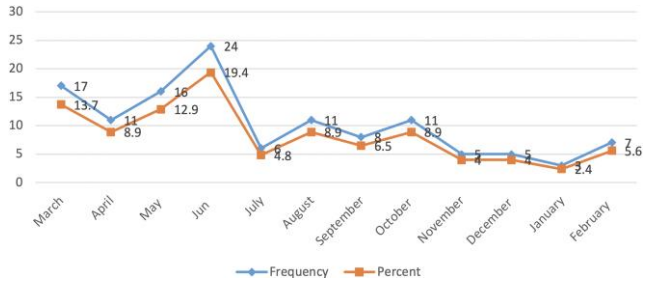



*Figure 2: Number and percentage of vehicle fire fatalities (VFFs) by month in Kerman Province, Iran (2007-2016)*

### *Environmental conditions*

In terms of air temperature at the place of the crash (county), the temperatures with the highest frequency were 31°C with 23 cases (18.5%) and 36°C with 12 cases (9.7%).

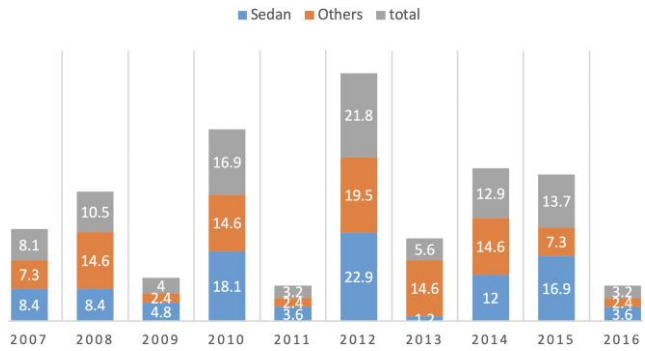



*Figure 3: percentage of vehicle fire fatalities (VFFs) by year and victim's vehicle in Kerman Province, Iran (2007-2016). The difference was not statistically significant(P > 0.05)*

In terms of mean temperature, the highest frequency, i.e. 38 cases (30.6%), was observed for the mean temperature of 30.70°C. In terms of wind speed, most crashes, i.e. 80 cases (64.5%), had occurred in the wind speeds of 10.8-14.4 km/h, with the mean speed being 17.65 ± 11.68. In terms of wind direction, the direction with the highest frequency was southwest with 25 cases (20.2%). From the horizontal visibility perspective, the visibility with the highest frequency was 10,000 meters, with 94 cases (75.8%), and the lowest frequency was 2,000 meters, with 2 cases (1.6%), with the mean visibility being 9,178.83 ± 1,779.82.

**Table 3: The number and rate of vehicle fire fatalities (VFFs) compared to RTI deaths in Kerman Province, Iran by year (2007-2016)**

| Year | Number of deaths from RTIs in Kerman province | Number of vehicle fire deaths in Kerman province | (Rate/1000) |
|---|---|---|---|
| 2007 | 1180 | 11 | (9.32) |
| 2008 | 1191 | 12 | (10.07) |
| 2009 | 1819 | 5 | (2.74) |
| 2010 | 1251 | 21 | (16.78) |
| 2011 | 1056 | 4 | (3.78) |
| 2012 | 1100 | 27 | (24.54) |
| 2013 | 904 | 7 | (7.74) |
| 2014 | 917 | 16 | (17.44) |
| 2015 | 1009 | 17 | (16.84) |
| 2016 | 920 | 4 | (4.34) |
| Total | 11347 | 124 | (10.92) |

## Discussion

Our investigation of RTIs occurring in Kerman province over 10 years from 2007 to 2017 found one 124 VFFs. This number equals 10.9/1000 killed of the total 11,347 RTIs deaths, an annual mean of 10 vehicle fire deaths and a quarter (24.55%) of the total 505 burns in Kerman Province, which is higher than the average in Iran and many countries in the world [8], [11], [21], [22]. More than ninety-six per cent of the deaths in men and women under 50 occurred in sedans. These findings showed that the lives of young and middle-aged people who are useful economically and culturally have been destroyed in sedans. This may be due to the speed of this type of car and its greater vulnerability. This finding needs special attention to determine its real cause and its prevention. This finding is consistent with past statistical reports about the road accident fatalities in Iran and the world [3], [23], [24]. Most victims of VFFs were married. Therefore accident's consequences affect their families, and this problem doesn't appear in the first phase of accidents [25].

Regarding the level of education, the highest rate of VFFs was observed among people less than a university education, which is consistent with the results of studies on road accident fatalities in Iran [25], [26], [27], [28], [29], [30]. This finding showed that despite the increasing number of post-revolutionary university education in Iran, still, most RTI victims are non-academic and less careful. This finding highlights the urgent need for effective, targeted education programs, before the issuance of driver's licenses, to reduce the rate of road accidents among these groups [27].

A major portion of victims was found to be urban dwellers, and the number of sedan fatalities among urban residents was higher than that of other vehicles, which suggests that these citizens travel more than rural citizens. This could be due to the greater tendency of urban citizens to vacation outside their home cities to escape urban traffic and pollution. Also, one-fourth of rural VFFs occurred in other vehicles. Therefore, a system must be established so that villagers do not have to travel to sell their crops and livestock products. This finding is consistent with the findings of other studies in Iran [24], [27], [31].

The self-employed, especially male self-employed, in sedans, and homemakers had the highest frequency of VFFs. This can be due to having a more flexible schedule and more free time. They have to travel more because of their occupation, so they were more exposed to crash due to fatigue and drowsiness. These findings are also consistent with other studies conducted in Iran [14], [22], [27], [31], [32].

In our study, the higher frequency of VFFs occurred among men whose job was driving, and the lowest frequency occurred among military personnel, which is understandable because most Iranian drivers are men. Because of proper training and observance of the law, military personnel had the lowest frequency of car crashes, a new point that should be addressed in educating people [4], [11], [27]. However, a study found that the highest fatality rate occurred among students [33]. This may be due to a gap between their knowledge about the risk, as other study also rely to fill this gap in order to promote the phenomena of interest.

In terms of nationality, nearly 92% of VFFS were Iranian, and about 10% were Afghan. Vehicles in high-speed chases carrying illegal immigrants in Iran and other countries have been shown to have an important effect on traffic fatalities [34], [35], [36].

The highest number of VFFs was related to night driving, especially between 1:00-6:00 a.m. and 18:01-24:00 p.m. This can be attributed to the peak traffic hours, less strict monitoring, limited police presence and less visibility as a result of limited lighting during these hours. Drivers are also more tired and sleepy in these hours [37]. Studies have suggested that good road lighting can reduce nighttime crashes [38], [39].

Most VFFs occurred in March, April, May and June and the highest frequency of VFFs occurred in spring and summer, especially on weekends, and the lowest occurred in winter. This is probably because

Iranians travel more in the summer and spring (Nowruz, weekends, school and university holidays). The police and coroner's reports, as well as other studies, showed that global road traffic control systems should pay particular attention to the management of family vacation trips [1], [27], [28], [37], [40]. One study showed that the rate of vehicle fires increased during school holidays, weekends, summer and autumn and decreased in winter [41].

Studies in Iran, as well as in other countries, reported a higher rate of traffic accidents on highways and extra-urban roads [13], [23]. We also found that most VFFs occur on extra-urban roads (freeways, highways, and trunk roads) and victim's sedans. This may be caused by increased traffic and the higher speed of sedans.

In this study, the highest frequency of VFFs was observed in southern Kerman Province. This area often experiences extreme temperatures during summer. The cities of Bam and Jiroft are centres of date and fruit production, car manufacturing, and tourism, especially due to Arg-e Bam, and are on the main route between the major cities of Kerman and other provinces [42]. These are the most accident-prone roads in Kerman province. Therefore, authorities in Iran and other countries should turn these kinds of roads into highways [43]. Based on our results, 64.5% (80 cases) of VFFs occurred with wind speeds of 10.8-14.4 km/h. One study indicated that wind speeds of 4-19.9 km/h had an impact on road accidents with vehicle fires [44, 45].

The study indicated most victims were transported to the hospital by ambulance. This finding is in agreement with other studies in Iran that's showed the importance of EMS [13], [27]. In terms of injury type, the most frequent class was multi-trauma. Multi-traumas were reported as the most frequent injuries among the victims of RTIs [46]. These results showed that in Iran, vehicles are unsafe and cause damage to different parts of the body. Although recently effective steps have been taken to ensure Iranian vehicle safety, they have not been enough. So, in addition to the upper airbag, which protects the head and face, vehicles must be equipped with an airbag or similar safety devices that also protect the torso and legs. Burns was the cause of 98% of fatalities. Vehicle fires caused by road accidents almost always led to the death of most passengers. Thus, the police officers and paramedics should be properly trained and equipped to handle these accidents.

Regarding the victim's status in the burning vehicle, we found that victims are frequent passengers, which is consistent with the reports of other studies [27], [31]. In the study that recorded by KLMO, drivers were most frequently victims in 2007 crashes, but in 2013, victims were most frequently passengers [15]. This finding is consistent with the results of a study conducted in Sweden [6].

In our study, the most frequent VFFs were related to the vehicle-to-vehicle collision, and the least one involved a vehicle falling off the road, which was consistent with other findings [6], [7], [12], [28]. Collision is still the main cause of accident fatality as well as VFFs, so serious prevention measures are needed to address it. The data showed that two-thirds of VFF victims were travelling in sedans. Several studies concluded that the greatest portion of traffic accident casualties involved sedans [15], [47]. Most deaths (87.8%) occurred at the scene of the accident. This is due to the severity of bodily injury and rapid death in vehicle fires. One study reported that 61.53% of victims died at the scene of accidents [13]. The year 2012 had the highest frequency of VFFs (about 25%), mostly related to sedans. This was due to a technical malfunction of the fuel system of certain domestic sedans, which were later recalled and remedied.

In conclusion, the VFFs is one of the most important causes of RTIs. There are a few types of research about VFFs thought worldwide. In Iran, our study is the first one. There are many different predisposing factors affected by VFFs. The significant ones which are extracted in our research are sex, age, marital status, education, Occupation, road type, crash location, transmission method, location of the body damage, Victim's status in the vehicle.

This study indicated that VFFs caused the death of young and middle-aged people. To prevent VFFs and promote car safety, the authors recommend equipping cars with fire extinguishers and other safety equipment, establishing speed management, enacting driving, manufacturing and vehicle importation laws, and construction of safe roads, identifying accident-prone areas, installing road warning signs at these points, improving access to fire and rescue services by establishing more roadside stations, and ensuring stricter police monitoring as well as improve sedan safety standards. Promotion of public awareness about the risks of speeding is also highly suggested.

*Research limitations:* The authors found information about the total number of RTIs in Kerman province. However, some details about some RTIs due to vehicle fire were missing. Also, it was difficult to access the data we needed for the study.

## Acknowledgements

We would like to thank the deputy director of research and staff of Shahid Beheshti University of Medical Sciences for their support, and express our sincere gratitude to Dr Amian, the head of Kerman Legal medicine organisation, and his colleagues, Mr Saeedi and Mrs Ebadi for their assistance and cooperation.

## References


1. Bazeli J, Aryankhesal A, Khorasani-Zavareh D. Epidemiology of special incidents: results from national mortality and morbidity registry and the associated factors in Iran in 2014. Electronic physician. 2017; 9(8):5113. https://doi.org/10.19082/5113 PMid:28979750 PMCid:PMC5614300

2. Delshad V, Sabzalizadeh S, Moradian MJ, Safarpour H, Malekyan L, Shemshadi H, et al. Epidemiology of accidents in Tehran emergency medical service during 2012 to 2013. Trauma Monthly. 2018; 23(1). https://doi.org/10.5812/traumamon.61871

3. Al-Risi A. Characteristics of road traffic injuries and potential risk factors in Oman: University of Otago; 2014.

4. Khorrami Z, Nazari SSH, Ghadirzadeh MR. An Epidemiology study of deaths from road traffic accidents. Safety Promotion and Injury Prevention. 2017; 4(4):217-24.

5. Tavakoli M, Ghadipasha M, Forozesh M. Management of Road Accident Victims in Iran. Journal of Orthopedic and Spine Trauma. 2015; 1(1).

6. Viklund A, Bjornstig J, Larsson M, Bjornstig U. Car crash fatalities associated with fire in Sweden. Traffic injury prevention. 2013; 14(8):823-7. https://doi.org/10.1080/15389588.2013.777956 PMid:24073770

7. Bunn T, Slavova S, Robertson M. Crash and burn? Vehicle, collision, and driver factors that influence motor vehicle collision fires. Accident Analysis & Prevention. 2012; 47:140-5. https://doi.org/10.1016/j.aap.2011.10.008 PMid:22405242

8. Ahrens M. Trends and patterns of US fire loss. National Fire Protection Association (NFPA) report Google Scholar. 2017.

9. Topical Fire Report Series Highway Vehicle Fires (2014-2016):|FEMA, 2018.

10. Brushlinsky Nikolai, Ahrens Marty, Sokolov Sergei , Peter WI. world-fire-statistics Center of Fire Statistics: the State Fire Academy of Emercom of Russia; 2017 .

11. Kashani AT, Shariat-Mohaymany A, Ranjbari A. Analysis of factors associated with traffic injury severity on rural roads in Iran. Journal of injury and violence research. 2012; 4(1):36. https://doi.org/10.5249/jivr.v4i1.67 PMid:21502788 PMCid:PMC3291279

12. Mahdieh Rad AL, Ansari-Moghaddam A, Mohammadi M, Rashedi F, Ghasemi A. The pattern of road traffic crashes in South East Iran. Global journal of health science. 2016; 8(9):149. https://doi.org/10.5539/gjhs.v8n9p149 PMid:27157159 PMCid:PMC5064071

13. Taravatmanesh S, Hashemi-Nazari SS, Ghadirzadeh MR, Taravatmanesh L. Epidemiology of fatal traffic injuries in the Sistan and Baluchistan province in 2011. Safety Promotion and Injury Prevention. 2015; 3(3):161-8.

14. ILMO. Statistics deaths and injuries from traffic accidents in 1395(2016) Iranian legal medicine organization, 2018.

15. Vaghefi SS, Ghadipasha M, Kazemi ES, Ohadi AR, Zareenejad M. Analysis type of injury in collisions' deceased referred to legal medicine in Kerman province during 2007 versus 2013. 2016; 21(4):291-96.

16. Ghadirzadeh M, Shojaei A, Khademi A, Khodadoost M, Kandi M, Alaeddini F, et al. Status and Trend of Deaths Due to Traffic Accidents From 2001 to 2010 in Iran. Iranian Journal of Epidemiology. 2015; 11(2):13-22.

17. Azami-Aghdash S, Abolghasem Gorji H, Sadeghi-Bazargani H, Shabaninejad H. Epidemiology of Road Traffic Injuries in Iran: Based on the Data from Disaster Management Information System (DMIS) Of the Iranian Red Crescent. Iran Red Crescent Med J. 2017; 19(1). https://doi.org/10.5812/ircmj.38743

18. Rad M, Mirhaghi A, Shomoossi N. Self-assertiveness interfacing incivility in student nurses: Possible outcomes. Nurse Education Today. 2015; 35(10):e6. https://doi.org/10.1016/j.nedt.2015.07.016 PMid:26242930

19. Balvardi M. The length of the highways of Kerman province kerman.mrud.ir 2017.

20. Kerman V. Desert Apitho. Kerman province map, 2014.

21. Haynes HJ. Fire loss in the United States during 2014: National Fire Protection Association. Fire Analysis and Research Division; 2015.

22. Moradi A, Rahmani K. Trend of traffic accidents and fatalities in iran over 20 years (1993-2013). J Mazandaran Univ Med Sci. 2014; 24(118):186-97.

23. Soroush D, Talebi DM, Reihani H, Vakili V, Mahmoodi GA, Tafakori A, et al. Characteristics of road traffic injuries in the second largest city of Iran. 2015.

24. Entezami N, Hashemi-Nazari SS, Soori H, Khosravi A, Ghadirzadeh MR. Epidemiology of fatal road traffic accidents in Northern provinces of Iran during 2009 to 2010. Safety Promotion and Injury Prevention. 2015; 3(1):1-8.

25. Sadeghi-Bazargani H, Samadirad B, Shahedifar N, Golestani M. Epidemiology of road traffic injury fatalities among car users; a study based on forensic medicine data in East Azerbaijan of Iran. Bulletin of Emergency & Trauma. 2018; 6(2):146. https://doi.org/10.29252/beat-060209 PMid:29719846 PMCid:PMC5928272

26. Samadirad B, Khodadoost M, Sadeghi-Bazargani H, Shahedifar N, Heydari ST, Gholamzadeh S. Epidemiology of Fatal Traffic Injuries Registered in East Azerbaijan and Fars Forensic Medicine Organizations: The Research Protocol. Journal of Clinical Research & Governance. 2016; 5(1).

27. Davoodi F, Hashemi-Nazari SS, Ghadirzadeh MR. An Epidemiology study of road traffic accidents resulting in death: in Lorestan province in 2012. Safety Promotion and Injury Prevention. 2016; 3(4):257-62.

28. Ahanchi NS, Hashemi NSS, Hasani J, Shojaei A. An epidemiology study of fatal road traffic accidents in Isfahan province in 2011.

29. Hashemi Nazari SS, Ghadirzadeh M. An epidemiology study of fatal road traffic accidents in khorasan razavi province in 2011. medical journal of mashhad university of medical sciences. 2016; 59(4):261-8.

30. Masoud MP, Asayesh H, Hoseini MHM, Jodaki K, Bagheri MJ, Jamshidi M. Epidemiology of Road Accident Death in Qom Province, 2014 (Iran). Qom Univ Med Sci J. 2017; 8(11):94-100.

31. Ahadi MR, Pejmanzad P, Ardebili PB. The epidemiology of accident fatalities in Iran (8-year review). Safety Promotion and Injury Prevention. 2015; 2(4):329-38.

32. Khazaie S, Mohammadian Ha, Mohammadian M, Salehiniya H, Afshari M. An Epidemiologic Study Of Traffic Accidents On Iran Drivers In 2013.

33. Sanyang E, Peek-Asa C, Bass P, Young TL, Daffeh B, Fuortes LJ. Risk factors for road traffic injuries among different road users in the Gambia. Journal of environmental and public health. 2017; 2017. https://doi.org/10.1155/2017/8612953 PMid:28512475 PMCid:PMC5420414

34. Perlman A, Radomislensky I, Peleg K, Group IT. Injury patterns among illegal migrants from Africa in Israel. Journal of immigrant and minority health. 2015; 17(4):1163-8. https://doi.org/10.1007/s10903-014-0068-x PMid:24972563

35. Sadeghi-Bazargani H, Ayubi E, Azami-Aghdash S, Abedi L, Zemestani A, Amanati L, et al. Epidemiological patterns of road traffic crashes during the last two decades in Iran: a review of the literature from 1996 to 2014. Archives of trauma research. 2016; 5(3). https://doi.org/10.5812/atr.32985 PMid:27800461 PMCid:PMC5078874

36. Lietuvnikė M, Vasiliauskas AV, Vasilienė-Vasiliauskienė V, Sabaitytė J. Peculiarities of illegal immigrant's intrusions into road freight transport units in the France-UK corridor. Entrepreneurship and Sustainability Issues. 2018; 5(3):634-47. https://doi.org/10.9770/jesi.2018.5.3(16)

37. Abbasi M, Sadeghi M, Azami AA, Esmaeili SM, Kavousi J, Aryafard A. Factors related to road traffic accidents leading to injury or death in Shahroud City. Safety Promotion and Injury Prevention. 2016; 4(2):83-90.

38. Jägerbrand AK, Sjöbergh J. Effects of weather conditions, light conditions, and road lighting on vehicle speed. SpringerPlus. 2016; 5(1):505. https://doi.org/10.1186/s40064-016-2124-6 PMid:27186469 PMCid:PMC4842190

39. Nabavi Niaki MS, Fu T, Saunier N, Miranda-Moreno LF, Amador L, Bruneau J-F. Road Lighting Effects on Bicycle and Pedestrian Accident Frequency: Case Study in Montreal, Quebec, Canada. Transportation Research Record: Journal of the Transportation Research Board. 2016(2555):86-94. https://doi.org/10.3141/2555-12

40. Bahadorimonfared A, Soori H, Mehrabi Y, Delpisheh A, Esmaili A, Salehi M, et al. Trends of Fatal Road Traffic Injuries in Iran (2004-2011). PLOS ONE. 2013; 8(5):e65198. https://doi.org/10.1371/journal.pone.0065198 PMid:23724132 PMCid:PMC3665536

41. Corcoran J, Higgs G, Rohde D, Chhetri P. Investigating the association between weather conditions, calendar events and socio-economic patterns with trends in fire incidence: an Australian case study. Journal of Geographical Systems. 2011; 13(2):193-226. https://doi.org/10.1007/s10109-009-0102-z

42. Moghbeli K. Most of the road casualties in the Kerman province occur at the Kerman-Bam Road, 2017/12/18.

43. Tavakkoli L, Khanjani N. The pattern of road crashes emphasizing the factors involved in their occurrence in Kerman City 2012-2015. Safety promotion and injury prevention (Tehran). 2016; 4(2):101-8.

44. Leffert MG. Effects of Wind Speed and Longitudinal Direction on Fire Patterns from a Vehicle Fire in a Compact Car. SAE Technical Paper; 2017:0148-7191. https://doi.org/10.4271/2017-01-1353

45. Perrels A, Votsis A, Nurmi V, Pilli-Sihvola K. Weather conditions, weather information and car crashes. ISPRS International Journal of Geo-Information. 2015; 4(4):2681-703. https://doi.org/10.3390/ijgi4042681

46. Hamzeh B, Najafi F, Karamimatin B, Ahmadijouybari T, Salari A, Moradinazar M. Epidemiology of traffic crash mortality in west of Iran in a 9 year period. Chinese journal of traumatology. 2016; 19(2):70-4. https://doi.org/10.1016/j.cjtee.2015.12.007 PMid:27140212 PMCid:PMC4897842

47. Katayama Y, Kitamura T, Kiyohara K, Iwami T, Kawamura T, Hayashida S, et al. Factors associated with prehospital death among traffic accident patients in Osaka City, Japan: A population-based study. Traffic injury prevention. 2018; 19(1):49-53. https://doi.org/10.1080/15389588.2017.1347645 PMid:28658590

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Jun 30; 7(12):2044-2049.
https://doi.org/10.3889/oamjms.2019.539
eISSN: 1857-9655
***Review Article***

# Oral Health Status of Athletes with Intellectual Disabilities: A Review

Shahd Omar Al-Shamlan, Mutaz Mohammad, Dimitrios Papandreou*

*Department of Health Sciences, CNHS, Zayed University, Dubai, United Arab Emirates*

**Citation:** Al-Shamlan SO, Mohammad M, Papandreou D. Oral Health Status of Athletes with Intellectual Disabilities: A Review. Open Access Maced J Med Sci. 2019 Jun 30; 7(12):2044-2049. https://doi.org/10.3889/oamjms.2019.539

**Keywords:** Oral health; Athletes; Intellectual disabilities; Special Olympics

***Correspondence:** Dimitrios Papandreou. Department of Health Sciences, CNHS, Zayed University, Dubai, United Arab Emirates. E-mail: dimitrios.papandreou@zu.ac.ae

**Received:** 11-May-2019; **Revised:** 11-Jun-2019; **Accepted:** 12-Jun-2019; **Online first:** 28-Jun-2019

**Copyright:** © 2019 Shahd Omar Al-Shamlan, Mutaz Mohammad, Dimitrios Papandreou. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

**Funding:** This research did not receive any financial support

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

**Abstract**

**BACKGROUND:** Oral health reflects the overall health of an individual; it impacts the mental and physical well-being, quality of life, and social relations of an individual. Special Olympics (SO) athletes have been found to have poorer oral health, and high unmet treatment needs globally. Nine articles are included in this systematic review to determine the oral health status of Special Olympics athletes with intellectual disabilities.

**AIM:** To identify the oral health status of athletes with intellectual disabilities who participated in the Special Olympics.

**METHODS:** Electronic bibliographic databases (PubMed, Biology database, Health management database, Science Direct, Health and medical collection (ProQuest), Mendeley, and Health reference centre academic) were used to search for eligible publications using "oral health," "special needs athletes," and "intellectual disabilities" terms. All included articles are in English and were published from 2000–2018. The whole process was conducted following PRISMA guidelines.

**RESULTS:** The search strategy yielded 4,090 articles. Only nine articles met the criteria and were included in the final analysis. All included articles reported outcome measurements of gingival signs, missing teeth, untreated decay, filled teeth and sealant.

**CONCLUSION:** The oral health status of athletes with intellectual disabilities can be considered poor compared with athletes without intellectual disabilities, which points to the need for oral health policies for this specific population.

## Introduction

Dental health is an essential aspect of general health and quality of life (World Health Organization [1], but it is also a major problem in public health, with strong links to people with health needs [2]. It has been reported that people with intellectual disabilities are more vulnerable to oral health issues [3]. Also, the oral treatment needs of intellectually disabled persons have been reported to be high in several studies [4], [5], [6]. According to the World Health Organization (WHO), around 10% of the world's population is disabled (approximately 650 million).

Gingival diseases and decay, in particular, are considered among the top ten secondary conditions within this population [7]. There is also strong evidence that poor oral hygiene is the primary cause of the increased prevalence of serious periodontal diseases [8]. Compared to the neurotypical population, people with intellectual disabilities have higher self-inflicted traumatic rates, poorer oral hygiene [9]. People with disabilities or their caregivers must seek dental care, but many factors, such as their living conditions or geographical location about dental services, influence access to care [10]. Likewise, low family income and lack of or inadequate dental health insurance may be a hindrance to oral care [11]. However, large amounts of oral health data

concerning persons with intellectual disabilities are scarce, although these data may be significant for the evaluation of existing policies [12]. Poor oral health can also cause sleep disturbances, difficulty eating, pain, and decreased self-esteem, which can all negatively impact an individual's quality of life [8].

Also, poor oral health negatively impacts the quality of life and athletic performance of special needs individuals. In 2015, Fernandez et al., [6] published data on the treatment need of oral health screening of athletes with intellectual disabilities in Belgium. He points out that an individual's cognitive and motor abilities can affect oral cleaning habits. The ability to perform oral hygiene is limited by the level of intellectual disability so that the assistance of a caregiver or supervision becomes necessary. Also, poor lip closure could be a predominant highlight among people with an intellectual disability that influences the cleansing of the oral cavity.

Moreover, Marks et al., [13] concluded that the high prevalence of oral health conditions among special needs individuals might be due to an inability to perform adequate personal oral hygiene, which leads to high levels of plaque, gingival inflammation, and periodontal disease. It has also been reported that individuals with ID are less cooperative with dentists and have more obstacles with the management of dental behaviour [14]. Currently, to the best of our knowledge, no systematic review has been done that examines this topic in depth, including Special Olympics (SO) athletes with intellectual disabilities, and evaluates these athletes' oral health status.

This systematic review aims to describe the oral health status (gingivitis, missing teeth, untreated decay, filled teeth, and sealant) of Special Olympics athletes with intellectual disabilities.

## Methods

### *Search strategy*

A systematic literature review was performed to identify existing articles that present data on the oral health status of athletes with intellectual disabilities between the years 2000 and 2018. Seven electronic databases accessed from the Zayed University library were carried out in PubMed, Biology database, Health management database, Science Direct, Health and medical collection (ProQuest), Mendeley, and Health reference centre academic. The search strategy consisted of terms and keywords of "oral health, athletes, intellectual disabilities and Special Olympics." The search specified two elements: the population of interest in athletes with intellectual disabilities, and the outcomes are gingivitis, missing teeth, untreated caries, filled teeth, and sealant.

### *Study outcomes*

The outcome of our study is presence gingivitis, missing teeth, untreated decay, filled teeth, and sealant in athletes with intellectual disabilities.

### *Sampling*

The initial search from online electronic databases yielded 4090 records, of which 1465 remained after duplicate articles were removed. With the screening of titles and abstracts, 75 publications were found to be not relevant, which left 19 publications eligible for full-text review. However, only nine of these publications met the inclusion criteria (Figure 1).

### *Study selection and eligibility criteria*

The systematic review was performed by the PRISMA (Preferred Reporting Items for Systematic review and Mata-Analyses) guidelines. All duplicate articles were removed. Titles and abstracts of searched records were screened to identify "potentially eligible" studies. The full texts of "potentially eligible" studies were retrieved and reviewed to determine whether all studies met the inclusion criteria.

### *Inclusion criteria*

1. Papers are written fully in English

2. Articles published between the years 2000 and 2018

3. Male and females included

4. Ages from 3 to 80 years old.

5. Included only athletes with ID who participated in the Special Olympics

6. Oral health outcomes were included (missing teeth, gingival signs, filled teeth, sealants, and untreated caries)

7. Not limited by study design (e.g., cohort and cross-sectional).

### *Exclusion Criteria*

1. Did not include specific disabilities such as Down syndrome.

2. The study failed to provide all oral health outcomes (missing teeth, gingival signs, filled teeth, sealants, and untreated caries).

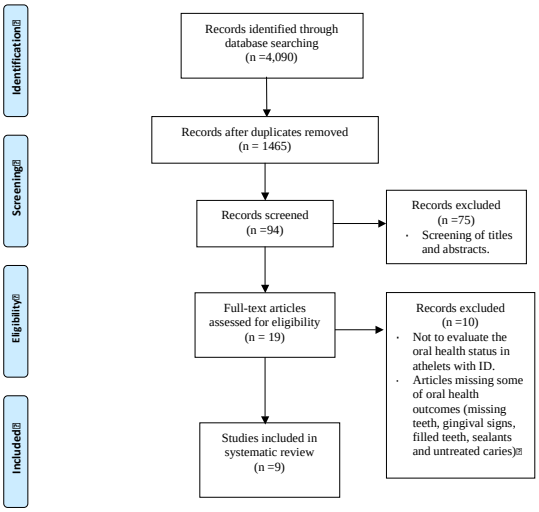



*Figure 1: PRISMA 2009 Flow Diagram-oral health status of athletes with intellectual disabilities*

### *Analytical tool*

Information extracted from eligible studies included participants, age range, sample size, and key outcome measurements (gingival signs, missing teeth, untreated decay, filled teeth, and sealant). Forest plot was used for the meta-analysis, and the R language tool was used for representing the meta-analysis. The forest plot represents the result of a set of studies and estimates the effect of each study with a line that represents the confidence interval. Another tool called the funnel plot was used to visually assess potential publication bias.

## Results

The main characteristics of the included studies are presented in Table 1. The included nine studies published within the past 18 years. One study was published in 2000, and the rest were published from 2010 to 2018. All articles evaluated the oral health status of athletes who participated in the Special Olympics from different regions in which the Special Olympics provided oral screening (SOSS) because the primary interest is the frequency of occurrence of oral conditions in athletes with intellectual disabilities. All studies included similar dental health outcomes (dental caries, filled teeth, untreated decay, sealants, and gingival signs).

### *Target population*

All studies evaluated participants from the Special Olympics. The total number of participants obtained from the included articles was 159,219. Each study collected data from different age groups; the first study focused on participant's aged 8 to more than 40 years old, the second study focused on those less than 18 to more than 26 years and the third from 9 to 80 years. The fourth study collected data from three countries, Poland, Romania, and Slovenia, and the participants were mainly adults, with an average age of 23.2 years (Poland), 22.9 years (Romania), and 27.8 years (Slovenia). The fifth study examined oral health status among 3 to 54-year-old. The sixth study focused only on participants who were less than 21 years of age, whereas the seventh study focused on ages 3 to 72 and the eighth study from 6 to 44. Finally, study number nine compared data between international and U.S. athletes, with the mean age of international athletes being 17.4 versus 24 years for U.S. athletes. Almost all studies recruited all age groups: children, adolescent, and adults.

**Table 1: Articles included in Systematic Review**

| Results of the prevalence of: | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Articles | *N* = athletes | Type of disability | Country | Age group | Missing teeth | Gingivitis | Untreated caries | Filled teeth | Sealant |
| 1. Marks et al. [24] | 149,272 | ID | Africa, Asia Pacific, East Asia, Europe/Eurasia, Latin America, Middle East North Africa (MENA)& North America. | *(8–11)<br>*(12–18)<br>*(19–39)<br>*(40+) | 28.20% | 46.40% | 36.60% | 49.80% | 14.20% |
| 2. Fernandez C et al. [6] | 132 | ID | Belgium | * < 18<br>*18–25<br>*26+ | 49.70% | 44.30% | 27.10% | 67.65 | 9.60% |
| 3. Leroy et al. [4] | 687 | ID | Belgium | Mean age 33 years (range 9–80 yrs) | 73% | 44% | 22% | 76% | 6% |
| 4. Fernandez C et al. [3] | 3,545 | ID | Poland<br>Romania<br>Slovenia | Mainly adult. Average: 23.2 years (Poland); 22.9 years (Romania); and 27.8 years (Slovenia) | 52.80%<br>38.40%<br>47.40% | 44.20%<br>70.40%<br>43.40% | 40.90%<br>19.10%<br>61.80% | 70.90%<br>33.90%<br>83% | 4.30%<br>3.80%<br>37.70% |
| 5. Hanke-Herrero et al. [17] | 930 | ID | Latin-American & Caribbean countries | 3-54 yrs | 23.02% | 48% | 51% | 52.50% | 40% |
| 6. Fernandez C et al. [3] | 503 | ID | 53 countries of Europe/ Eurasia | < 21 yrs | 25.20% | 38.70% | 33.40% | 47.70% | 9.90% |
| 7. Oretugba Folakemi et al. [19] | 1,286 | ID | Nigeria | 3–71 yrs | 4.30% | 48.10% | 21.10% | 0.30% | Non had |
| 8. Fernandez JB et al. [16] | 664 | ID | New York | Children 6–8 Adolescent 12–19 Adult 35–44 | 29% | 32% | 28% | 60% | 11% |
| 9. Reid et al. [18] | 2200 | ID | International (China, Lebanon, Poland, south Africa, Turkey) | Mean age 17.4 yrs | 17.70% | 27.80% | 41.60% | 19.60% | 1.80% |

### *Study location*

Regions of the world where studies on oral health status among athletes with intellectual disabilities were conducted include 1. Africa & North Africa (MENA); 2. East Asia & Pacific; 3. Europe & Eurasia; 4. Middle East; 5. North America; 6. Latin America and the Caribbean.

### *Assessments of oral health*

A standardized Special Smile screening form and procedure (SOSS program) was used to guide oral assessment in all nine studies [3], [12], [13], [15], [16], [17], [18], [19], [20].

### *Data Analysis*

The main results of the meta-analyses regarding the prevalence of oral health issues are shown in Figure 2.

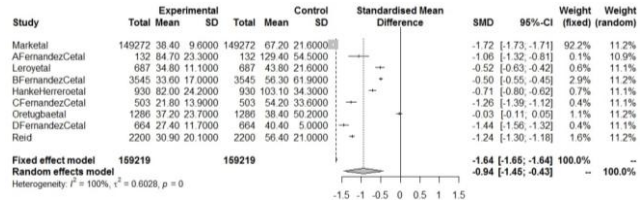



*Figure 2: Forest plot on the fixed effect model of the dental health status of athletes with intellectual disabilities*

As illustrated, by the meta-analysis, the R package Meta from the fixed-effects model fitting was used. We noted from the 95% confidence intervals (CIs) that the overall analysis of the included studies from the fixed-effects model with a P = 0.0 with a corresponding 95% CI of [-043, -1.45]. Another test was performed, the funnel plot, which is shown in Figure 3 and visually assesses potential publication bias.

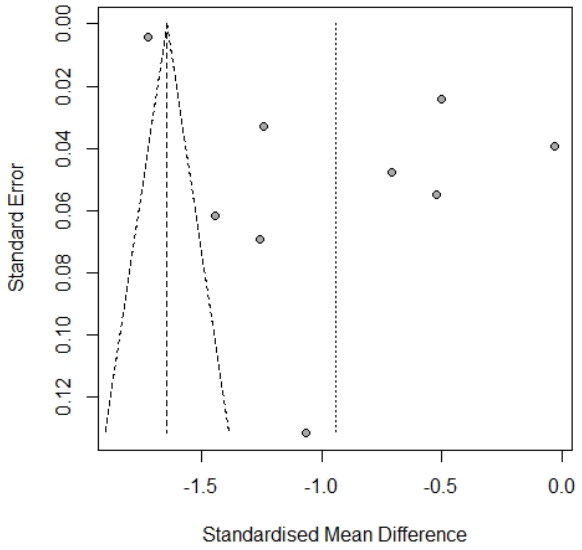



*Figure 3: Funnel plot of the nine studies*

## Discussion

The results of this study support the hypothesis by providing evidence that athletes with intellectual disabilities have a high level of oral health problems compares with the healthy ones. In all included studies, a significant number of athletes were diagnosed with oral health problems such as missing teeth, gingivitis, filled teeth, sealants, and untreated decays. The prevalence of filled teeth had the highest score among the seven studies compared to other dental conditions. However, it had a wide variation, showing values between 47.7 and 83%. Also, the risk of gingival diseases was found to be 40% or greater in seven studies. Needleman et al., [21] found that athletes who participated in the London 2013 Special Olympics also had a high prevalence of gingivitis. This study also showed that untreated decay occurred in 18.5 – 61.8% of the participant subjects. Likewise, at the 2005 Glasgow Special Olympics [22], more untreated dental diseases were reported in participants than in the general population.

The eight studies indicated that the prevalence of sealant among athletes with intellectual disabilities was the lowest of the found oral conditions, with values between 0 and 37.7%; similarly a cross-sectional study of dental health and odontogenic infections among German students with intellectual/learning disabilities (ID/LD), sensory disorders (SD), and physical disabilities (PD) revealed that fissure sealants had the lowest prevalence among all students with ID/LD [23].

Another point to be highlighted is the prevalence of dental issues among regions, as a study found that people with intellectual disabilities in Europe/Eurasia, MENA, and Latin America have higher rates of gingivitis than other regions at all age groups [24].

In this review, only one study conducted oral health status research on individuals with intellectual disabilities in MENA and two studies in Latin American, with five studies being undertaken in Europe/Eurasia. These results agree with our findings that the prevalence of gingival diseases was highest in the regions of MENA, Europe/Eurasia, and Latin America, showing values between 38.7 and 70.4%. Instead, a study in Africa, particularly in Nigeria [19], showed a value of 48.1%, which is considered high.

The high prevalence of oral health issues in athletes with intellectual disabilities can be attributed to an association of several factors; for instance; their living conditions or geographical location can influence their actual access to health care institutions [10]. Low family income and lack of or inadequate dental health insurance may also be a hindrance to oral care [11].

Moreover, other researchers demonstrated that individuals with intellectual disabilities had more dental conditions, partly due to the cognitive and motor abilities that can affect their oral cleaning habits in performing oral hygiene [6], [23]. The side effects of medication use may also have contributed to lower dental health status [25]. Another important factor that should be considered is that individuals with intellectual disabilities are less cooperative with dentists and have more obstacles with the management of dental behaviour [14].

### *Study limitations and Recommendations for the future research*

This review has several limitations that can affect the generalizability of this study. First, one of the main limitations is the heterogeneity of the studies. High heterogeneity was found based on an $I^2$ test, the value of which was $I^2$ = 100%, when the recommended value is less than 50%. The high value of heterogeneity was expected, as the included studies have a wide age range. The second limitation is that this review does not include all oral health problems; it includes only the gingivitis, filled teeth, untreated decays, sealants, and missing teeth. This is because each study examined different oral health outcomes, and in this article were only included studies that used similar oral health problems.

Also, the sample characteristics in all studies were not matched with the same age group, and each study had a different wide age range. Studies that evaluate individuals with special needs commonly use convenience sampling, which justifies the failure at presenting the relatively small number of the participants and their wide age range in the included studies. This could be related to the difficulties of intellectually disabled athletes in cooperating with the examiners. Also, the findings of the eligible studies might represent the oral health status of athletes with mild or less severe intellectual disabilities, as athletes with severe intellectual disabilities may not tolerate dental examination.

The recommendation for the future research should include the oral hygiene level with the outcomes we measured (missing teeth, untreated decays, filled teeth, gingival signs, and sealant) assessing of the hygiene factor that could be associated with these conditions in individuals with intellectual disabilities. This is because the cognitive and motor abilities of intellectually disabled individuals can affect their oral hygiene performance.

## Conclusion

The findings from this systematic review of the nine studies indicated that the oral health status of Special Olympics athletes with intellectual disabilities is below expectations. The meta-analysis provided an estimate of the prevalence of oral health issues of athletes with an intellectual disability that indicates a significant unmet treatment need among this population. The consistency of the results in this review supports the necessity for better dental preventive care of athletes with intellectual disabilities, even though this study sample is not representative of the whole population of athletes with intellectual disabilities. Moreover, research in this area should focus on strategies that promote self-care, in particular, improving the daily hygiene of individuals with intellectual disabilities.

## References


1. World Health Organization. (2007). Atlas: Global resources for persons with intellectual disabilities: 2007.

2. Howell R, Brimble M. Dental health management for children with special healthcare needs. Nurs Child Young People. 2013; 25(5):19-22. https://doi.org/10.7748/ncyp2013.06.25.5.19.e201 PMid:23988072

3. Fernandez Rojas C, Wichrowska-Rymarek K, Pavlic A, Vinereanu A, Fabjanska K, Kaschke I, Marks LA. Oral health needs of athletes with intellectual disability in Eastern Europe: Poland, Romania and Slovenia. International dental journal. 2016; 66(2):113-9. https://doi.org/10.1111/idj.12205 PMid:26601920

4. Leroy R, Declerck D. Objective and subjective oral health care needs among adults with various disabilities. Clinical oral investigations. 2013; 17(8):1869-78. https://doi.org/10.1007/s00784-012-0879-x PMid:23192286

5. Trihandini I, Wiradidjaja Adiwoso A, Erri Astoeti T, Marks L. Oral health condition and treatment needs among young athletes with intellectual disabilities in I Indonesia. International journal of paediatric dentistry. 2013; 23(6):408-14. https://doi.org/10.1111/ipd.12010 PMid:23163957

6. Fernandez C, Declerck D, Dedecker M, Marks L. Treatment needs and impact of oral health screening of athletes with intellectual disability in Belgium. BMC oral health. 2015; 15(1):170. https://doi.org/10.1186/s12903-015-0157-9 PMid:26714613 PMCid:PMC4696164

7. Newacheck PW, Hughes DC, Hung YY, Wong S, Stoddard JJ. The unmet health needs of America's children. Pediatrics. 2000; 105(Supplement 3):989-97. https://doi.org/10.1542/peds.105.4.760 PMid:10742317

8. Anders PL, Davis EL. Oral health of patients with intellectual disabilities: a systematic review. Special Care in Dentistry. 2010; 30(3):110-7. https://doi.org/10.1111/j.1754-4505.2010.00136.x PMid:20500706

9. Bhat M, Nelson KB. Developmental enamel defects in primary teeth in children with cerebral palsy, mental retardation, or hearing defects: a review. Advances in dental research. 1989; 3(2):132-42. https://doi.org/10.1177/08959374890030021101 PMid:2701156

10. Guay AH. Access to dental care: the triad of essential factors in access-to-care programs. The Journal of the American Dental Association. 2004; 135(6):779-85. https://doi.org/10.14219/jada.archive.2004.0307 PMid:15270163

11. Al Agili DE, Roseman J, Pass MA, Thornton JB, Chavers LS. Access to dental care in Alabama for children with special needs: Parents' perspectives. The Journal of the American Dental Association. 2004; 135(4):490-5. https://doi.org/10.14219/jada.archive.2004.0216 PMid:15127874

12. Fernandez C, Descamps I, Fabjanska K, Kaschke I, Marks L. Treatment needs and predictive capacity of explanatory variables of oral disease in young athletes with an intellectual disability in Europe and Eurasia. European journal of paediatric dentistry: official journal of European Academy of Paediatric Dentistry. 2016; 17(1):9-16.

13. Marks L, Fernandez C, Kaschke I, Perlman S. Oral cleanliness and gingival health among Special Olympics athletes in Europe and Eurasia. Medicina oral, patologia oral y cirugia bucal. 2015; 20(5):e591. https://doi.org/10.4317/medoral.20396 PMid:26241452 PMCid:PMC4598929

14. Blomqvist M, Holmberg K, Fernell E, Dahllöf G. A retrospective study of dental behavior management problems in children with

attention and learning problems. European journal of oral sciences. 2004; 112(5):406-11. https://doi.org/10.1111/j.1600-0722.2004.00150.x PMid:15458498

15. Leroy R, Declerck D, Marks L. The oral health status of special olympics athletes in Belgium. Community dental health. 2012; 29(1):68.

16. Fernandez JB, Lim LJ, Dougherty N, LaSasso J, Atar M, Daronch M. Oral health findings in athletes with intellectual disabilities at the NYC Special Olympics. Special Care in Dentistry. 2012; 32(5):205-9. https://doi.org/10.1111/j.1754-4505.2012.00268.x PMid:22943773

17. Hanke-Herrero R, Lopez del Valle LM, Sánchez C, Waldman HB, Perlman SP. Latin-American Special Olympics athletes: evaluation of oral health status, 2010. Special Care in Dentistry. 2013; 33(5):209-12. https://doi.org/10.1111/scd.12019 PMid:23980552

18. Reid BC, Chenette R, Macek MD. Special Olympics: the oral health status of US athletes compared with international athletes. Special Care in Dentistry. 2003; 23(6):230-3. https://doi.org/10.1111/j.1754-4505.2003.tb00317.x PMid:15085960

19. Oretugba FA, Perlman SP. Oral health condition and treatment needs of Special Olympics athletes in Nigeria. Special Care in Dentistry. 2010; 30(5):211-7. https://doi.org/10.1111/j.1754-4505.2010.00155.x PMid:20831740

20. Fernandez C, Kaschke I, Perlman S, Koehler B, Marks L. A multicenter study on dental trauma in permanent incisors among Special Olympics athletes in Europe and Eurasia. Clinical oral investigations. 2015; 19(8):1891-8. https://doi.org/10.1007/s00784-015-1403-x PMid:25600092

21. Needleman I, Ashley P, Petrie A, Fortune F, Turner W, Jones J, Niggli J, Engebretsen L, Budgett R, Donos N, Clough T. Oral health and impact on performance of athletes participating in the London 2012 Olympic Games: a cross-sectional study. Br J Sports Med. 2013; 47(16):1054-8. https://doi.org/10.1136/bjsports-2013-092891 PMid:24068332 PMCid:PMC3812828

22. McPherson G, Finn J, Barclay S, Mclntosh A. The Special Olympics Great Britain Games 2005: Glasgow City Counsil and the case of voluntary management. 2006:306-35601.

23. Dziwak M, Heinrich-Weltzien R, Limberger K, Ifland S, Gottstein I, Lehmann T, Schüler IM. Dental health and odontogenic infections among 6-to 16-year-old German students with special health care needs (SHCN). Clinical oral investigations. 2017; 21(6):1997-2006. https://doi.org/10.1007/s00784-016-1988-8 PMid:27815794

24. Marks L, Wong A, Perlman S, Shellard A, Fernandez C. Global oral health status of athletes with intellectual disabilities. Clinical oral investigations. 2018; 22(4):1681-8. https://doi.org/10.1007/s00784-017-2258-0 PMid:29119318 PMCid:PMC5906489

25. Elliot I, Nunn J, Sadlier D. Oral health & disability: the way forward. National Disability Authority, Dublin. 2005.

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Jun 30; 7(12):2050-2056.
https://doi.org/10.3889/oamjms.2019.586
eISSN: 1857-9655
***Review Article***

# Association between the Gut Microbiota and Obesity

Kiril Mitev*, Vaso Taleski

*Faculty of Medical Sciences, University "Goce Delchev", Shtip, Republic of Macedonia*

**Citation:** Mitev K, Taleski V. Association between the Gut Microbiota and Obesity. Open Access Maced J Med Sci. 2019 Jun 30; 7(12):2050-2056. https://doi.org/10.3889/oamjms.2019.582

**Keywords:** gut; microbiota; association; obesity

***Correspondence:** Kiril Mitev. Faculty of Medical Sciences, University "Goce Delchev", Shtip, Republic of Macedonia. E-mail: mitev_kiril@hotmail.com

**Received:** 09-May-2019; **Revised:** 25-Jun-2019; **Accepted:** 28-Jun-2019; **Online first:** 29-Jun-2019

**Copyright:** © 2019 Kiril Mitev, Vaso Taleski. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

**Funding:** This research was financially supported by the Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia

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

**Abstract**

**BACKGROUND:** People co-evolved with members of the microbiota and developed, used and adapted many complex immune mechanisms, which are used for monitoring and control of the microbiota. The gut microbiota in cooperation with humans became its essential part, so-called "hidden organ" with many important and indispensable functions. Quantitative and/or qualitative deficiency of the gut microbiota (dysbiosis) probably is a basis of many disorders, including obesity.

**AIM:** To present an overview of the possible association between gut microbiota and obesity.

**METHODS:** Meta-analysis of available scientific and published data including PubMed, Web of Science, Scopus and Cochrane Library.

**RESULTS:** In the intestinal microbiota at obese people is detected a specific increase in the proportion between class *Firmicutes* and *Bacteroidetes* despite the non-obese people. Also, it is detected a decrease in this proportion if the person lost weight. These facts may be secondary to obesity. The colonisation of germ-free mice with microbiota from ordinarily feed or obese mice, without changes in the feed style leads to increase body fat to more than 50%.

**CONCLUSION:** The human gut microbiota directly affects the food digestion, absorption and metabolism. The gut microbiota of obese people has a higher capacity for receiving energy from the food than the microbiota at slim people. The gut microbiota affects appetite control and energy balance. Lifestyle and food regimen affect the diversity of the gut microbiota and the presence of dysbiosis.

## Introduction

Malnutrition is a wide term, which embraces obesity and undernutrition and is featuring with an imbalance between energy input and output. In 2014 in the World, more than 600 million were obese [1]. Obesity is a condition with excess body fat and increased body weight. There are some disorders, which are associated with obesity; such are atherosclerosis, diabetes, non-alcoholic fatty liver disease and some types of cancer, which are leading causes of death in the USA [2].

Some studies on GFM (germ-free mice) indicate that GFM are resistant to high-calorie foods and fattening besides feeding with high-calorie foods [3]. Colonisation the GFM with microbiota derived from "obese donor" results with an increase of the full body fat and body weight, backwards if GFM is colonised with microbiota derived from "slim donor", they won't get fat beside feeding with high-calorie foods [4]. GFM, which received faecal transplantation from “obese donor”, will increase their body weight [5]. All of these studies impose an opinion that the human gut microbiota may have a role in the development of obesity and obesity-associated disorders.

## Results

### *Composition and taxonomic distribution of gut microbiota*

Human Microbiome Project included 554 individuals, selected by many criteria. The average age of them was 26, and the average Body Mass

Index (BMI) was 24 kg/m$^2$. Samples were collected from 18 regions of the body and gut sample just from faeces [6]. Compositions of the microbiota were determined by 16S rRNA genetic sequencing performing metagenomics profiles of all sequenced genomes. All knowledge about gut microbiota was published in 2012 in two studies [7], [8]. The conclusion was that the healthy human gut microbiota consists of 70-100 known bacterial species [7]. However, 16S rRNA gene sequencing techniques guessed that the number of bacteria in the human gut microbiota might exceed 1000 species because of consisting some very rare, thin and non-cultivable or non-classified categories of bacteria [9].

**Table 1: Taxonomic review of the members of gut microbiota into divisions, classes, orders, families and species**

| Division | Class | Order | Family | Species |
|---|---|---|---|---|
| *Firmicutes* | *Clostridia* | *Clostridiaels* | *Clostridiaceae* | *Clostridium* |
| | | | *Ruminococcaceae* | *Ruminococcus* |
| | | | | *Faecalibacterium* |
| | | | *Eubacteriaceae* | *Eubacterium* |
| | *Bacilli* | *Lactobacilliales* | *Lactobacillaceae* | *Lactobacillus* |
| | | | *Streptococcaceae* | *Streptococcus* |
| | | | | *Enterococcus* |
| | *Erysiphelotrichia* | *Erysiphelotrichales* | *Erysiphelotrichaceae* | *Turicibacter* |
| | | | | *Catenibacterium* |
| | | | | *Coprobacillus* |
| | | | | *Allobaculum* |
| | *Negativicutes* | *Selemonadales* | *Selenomonadaceae* | *Megamonas* |
| | | *Veillonellales* | *Veillonellaceae* | *Dialister* |
| | | | | *Megasphaera* |
| | | | | *Veillonella* |
| *Bacteroidetes* | *Bacteroidia* | *Bacteroidales* | *Prevotellaceae* | *Prevotella* |
| | | | *Bacteroidaceae* | *Bacteroides* |
| *Actinobacteria* | *Coriobacteria* | *Coriobacteriales* | *Coriobacteriaceae* | *Collinsella* |
| | | | *Atopobiaceae* | *Olsenella* |
| | | *Egerthellales* | *Eggerthellaceae* | *Slackia* |
| | | | | *Eggerthella* |
| | *Actinobacteria* | *Bifidobacteriales* | *Bifidobacteriaceae* | *Bifidobacterium* |
| *Fusobacteria* | *Fusobacteria* | *Fusobacteriales* | *Fusobacteriaceae* | *Fusobacterium* |
| *Proteobacteria* | *Gammaproteo-bacteria* | *Enterobacteriales* | *Enterobacteriaceae* | *Escherichia* |
| | | | | *Shigella* |
| | | *Aeromonadales* | *Succinivibrionaceae* | *Succinivibrio* |
| | | | | *Anaerobiospirillum* |

### *Oesophagus, stomach and small intestine*

There are no data for permanent microbiota in the proximal part of the oesophagus. Studies have detected that in proximal oesophagus, the diversity of microbes is low. Studies for the distal part of the oesophagus say that there is reduced diversity and a dominant species of it is *Streptococcus*. Also, it is known that the reduced diversity of microbes in the oesophagus is good to sustain the wellbeing of the oesophagus [9]. The low pH of stomach limits many types of microbes, which get there. Attendance or absence of *Helicobacter pylori* affects the composition of the stomach microbiota. When *H. pylori* are there, it is a dominant species. Otherwise, *Streptococcus* is dominant [10]. *H. pylori* can be commensal or pathogen species of the stomach [4]. Besides this, stomach microbiota consists of other species as *Prevotella*, *Veillonella* and *Rothia*.

It is very hard to derive data about the microbiota in the small intestine because of insufficiency of healthy volunteers. Based on some routine microbiological examinations and molecular studies, it is known that *Streptococcus* is a dominant species in duodenum and jejunum. Generally, it is accepted that the diversity and complexity of bacterial communities increases in proximal-distal direction (from the duodenum to ileum).

### *Large intestine and faeces*

The composition of microbiota in the large intestine is very similar to those in the distal part of the small intestine, actually ileum. It is much easier to examine the composition of the large intestine microbiota than other parts of the gut. Five divisions of bacteria are present; such are *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Fusobacteria* and *Proteobacteria*. Divisions *Firmicutes* and *Bacteroidetes* are more numerous than the others. They take 90% of the number of all bacteria in the large intestine [9]. Less than 0.1% of human gut microbiota are some pathogens as *Campylobacter jejuni*, *Salmonella enterica* and *Vibrio cholerae* (Figure 1).

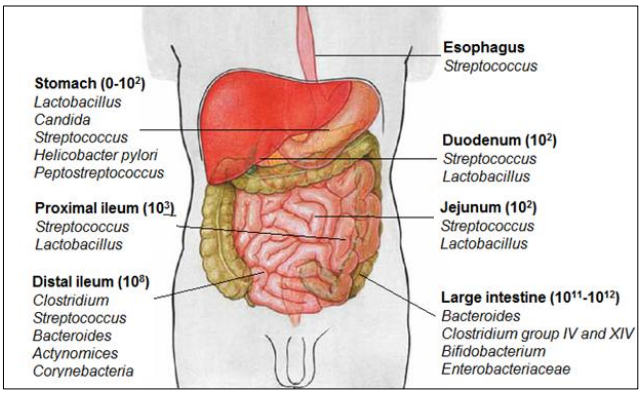



*Figure 1: Composition and number of major gut microbial species*

### *Factors that influence the human gut microbiota*

The composition and count of gut microbiota are dynamic and variable, even under the subtle influences of some environmental factors [11]. Some of these factors are:

**Genetics** - Studies have revealed that host genetics has a small but statistically significant effect on the microbiota's composition and count. Studies on mice with variation in the MHC (Major Histocompatibility Complex) genes have shown that the host's genetics influences the microbiota. These mice had a higher susceptibility to autoimmune disease because of influences of the gut microbiota.

**Age** - There are some acknowledgements that the humans are exposed to microbes even during the intrauterine period, and there were identified bacterial DNA in healthy placentas, amniotic liquid and meconium of term newborns. The delivery mode (vaginal or cesarean section), the feeding style (breast milk or baby milk) are the main factors which

determine the microbiota at newborns. During the first three years of life, the microbiota of children is getting more numerous and more complex.

**Diet** - Food, which people eat, obtain nutrients for the host, but also the gut microbiota. This fact is remarkable at children who are weaning from baby milk and are starting to eat some different food, which is getting a low number of *Bifidobacterium* species in their intestines. Domination of gut bacteria which are metabolising vegetative polysaccharides (*Roseburia, Eubacterium rectangle* and *Ruminococcus bromii*) is marked in veggies. People who eat meat have bile-tolerant microbes; such are *Alistipess*, *Bilophila* and *Bacteroides*.

**Drugs** - As it is well known, all the medications affect the gut microbiota. The most studied affections are connected with the use of antibiotics. For example, during the treatment with Ciprofloxacin, which does not influence anaerobic bacteria, reduction of 1/3 of anaerobic gut bacteria may be detected. This effect is a result of the elimination of some basic bacterial species, which are necessary for the survival of the others.

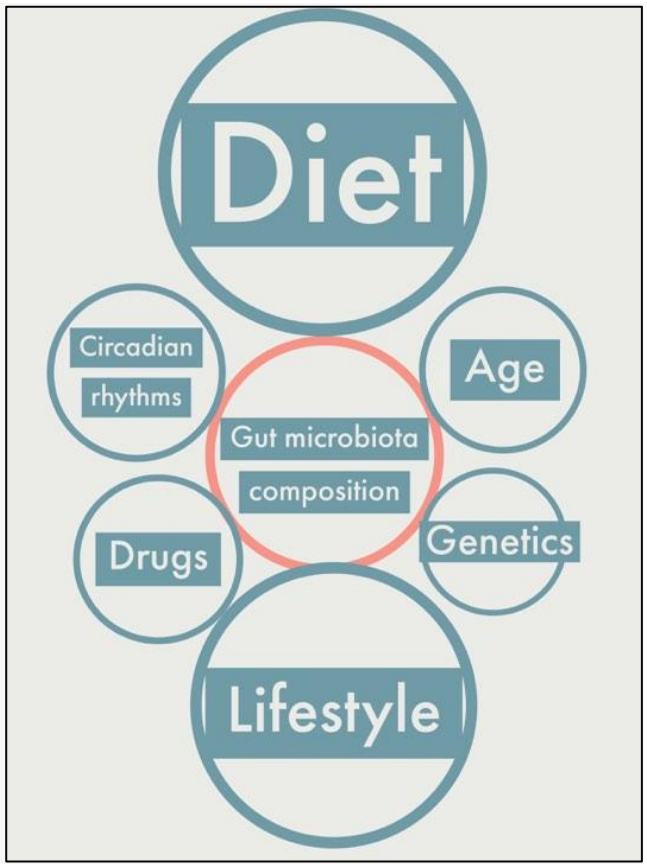



*Figure 2: Main factors affecting the gut microbiota composition highlighting the great influence of diet and lifestyle on the composition*

**Lifestyle** - Many everyday activities can affect the gut microbiota. For example, the microbiota on the skin and in the intestine are similar between family members, especially if they have a pet, which is some vector transferring microbes between family members. Also, travelling in different parts of the World derives variation in the gut microbiota, very similar to those of the domestic people.

**Circadian rhythms** - Period of the day, ambient of lights and temperature and food availability affects the human physiological processes and the human gut microbiota. Some oscillations in the microbiota in people, who work in shifts or work hard, can because of some metabolic disorders.

## Discussion

### *Dysbiosis*

Dysbiosis is a condition, determined by quantitative and/or qualitative (functional) reduction of the composition of microbiota [12]. Dysbiosis has two main characteristics:

- Reduced diversity of microbial community against healthy individuals;

- Some degree of the inflammatory state, despite the primary process of the disease/condition.

Many daily activities, such as feeding style, hygiene habits, physical activity, medications, host genetics, living region, etc., probably cause dysbiosis.

### *Obesity*

Malnutrition encompasses obesity and undernutrition are characterised by an imbalance between energy input and output. In 2014, assessments were about more than 600 million obese people, and more than one billion suffering from undernutrition [1]. The epidemic of obesity has spread in more than 1/3 of the elderly population, and the average costs for obesity treatment complications are 1429 dollars higher than costs for slim people [2]. Data from National Health and Nutrition Examination Surveys point than 64% from elderly Americans (>20 years old) was with BMI (Body Mass Index) higher than 25 between the years 1999-2000 [13].

Obesity is a condition of the excess of body fat and scarcely ever is a condition of higher body mass, because the body mass can be increased at people with higher muscle volume or women who are pregnant. Obesity-associated diseases, like atherosclerosis, diabetes, non-alcoholic liver disease and some kinds of cancer are the leading causes of death in the USA and the World [2]. Also, the distribution of body fat influences morbidity. For example, intra-abdominal and abdominal fatty tissue is more implicated in the initiation of obesity complications than the subcutaneous fatty tissue on the thigh and arm.

### *Appetite control and energy balance*

Body weight is controlled by endocrine and neural mechanisms, which influence the energy input and output. This complex regulatory system is essential because even small balance disorders lead to big bodyweight disturbances. By the weight loss, the appetite is getting up, and consumption of energy decreases, but this mechanism does not work very often.

The appetite is controlled by many factors, which are integrated into the central nervous system (CNS), rather the hypothalamus. Signals, which arrive, to the hypothalamus include afferent neural, hormone and metabolic signals. Neural signals are derived by the Vagus nerve, which is produced by the distension of the digestive system walls. Hormone signals include leptin, insulin, cortisol, ghrelin, peptide YY (PYY), cholecystokinin and some peptides from the digestive system. Also, a lot of physiological and culturally factors play a role in appetite control (Figure 3).

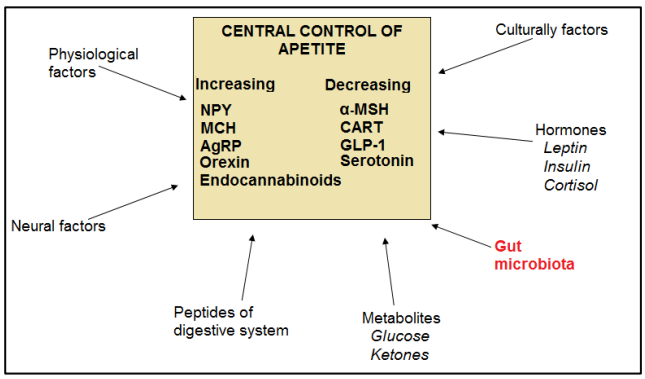



*Figure 3: Factors that regulate appetite through effects on CNS. (NPY-neuropeptide Y; MCHmelanin-concentrating hormone; AgRP-agouti-related transcript; MSH-melanocyte stimulating hormone; CART-cocaine and amphetamine-related transcript; GLP-1-glucagon-like peptide; CCK-cholecystokinin; PYY-peptide YY)*

### *Gut microbiota and appetite control*

Gut microbiota is included in the regulation of food intake by the influence to the hormones, which regulate the metabolic function and some brain regions responsible for the food intake, also defined as gut-brain axis [1].

The brain constantly comes up to different neural and hormone signals, which monitor the energy level in the body. Most of these signals are evolved in the gut and are part of the gut-brain axis. The axis is affected by the feeding style, genetics, anatomy of the gut and the gut microbiota. The food intake influences the releasement of some hormones and makes sense of fullness and appetite decrease. Distension of the stomach evolves Vagus nerve afferent signal to the brain (rhomb encephalon) and release of hormones from the stomach mucosa, such as CCK, GLP-1, PYY and leptin. All of these neural and hormone factors influence the gut-brain axis affecting the hypothalamic arcuate nucleus (Figure 4).

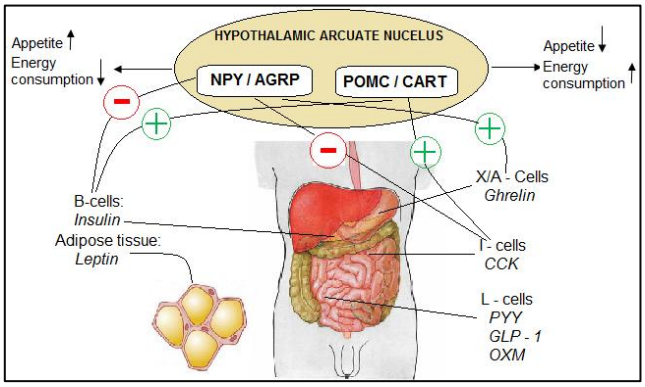



*Figure 4: Central hormonal control of appetite and energy expenditure. (NPY-neuropeptide Y; AgRP-agouti-related peptide; POMC-proopiomelanocortin; CART-cocaine and amphetamine-related peptide; CCK-cholecystokinin; GLP-1-glucagon-like peptide; OXM-oxyntomodulin)*

The hypothalamus plays the central role in the energy homeostasis by influencing appetite and energy consumption. Two different groups of neurons are responsible for the interpretation of the peripheral signals. Appetite suppressing neurons (anorexigenic), proopiomelanocortin (POMC) and cocaine and amphetamine-regulated transcript (CART) are located in the lateral part of the hypothalamic arcuate nucleus (Fig. 4) which expresses α-melanocyte stimulating hormone. Melanocortin and α-melanocyte stimulation hormone induce a negative energy balance. These peptides are synthesised because of the increase in fatty tissue. In the medial part of the hypothalamic arcuate nucleus are located the orexigenic neurons which express the neuropeptide Y (NPY) and agouti-related protein (AGRP). NPY and AGRP are the main central neurotransmitters, which stimulate appetite and decrease energy consumption.

The gut microbiota composition and dysbiosis influence on the hormone orchestration and indirectly affect the appetite. Breton et al., [20] studied how the intestinal infusion of *Escherichia coli* proteins in mice affects the increase of plasmatic levels of GLP-1 and PYY. They found that the changes in the gut microbiota (dysbiosis) induced changes in the plasmatic levels of GLP-1 and PYY, which are hormones that affect the hypothalamic arcuate nucleus and the appetite.

Queipo-Ortuño et al., [21] studied the association between some genus from the gut microbiota and the plasmatic levels of ghrelin and leptin. They noticed that there is a significant positive correlation between the number of *Bifidobacterium* and *Lactobacillus* and a significant negative correlation between the genus *Clostridium*, *Bacteroides* and *Prevotella* with the plasmatic levels of leptin. Also, they noticed that the plasmatic levels of ghrelin are negatively correlated with the genus *Bifidobacterium*, *Lactobacillus* and *B. coccoides* and

positively correlated with *Bacteroides* and *Prevotella* (Table 2).

**Table 2: Relations between plasmatic levels of leptin and ghrelin with gut microbiota composition**

| | |
|---|---|
| ↑Leptin | ↑*Lactobacillus* and *Bifidobacterium* |
| | ↓*Prevotella* and *Clostridium* |
| ↑Ghrelin | ↑*Prevotella* and *Bacteroides* |
| | ↓*Lactobacillus* and *Bifidobacterium* |

All these acknowledgements indicate that the differences in feeding style and physical activity influence the gut microbiota composition, affecting its diversity. Generally, the food restriction and increase of physical activity have a potentially negative influence on the number and composition of the intestinal bacteria, which are health promoters.

Generally, there is a trend of eating high-calorie foods, which affect the gut microbiota. In a way, the gut microbiota is described as an environmental factor, which activity results with an increased depot of adipose tissue and obesity [1].

In the past fifteen years, many scientists were exploring the association of the gut microbiota and some diseases, and the possibility for treatment affecting the gut microbiota. Conclusions from these studies resulted in inconsistent findings of the association between gut microbiota and obesity. The association was studied mainly on animal models. GFM (germ-free mice) are resistant to high-calorie food and high food intake, which by science are the main promoters of obesity. Additionally, colonisation of GFM with "obese gut microbiota" increased the fatty tissue and body weight, in contrast to colonisation with "slim gut microbiota" [14].

Contact between microbes and the intestinal epithelial cells determine which signals are good and which are bad. Enhanced pilling up to energy by the "obese gut microbiota" is the most common explanation for obesity. Reduced number of the members of the division *Bacteroidetes* and proportionally increased number of the members of the division *Firmicutes*, which are associated with a bigger capacity to supply energy from food, such as degradation and fermentation to complex carbohydrates [15], characterise “obese gut microbiota”.

The "obese gut microbiota" has a bigger capacity to derive energy from the food, and to stimulate gene reprogramming in the large intestine, to change the secretion of polypeptide hormones and other bioactive molecules from enteroendocrine cells and too weak the defence and immunologic homeostasis in the intestine. The gut microbiota also communicates with the adipose tissue, the liver and the brain [16], (Figure 5).

***Axis gut microbiota-adipose tissue:*** The gut microbiota is included in the regulation of adipose tissue through different mechanisms. LPS (lipopolysaccharide) incites immune response with inflammation and immune cells penetration in the adipose tissue. SCFAs (short-chain fatty acids) are included in insulin-related fat accumulation in the adipose tissue through activation of GPR43 and GPR41, which inhibit the lipolysis and enhance the differentiation of the adipose cells.

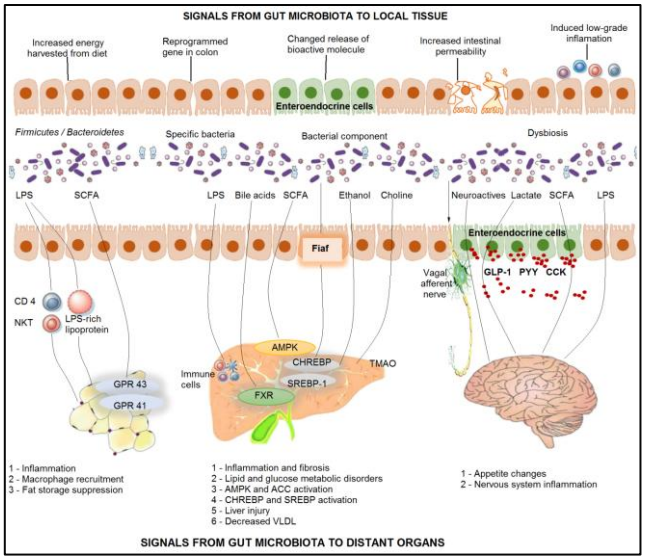



*Figure 5: Local and distant effects of gut microbiota in the pathogenesis of obesity [16]. (LPS-lipopolysaccharide; SCFAsshort-chain fatty acids; Fiaf-fasting-induced adiposity factor; NKT-natural killer cell; GPR 43/41-G-protein-related receptor; AMPKAMP-activated protein kinase; CHREBP-carbohydrate related element-binding protein; SREBP-1-sterol regulating element-binding protein; FXR-farnesoid X receptor; TMAO-trimethylamine N-oxide; GLP-1-glucagon-like peptide; PYY-peptide YY; CCK-cholecystokinin)*

***Axis gut microbiota-liver:*** Dysbiotic microbiota induces increased penetration at the intestinal mucosa for some pathogens and parts or metabolites from intestinal bacteria, such as LPS and ethanol. In the liver, LPS induces inflammation through stimulation the immune cells. Some metabolites as the bile acids, SCFAs and trimethylamine-N-oxide (TMAO) play a role in the pathogenesis of the non-alcoholic fatty liver disease (NAFLD).

***Axis gut microbiota-brain:*** Afferent neurons from the digestive system and intestinal hormones are key signal molecules included in the communication between gut and brain and the metabolism of the host. Bioactive molecules which are included in the process are LPS, intestinal peptides, SCFAs, lactates etc. [16], (Figure 5).

Fiaf (Fasting-induced adipose factor) is a circulating inhibitor to the lipoprotein lipase, which is normally suppressed by the gut microbiota in the intestinal epithelium, and play a central role in the metabolism of triglycerides through inhibition the lipoprotein lipase production in the adipose tissue [3]. From this, it can be concluded that GFM are protected from obesity because of increased levels of Fiaf and

increased activity of AMPK (AMP-activated protein kinase).

Polypeptide hormones and other bioactive molecules released from enteroendocrine intestinal cells are included in the food intake regulation. Different TLRs (Toll-like receptors) expressed on enteroendocrine cells recognise different PAMPs (Pathogen-associated molecular patterns) and influence the polypeptide hormones release. For example, LPS from Gram-negative bacteria is recognised by TLR4 and induce secretion of CCK (satiety hormone) [16], which decrease the appetite and increases energy consumption [1].

Injured intestinal barrier and penetration of bacteria or bacterial products through the barrier is an important mechanism to the pathogenesis of obesity. Some bacterial products play a role in the regulation of the intestinal barrier, associated with SCFAs. Also, obesity is related to mild chronic inflammation, and some antigens from the gut may be inducers of this activity. Dysbiosis affects the innate and adaptive immunity through some microbe cell components and metabolic signals.

Besides the local influence, the gut microbiota influences distant organs, mainly the adipose tissue. Obesity is characterised by an increase of the volume of adipose tissue, and the gut microbiota enhances the mechanisms of metabolic disorders in the axis gut microbiota-adipose tissue. For example, LPS is identified as an inducing factor to the insulin resistance of the adipose tissue, which is transported to the adipose cells together with other lipoproteins through translocases. LPS-reach lipoproteins are absorbed by the bigger adipose cells, which has high metabolic activity. SCFAs synthesised by the gut microbiota participate in the insulin-mediated accumulation of lipids in the adipose cells, through activation of the SCFA-receptors (GPR43 and GPR41) which inhibits the lipolysis and induces differentiation of adipose cells. Mice with GPR43 deficiency are obese besides the normal feeding, while the mice, which has a high expression of GPR43, are lean, besides feeding with high-calorie foods. GPR43 activation by SCFAs suppresses insulin signals to the adipose cells, which enhances the metabolism of lipids and carbohydrates in the other tissues. Suspect that GPR43 is a sensor for excessively receiving energy from the foods and keep the metabolic homeostasis [18]. A conclusion may be that GPR43 activation causes insulin resistance in the adipose tissue, but increased sensitivity in the muscles and the liver.

The liver is constantly exposed to signals by the digestive system, gut microbes and their particles. Alterations in the gut microbiota composition are firmly connected with risk for disorders in the liver associated with obesity, such as non-alcoholic fatty liver disease (NAFLD) [4]. The NAFLD severity is associated with the degree of dysbiotic gut microbiota, especially with the number of *Bacteroidetes* related to NAFLD and *Ruminococcus* related to liver fibrosis [19].

Gut microbiota has a deep influence on the metabolism of bile acids through deconjugation, dehydrogenation and dihydroxylation of primary bile acids. Alteration in the gut microbiota gets changes in the bile-acid pool, which influences the nuclear antagonist of bile-acid X receptor (FXR) included in the regulation of bile acids, as the regulation of lipid and carbohydrate metabolism and may induce metabolic dysfunction, obesity and insulin resistance.

Also, Fiaf is included in the mechanisms which relate the gut microbiota and NAFLD, where the dysbiotic gut microbiota inhibits secretion of Fiaf from the intestinal cells and activates LPL, CHREBP and SREBP-1 and resulted in accumulation of triglycerides in the liver [3]. Ethanol and other bacterial products included in the NAFLD progression may be related to a high amount of alcohol producing bacteria from the *Proteobacteria* genus.

Gut microbiota has impinged upon distant organs and CNS, which recipes the abundance of neural and hormone signals from the digestive system. Bacteria and their metabolites may affect directly through vagus nerve stimulation or indirectly through immune-neuroendocrine mechanisms. Vagus nerve activation particularly is depended from the secretion of signal molecules as PYY, GLP- 1 and CCK. SCFAs is not just a source of energy, but also plays a role as a chemical transmitter [18].

## Conclusion

Gut microbiota directly affects the metabolism of nutrients and vitamins, essential for the host organism. It is noted that the gut microbiota of obese people has a bigger capacity for getting energy from the food than the gut microbiota of lean people and the division *Firmicutes* is more effective in that. The gut microbiota is included in the control of appetite through hormones, which influence the metabolism and some parts of the brain responsible for the eating behaviour. Feeding style and physical activity affect the gut microbiota composition and its diversity - lower diversity relates to obesity. "Obese gut microbiota" has a lower number of members of the division *Bacteroidetes* and a high number of member of the division *Firmicutes*. Diet has maybe the biggest influence on the gut microbes, which is known through observation of the composition of microbiota after permanently taking some food. Undoubtedly, it may be concluded that the gut microbiota has a big influence in the pathogenesis of obesity and other obesity-related disorders.

## Acknowledgements

The paper was awarded the Best Poster Presentation at the 2nd Students Congress of General Medicine, May 9-10, 2019, Shtip, the Republic of Macedonia by the Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia.

## References

1. De Clercq NC, Groen AK, Romijn JA, Nieuwdorp M. Gut Microbiota in Obesity and Undernutrition. The Advances in Nutrition journal. 2016; 15; 7(6):1080-1089. https://doi.org/10.3945/an.116.012914 PMid:28140325 PMCid:PMC5105041

2. Parekh PJ, Arusi E, Vinik AI, Johnson DA. The role and influence of gut microbiota in pathogenesis and management of obesity and metabolic syndrome. The Frontiers in endocrinology journal. 2014; 5:47. https://doi.org/10.3389/fendo.2014.00047 PMid:24778627 PMCid:PMC3984999

3. Bäckhed F, Manchester JK, Semenkovich CF, Gordon JI. Mechanisms underlying the resistance to diet induced obesity in germ-free mice.Proceedings of the National Academy of Sciences of the United States of America. 2007; 104(3):979-84. https://doi.org/10.1073/pnas.0605374104 PMid:17210919 PMCid:PMC1764762

4. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006; 444(7122):1027-31. https://doi.org/10.1038/nature05414 PMid:17183312

5. Ridaura VK, Faith JJ, Rey FE, Cheng J, Duncan AE, Kau AL, Griffin NW, Lombard V, Henrissat B, Bain JR, Muehlbauer MJ, Ilkayeva O, Semekovich CF, Funai K, Hayashi DK, Lyle BJ, Martini MC, Ursell LK, Clemente JC, Van Treuren W, Walters WA, Knight R, Newgard CB, Heath AC, Gordon JI. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science. 2013; 341(6150):1241214. https://doi.org/10.1126/science.1241214 PMid:24009397 PMCid:PMC3829625

6. Aagard K, Petrosino J, Keitel W, Watson M, Katancik J, Garcia N, Patel S, Cutting M, Madden T, Hamilton H, Harris E. The Human Microbiome project strategy for comprehensive sampling of the human microbiome and why it matters. The FASEB Journal. 2013; 27(3): 1012-22. https://doi.org/10.1096/fj.12-220806 PMid:23165986 PMCid:PMC3574278

7. Huttenhower C. et collaborators. Structure, function and diversity of the healthy human microbiome. Nature. 2012; 486(7402):207-14. https://doi.org/10.1038/nature11234 PMid:22699609 PMCid:PMC3564958

8. Methe BA, et collaborators. A framework for human microbiome research. Nature. 2012; 486(7402):215-21. https://doi.org/10.1038/nature11209 PMid:22699610 PMCid:PMC3377744

9. Hollister EB, Gao C, Versalovic J. Compositional and functional features of the gastrointestinal microbiome and their effects on human health. The Gastroenterology journal. 2014; 146(6):1449-58. https://doi.org/10.1053/j.gastro.2014.01.052 PMid:24486050 PMCid:PMC4181834

10. Andersson AF, Lindberg M, Jakobsson H, Bäckhed F, Nyrèn P, Engstrand L. Comparative analysis of human gut microbiota by barcoded pyrosequencing. The PloS One Journal. 2008; 3(7):e2836. https://doi.org/10.1371/journal.pone.0002836 PMid:18665274 PMCid:PMC2475661

11. Jameson JL, Fauci AS, Kasper DL, Hauser SL, Longo DL, Loscalzo J.Harrison's principles of Internal medicine 20th edition. The Human Microibiome: McGraw-Hill Education, 2018: 3379-3390.

12. Passos MDCF & Moraes-Filho JP. Intestinal microbiota in digestive diseases. The Arquivos de gastroenterologia. 2017; 54(3):255-262. https://doi.org/10.1590/s0004-2803.201700000-31 PMid:28723981

13. Fauci AS, Braunwald E, Kasper DL, Stephen LH, Longo DL, Jameson JL, Loscalzo J. Harrison's principles of internal medicine 17th edition: Biology of obesity. McGrawHill Medical. 2008; 462-468.

14. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, Egholm M, Henrissat B, Heath AC, Knight R, Gordon JI. A core gut microbiome in obese and lean twins. Nature. 2009; 457(7228):480-4. https://doi.org/10.1038/nature07540 PMid:19043404 PMCid:PMC2677729

15. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. Nature. 2006; 444(7122):1022-3. https://doi.org/10.1038/4441022a PMid:17183309

16. Sun L, Ma L, Ma Y, Zhang F, Zhao C, Nie Y. Insights into the role of gut microbiota in obesity: pathogenesis, mechanisms and therapeutic perspectives. The Proetin&Cell journal. 2018; 9(5):397-403. https://doi.org/10.1007/s13238-018-0546-3 PMid:29725936 PMCid:PMC5960470

17. Kimura I, Ozawa K, Inoue D, Imamura T, Kimura K, Maeda T, Terasawa K, Kashihara D, Hirano K, Tani T, Takahashi T, Miyauchi S, Shioi G, Inoue H, Tsujimoto G. The gut microbiota suppresses insulin-mediated fat accumulation via short-chain fatty acid receptor GPR43. The Nature communications journal. 2013; 4:1829. https://doi.org/10.1038/ncomms2852 PMid:23652017 PMCid:PMC3674247

18. Nohr MK, Pedersen MH, Gille A, Egerol KL, Engelstoft MS, Husted AS, Sichlau RM, Grunddal KV, Poulsen SS, Han S, Jones RM, Offermanns S, Schwartz TW.GPR41/FFAR3 and GPR43/FFAR2 as cosensors for short-chain fatty acids in enteroendocrine cells vs FFAR3 in enteric neurons and FFAR2 in enteric leukocytes. The Endocrinology journal. 2013; 154(10):3552-64. https://doi.org/10.1210/en.2013-1142 PMid:23885020

19. Boursier J, Mueller O, Barret M, Machado M, Fizanne L, Araujo-Perez F, Guy CD, Seed PC, Rawls JF, David LA, Huanault G, Oberti F, Calès P, Diehl AM. The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota. The Hepatology journal. 2016; 63(3):764-75. https://doi.org/10.1002/hep.28356 PMid:26600078 PMCid:PMC4975935

20. Breton J, Tennoune N, Lucas N, Francois M, Legrand R, Jacquemont J, Goichon A, Guèrin C, Peltier J, Pestel-Caron M, Chan P, Vaudry D, do Rego JC, Lienard F, Pènicaud L, Fioarmonti X, Ebenezer IS, Hökfelt T, Dèchelotte P, Fetissov SO.Gut Commensal E.coli Proteins Activate Host Satiety Pathways following Nutrient-Induced Bacterial Growth.The Cell Metabolism journal. 2016; 23(2):324-34. https://doi.org/10.1016/j.cmet.2015.10.017 PMid:26621107

21. Queipo-Ortun˜o MI, Seoane LM, Murri M, Pardo M, Gomez-Zumaquero JM, Cardona F, Casanueva F, Tinahones FJ. Gut microbiota composition in male rat models under different nutritional status and physical activity and its association with serum leptin and ghrelin levels. The PloS one journal. 2013; 8(5):e65465. https://doi.org/10.1371/journal.pone.0065465 PMid:23724144 PMCid:PMC3665787

22. Blaser MJ. Hypothesis: the changing relationship of Helicobacter pylori and humans: implications for health and disease. The journal of infectious diseases. 1999; 179(6):1523-30. https://doi.org/10.1086/314785 PMid:10228075

23. Cho I, Yamanishi S, Cox L, Methè BA, Zavadil J, Li K, Gao Z, Mahana D, Raju K, Teitler I, Li H, Alekseyenko AV, Blaser MJ. Antibiotics in early life alter the murine colonic microbiome and adiposity. Nature. 2012; 488(7413):621-6. https://doi.org/10.1038/nature11400 PMid:22914093 PMCid:PMC3553221

24. Neish AS. Microbes in gastrointestinal health and disease. The Gastroenterology journal. 2009; 136(1):65-80. https://doi.org/10.1053/j.gastro.2008.10.080 PMid:19026645 PMCid:PMC2892787

25. Vulevic J, Juris A, Tzortzis G, Gibson GR. A mixture of trans-galactooligosaccharides reduces markers of metabolic syndrome and modulates the fecal microbiota and immune function of overweight adults. The Journal of nutrition. 2013; 143(3):324-31. https://doi.org/10.3945/jn.112.166132 PMid:23303873