ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Apr 15; 7(7):1053-1058.
https://doi.org/10.3889/oamjms.2019.242
eISSN: 1857-9655
Basic Science

Efficacy of Probiotic Therapy on Atopic Dermatitis in Adults Depends on the C-159T Polymorphism of the *CD14* Receptor Gene - A Pilot Study

Oleksandr Litus, Nadiya Derkach, Viktor Litus, Yuriy Bisyuk, Bohdan Lytvynenko*

Shupyk National Medical Academy of Postgraduate Education, Kiev, Ukraine

Citation: Litus O, Derkach N, Litus V, Bisyuk Y, Lytvynenko B. Efficacy of Probiotic Therapy on Atopic Dermatitis in Adults Depends on the *C-159T* Polymorphism of the *CD14* Receptor Gene - A Pilot Study. Open Access Maced J Med Sci. 2019 Apr 15; 7(7):1053-1058. https://doi.org/10.3889/oamjms.2019.242

Keywords: Atopic dermatitis; Probiotics; C-159T polymorphism; Cytokines

***Correspondence:** Bohdan Lytvynenko. Shupyk National Medical Academy of Postgraduate Education, Kiev, Ukraine. E-mail: libovi@gmail.com

Received: 14-Feb-2019; **Revised:** 22-Mar-2019; **Accepted:** 23-Mar-2019; **Online first:** 10-Apr-2019

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: The C-159T polymorphism of the *CD14* receptor gene can be associated with the development of atopic dermatitis. Probiotics can modulate chronic inflammation through activation of the CD14 receptor. So, the efficacy of probiotic therapy can be dependent on this genetic polymorphism.

AIM: The purpose of the study was to investigate the efficacy of adding probiotic (*Lactobacillus acidophilus, LA-5 and Bifidobacterium animalis subsp. lactis, BB-12*) to standard treatment (ointment of fluticasone propionate 0.005% and emollient) of atopic dermatitis in adults during 28 days, depending on the stratification of patients on CC or TT genotypes of the *CD14* receptor gene.

MATERIAL AND METHODS: The study included 37 adult patients with AD. There were identified 19 patients with exogenous (IgE-dependent) and 18 with endogenous (IgE-dependent) AD. To evaluate the efficacy of the probiotics all patients were divided into three groups for both exogenous and endogenous AD. The first group was selected from patients with CC genotype (C-159T) who received standard therapy (ointment of fluticasone propionate 0.005% – 2 times a day, emollients – 2 times a day) and probiotic (*Lactobacillus acidophilus*, LA-5 and *Bifidobacterium animalis subsp. lactis*, BB-12 - 1 capsule 2 times per day) The second group included patients with CC genotype, who received only standard therapy. The third group was presented by patients with TT genotype (C-159T) who received standard therapy and probiotic. The SCORAD and DLQI parameters were evaluated on Day 0, 14 and 28. The level of IL-4, IL-5, IL-10, TGF-β cytokines was determined on Day 0 and Day 28.

RESULTS: The results of our study found that the addition of probiotics (*Lactobacillus acidophilus*, LA-5 and *Bifidobacterium animalis subsp. lactis,* BB-12) to standard treatment (ointment of fluticasone propionate 0.005%, emollient) significantly increased the effectiveness of treatment of atopic dermatitis in adults with exogenous form and CC genotype (C-159T), confirmed by clinical (a significant decrease of SCORAD and DLQI indices) and immunological criteria (a significant decrease of IL-4 and an increase of TGF-β).

CONCLUSION: Simultaneous determination of the exogenous or endogenous form, identification of the C-159T genotypes, evaluation of the serum level of IL-4 and TGF-β can serve as an algorithm for the personalised treatment of patients with atopic dermatitis.

Introduction

To date, there are practically no drugs for the treatment of atopic dermatitis (AD) that can modify chronic inflammation or activate natural suppressor immune mechanisms, especially by activating regulatory T-lymphocytes. However, probiotics themselves may have such properties.

Probiotics are living microorganisms that can be of benefit to health when administered in adequate doses [1]. The most commonly used are *Lactobacillus, Bifidobacterium, Enterococcus, Propionibacterium* and some yeasts such as *Saccharomyces boulardii*. Their benefit is proven in the treatment of diarrhoea after taking antibiotics, irritable bowel syndrome and inflammatory bowel disease [2]. Genetic polymorphism and the effect of microorganisms on the immune system are closely related to the modulation of skin inflammation in AD, including the activation of the CD14/TLR-4 receptor complex by endotoxin of gram-negative bacteria.

The *CD14* receptor gene is located on chromosome 5q31.1, has two exons and 3900

nucleotides [3]. In the same locus, there are genes responsible for the synthesis of IgE. There are many studies of the C-159T polymorphism (rs2569190) of the *CD14* receptor gene in atopic patients [4]. For this polymorphism, cytosine (C) is replaced by thymine (T) at position 159 of the promoter region, resulting in the population presence of homozygotes of cytosine and thymine (CC, TT) and heterozygotes cytosine-thymine (CT) [3]

This polymorphism can affect the development of various diseases in different ways. The risk of nasal allergies and atopy was the most reduced in the subjects who combined both an early-life exposure to a farming environment and the -159TT genotype [5]. Although children with C/C variant of *CD14* C-159T had a significantly lower prevalence of croup [6].

It has been shown that the number of positive skin tests was significantly higher in patients with CC genotype compared with TT [3]. In the Netherlands, it was found that in patients with positive skin tests, the level of total IgE was significantly ($p < 0.05$) higher in CC compared to TT genotype [7]. In Australia, it was found that the risk of atopy in children is significantly higher in CC genotype (OR = 2.0, P = 0.04) [8]. In China, one study has shown that atopic subjects with CC genotype had the highest serum total IgE levels compared with CT and TT genotypes [9]. Another research has shown that TT homozygotes are more common in adult patients with allergic rhinitis among the Chinese population and the C-159T polymorphism was not associated with serum IgE levels [10]. Other studies indicate that C-159T gene polymorphism can be associated with elevated levels of soluble CD14 [11], [12].

In turn, probiotics have antagonistic properties regarding activation mechanisms of inflammation, including endotoxin-dependent ones. So, probiotics stimulate regulatory T-lymphocytes, increase the synthesis of IF-β and TGF-β, inhibit the function of T-helper type 2, reduce the secretion of TNF-α and eosinophilic cationic protein, reduce the concentration of total and specific IgE, reduce colonisation of the skin by *Staphylococcus aureus* and restore its barrier function [13].

Identifying phenotypes and genotypes, on the one hand, and potential biomarkers on the other, are vital elements for the successful development of new and personalised therapeutic approaches in patients with AD [14].

The purpose of the study was to investigate the efficacy of adding probiotic (*Lactobacillus acidophilus, LA-5 and Bifidobacterium animalis* subsp. *lactis, BB-12*) to standard treatment (ointment of fluticasone propionate 0.005% and emollient) of atopic dermatitis in adults during 28 days, depending on the stratification of patients on CC or TT genotypes of the CD14 receptor gene.

Material and Methods

The study included 37 adult patients with AD. For the diagnosis of atopic dermatitis, we used the recommendations provided by the European Academy of Dermatology and Venereology [15].

All patients with atopic dermatitis, depending on the level of total IgE and at least one allergen's positivity by skin prick tests, were divided into two main groups: exogenous or IgE-dependent AD (total IgE level greater than 100 IU/ml) and endogenous IgE-independent AD (total IgE level less than 100 IU/ml). The level of total IgE was determined by ELISA method.

There were identified 19 patients with exogenous (IgE-dependent) and 18 with endogenous (IgE-dependent) AD.

Allele-specific PCR with electrophoretic detection was used to access gene polymorphisms of the CD14 receptor (C-159T, rs2569190). Allele-specific PCR was performed using kit: "Mutation of monocyte differentiation antigen (CD14) C-159T" (Lytech, Russia) according to the manufacturer's instructions.

The level of IL-4, IL-5, IL-10, TGF-β in serum was determined by ELISA method.

Determination of the severity of the disease was carried out using SCORAD (SCORing Atopic Dermatitis) index [16]. The quality of life of the patients was assessed using the DLQI questionnaire (Dermatitis Quality of Life Index) [17].

The clinical trial was conducted as open, controlled, randomised, in 6 parallel groups during 28 days at the departments of dermatology and clinical, laboratory immunology and Allergology of the Shupyk National Medical Academy of Postgraduate Education.

To study the efficacy of the probiotics (*Lactobacillus acidophilus, LA-5 and Bifidobacterium animalis* subsp. *lactis, BB-12*) on the severity of the disease, quality of life and immune parameters, genotypes of CC and TT, all patients were divided into three groups for both exogenous and endogenous AD. The first groups were selected from patients with CC genotype (C-159T) who received standard therapy (ointment of fluticasone propionate 0.005% – 2 times a day, emollients – 2 times a day) and probiotic (*Lactobacillus acidophilus, LA-5 and Bifidobacterium animalis* subsp. *lactis, BB-12* - 1 capsule 2 times per day) The second group included patients with CC genotype, who received only standard therapy. The third group was presented by patients with TT genotype (C-159T) who received standard therapy and probiotic. The SCORAD and DLQI parameters were evaluated on Day 0, 14 and Day 28. The level of IL-4, IL-5, IL-10, TGF-β cytokines was determined on

Day 0 and Day 28.

All results were analysed using "Minitab 16" statistical software. In the analyses, the normality test was done using the Kolmogorov-Smirnov test. The comparison of central tendencies of two independent samples was performed using the U-Mann-Whitney test. Comparison of the average of two independent samples used Student's criterion for non-normally and normally distributed samples, respectively. Quantitative variables are presented as mean values and standard deviation (SD) or 95% confidence intervals for normally distributed data, and the median with first (Q1) and third (Q3) quartile or 95% confidence intervals for non-normally distributed data. For multiple comparisons, the Kruskal-Wallis test and ANOVA (Bonferroni and Sheffe correction) were used.

All study subjects provided written informed consent to participate in this research. Ethics approval was received from the Bioethics Committee of the Shupyk National Medical Academy of Postgraduate Education, Kyiv, Ukraine.

Results

The average age of patients with exogenous AD (28.32 ± 11.70 years) did not significantly differ (p = 0.520) from endogenous (29.11 ± 9.99 years) one. Groups did not differ significantly (P = 0.851) by gender (exogenous AD, male to male ratio 11/8; endogenous AD, female/male ratio – 10/8). The duration of the disease (exogenous AD – 17.68 ± 6.39 years, endogenous AD – 16.44 ± 7.04, p = 0.676) and the number of exacerbations in the last year (exogenous AD – 3.00 ± 0.94, endogenous AD – 2.78 ± 1.06, p = 0.625) also did not significantly differ.

The assessment of the SCORAD index on Day 0, 14 and 28 are provided in Table 1.

Table 1: The dynamics of SCORAD scores in patients that underwent the different treatments

Groups		Day 0	Day 14	Day 28	P1
Exogenous AD	CC genotype, standard therapy + probiotics, n = 7	12.00 (10.50-21.52)	6.00 (2.50-10.02)#	3.00 (2.00-5.51)#	0.001
	CC genotype, standard therapy, n = 6	12.50 (8.19-18.61)	7.50 (6.19-16.61)	5.50 (4.19-14.81)*	0.121
	TT genotype, standard therapy + probiotics, n = 6	14.00 (10.19-22.42)	8.50 (6.39-14.81)	6.50 (4.19-11.23)* #	0.015
	P2	0.786	0.132	0.022	
Endogenous AD	CC genotype, standard therapy + probiotics, n = 6	14.50 (9.19-21.42)	9.00 (6.27-12.73)#	6.00 (4.19-7.81)#	0.006
	CC genotype, standard therapy, n = 6	14.00 (11.19-20.61)	11.00 (8.39-16.03)	9.50 (7.19-13.42)*#	0.039
	TT genotype, standard therapy + probiotics, n = 6	16.00 (10.00-23.42)	11.00 (7.39-13.61)	8.50 (6.19-9.81)*#	0.011
	P2	0.891	0.558	0.020	

Note: P1 – reliability in multiple comparison with the use of the Kruskal-Wallis test in the group on Day 0, 14 and 28; P2 – reliability in multiple comparisons by using the Kruskal-Wallis test between groups; * – significance of differences between the group with the CC genotype (standard therapy + probiotics) from the groups with the CC genotype (standard therapy) and TT genotype (standard therapy + probiotics), p < 0.05; # – significance of differences between groups on Day 0 compared with 14 and 28, p < 0.05; Me – median, 95% CI – 95% confidence interval of the median.

There was estimated that standard treatment with probiotics during 28 days significantly reduced the SCORAD index in exogenous AD patients with CC and TT genotypes (Table 1). Patients with CC genotype who received standard therapy with probiotics had the most significant reduction of SCORAD index on Day 28.

Table 2: The dynamics of DLQI scores in patients that underwent the different treatments

Groups		Day 0	Day 14	Day 28	P1
Exogenous AD	CC genotype, standard therapy + probiotics, n = 7	10.00 (7.50-15.53)	7.00 (4.50-10.01)#	3.00 (2.00-3.50)#	0.001
	CC genotype, standard therapy, n = 6	9.50 (7.19-19.06)	6.50 (5.19-15.25)	4.50 (3.19-12.45)*#	0.037
	TT genotype, standard therapy + probiotics, n = 6	12.50 (6.19-21.42)	9.50 (4.19-14.81)	6,00 (3.19-11.61)*	0.150
	P2	0.841	0.715	0.012	
Endogenous AD	CC genotype, standard therapy + probiotics, n = 6	12.50 (6.39-19.42)	8,50 (5.00-13.42)	5,50 (3.19-8.42)#	0.034
	CC genotype, standard therapy, n = 6	11.00 (7.39-17.42)	7.00 (4.19-11.81)	4.50 (3.19-8.81)#	0.027
	TT genotype, standard therapy + probiotics, n = 6	13.50 (8.19-19.42)	10.00 (6.19-13.81	6.00 (4.19-9.81)#	0.031
	P2	0.816	0.442	0.544	

Note: same as in Table 1.

There was a significant decrease in SCORAD index (Table 1) in patients of all groups who had endogenous AD. It was significantly lower (p = 0.020) in patients with the CC genotype who received standard treatment with probiotics compared to other groups.

The next step was to analyse the quality of life of patients during treatment. The assessment of the DLQI index is provided in Table 1.

Table 3: The serum level of IL-4 (pg/ml) in patients that underwent different treatments

Groups		Day 0	Day 28	P1
Exogenous AD	CC genotype, standard therapy + probiotics, n = 7	41.70 (19.98-43.81)*	12.90 (11.50-18.43)	0.004
	CC genotype, standard therapy, n = 6	30.20 (24.34-42.51)*	24.15 (14.16-34.77)*	0.240
	TT genotype, standard therapy + probiotics, n = 6	9.80 (8.12-24.58)	9.85 (6.73-19.47)	0.699
	P2	0.014	0.010	
Endogenous AD	CC genotype, standard therapy + probiotics, n = 6	29.05 (18.87-42.11)*	23.00 (16.32-30.39)*	0.240
	CC genotype, standard therapy, n = 6	29.90 (25.13-39.56)*	24.20 (18.07-34.08)*	0.078
	TT genotype, standard therapy + probiotics, n = 6	16.35 (9.91-24.43)	12.90 (8.53-19.42)	0.310
	P2	0.017	0.023	

Note: P1 – comparison of central tendencies between 0 and day 28 using the Mann-Whitney U-test; P2 – reliability in multiple comparisons using the Kruskal-Wallis test between groups; * – significance of differences between groups with TT genotype (standard therapy + probiotics) from groups with CC genotype (standard therapy) and CC genotype (standard therapy + probiotics), p <0.05; # – significance of differences between the group with CC genotype (standard therapy + probiotics) from the group with the genotype CC (standard therapy), P <0.05.

For the exogenous form of AD (Table 2), there was a significant decrease in DLQI scores on Day 28 for patients in both treatment groups who had CC genotype. For patients with TT genotype, results did not reach the level of statistical significance (p = 0.150). On the contrary, for endogenous AD there was a significant decrease in DLQI score for all groups.

The lowest score was observed in patients with CC genotype (standard treatment + probiotics), which significantly differed (p = 0.012) from other groups in the exogenous form of AD.

Dynamics of the concentration of IL-4 is provided in Table 3.

On Day 0, the serum level of IL-4 (Table 3) in patients with TT genotype was significantly lower compared to CC genotype in both groups for exogenous and endogenous AD. On Day 28 (Table 3), there was a significant decreased (p = 0.004) of this cytokine only in patients with CC genotype and exogenous form of AD who received standard therapy with probiotics. For endogenous AD, no such significant changes have been observed.

Table 4: The serum level of IL-5 (pg/ml) in patients that underwent different treatments

Groups		Day 0	Day 28	P1
Exogenous AD	CC genotype, standard therapy + probiotics, n = 7	30.70 (23.18-43.16)*	23.10 (16.74-35.14)*	0.074
	CC genotype, standard therapy, n = 6	28.80 (20.68-44.06)*	24.50 (15.72-34.93)*	0.132
	TT genotype, standard therapy + probiotics, n = 6	20.50 (10.64-25.46)	13.85 (9.62-21.56)	0.240
	P2	0.023	0.048	
Endogenous AD	CC genotype, standard therapy + probiotics, n = 6	28.10 (20.59-45.97)*	24.65 (15.64-37.95)*	0.394
	CC genotype, standard therapy, n = 6	35.40 (29.87-48.62)*	29.85 (24.59-39.20)*	0.240
	TT genotype, standard therapy + probiotics, n = 6	15.80 (9.77-23.45)	12.40 (8.86-18.91)	0.379
	P2	0.003	0.004	

Note: same as in Table 3.

In patients with TT genotype, the level of IL-5 (Table 4) was significantly lower in comparison to other groups on Day 0 and on Day 28. During treatment, the concentration of IL-5 (Table 4) did not significantly change in any of the studied groups.

Table 5: The serum level of IL-10 (pg/ml) in patients that underwent different treatments

Groups		Day 0	Day 28	P1
Exogenous AD	CC genotype, standard therapy + probiotics, n = 7	15.40 (10.15-26.45)*	22.20 (13.65-30.20)*	0.210
	CC genotype, standard therapy, n = 6	19.25 (11.98-26.52)*	18.90 (14.69-32.67)*	0.589
	TT genotype, standard therapy + probiotics, n = 6	44.65 (39.99-56.23)	46.75 (37.01-62.68)	0.818
	P2	0.003	0.003	
Endogenous AD	CC genotype, standard therapy + probiotics, n = 6	25.70 (16.34-37.96)*	27.70 (19.87-38.24)*	0.749
	CC genotype, standard therapy, n = 6	23.55 (16.68-43.63)*	25.20 (21.07-46.01)*	0.589
	TT genotype, standard therapy + probiotics, n = 6	58.45 (37.61-70.55)	67.85 (47.97-75.34)	0.485
	P2	0.008	0.004	

Note: same as in Table 3.

In turn, the concentration of IL-10 (Table 5) was significantly higher at the beginning and the end of treatment in the groups of patients with TT genotype compared to CC genotype. The treatment of exogenous and endogenous AD did not lead to significant changes in the level of IL-10.

Table 6: The serum level of TGF-β (pg/ml) in patients that underwent different treatments

Groups		Day 0	Day 28	P1
Exogenous AD	CC genotype, standard therapy + probiotics, n = 7	16.10 (13.69-21.61)*	35.90 (28.64-47.48)#	0.001
	CC genotype, standard therapy, n = 6	17.40 (12.91-25.43)*	18.30 (13.84-20.10)*#	0.998
	TT genotype, standard therapy + probiotics, n = 6	41.90 (31.81-50.34)	39.85 (32.66-52.83)	0.998
	P2	0.003	0.002	
Endogenous AD	CC genotype, standard therapy + probiotics, n = 6	16.70 (11.12-32.59)*	18.20 (12.84-29.77)*	0.818
	CC genotype, standard therapy, n = 6	17.75 (13.17-26.64)*	17.95 (12.12-29.88)*	0.810
	TT genotype, standard therapy + probiotic, n = 6	46.90 (34.86-50.23)	46.85 (37.12-53.04)	0.631
	P2	0.003	0.003	

Note: same as in Table 3.

The dynamics of TGF-β in contrast to IL-10 was different. Thus, in patients with CC genotype and exogenous form of AD (Table 6) who received standard treatment and probiotics, the level of TGF-β (p = 0.001) was significantly increased by Day 28 compared to Day 0. Also, the level of TGF-β in this group on Day 28 did not significantly differ from the group with TT genotype. In all other cases, the concentration of this cytokine in TT genotype (Table 6) was significantly higher compared to CC genotype.

Discussion

A possible explanation is that there is a high activity of type 2 immune response in patients with an exogenous form of AD with CC genotype. Probiotics are likely to cause the most active suppression of inflammation precisely in such a cohort of patients.

A randomised double-blinded placebo-controlled pilot study showed significantly improved oxidative stress values during probiotics treatment in inflammatory bowel disease [18]. In another clinical trial, it was shown that probiotics supplementation reduced the incidence of infections in the oral cavity and respiratory tracts without any drugs-related adverse effects [19].

It has been investigated that the positive effect of probiotics comes from several possible immunological mechanisms, including the modulation of Tx cell activation, the induction of regulatory T-lymphocytes (Treg), and improved restoration of the barrier function [20]. Probiotic bacterial strains have been shown to inhibit Tx-2 cell responses and stimulate the production of cytokines by T-helper type 1 [21]. Also, the use of probiotics increases the number of populations of T-regulatory lymphocytes in experimental models of allergic diseases, including AD [22], [23], most likely inducing regulatory dendritic cells [24].

In a randomised, double-blind, placebo-controlled study, clinical efficacy in the administration of probiotic strains (Lactobacillus salivarills LS01) in the treatment of adult AD patients was evaluated. There were included 38 AD patients who took probiotics for 16 weeks. Patients receiving probiotics showed a statistically lower SCORAD ($p < 0.0001$) and DLQI ($p = 0.021$) indices at the end of treatment compared to the placebo group. It has also been shown that the treatment of L.salivarius LSO1 reduces the production of cytokines Th2 type while maintaining a stable concentration of Th1 cytokines. At the end of treatment, there was also a statistically significant decrease in staphylococci in faeces in patients taking probiotics [25].

In this pilot study we found that the addition of probiotics (*Lactobacillus acidophilus, LA-5 and Bifidobacterium animalis* subsp. *lactis, BB-12*) to standard treatment protocol of AD (ointment of fluticasone propionate 0.005%, emollient) significantly increased the effectiveness of treatment of atopic dermatitis in adults with exogenous form and CC genotype (C-159T). Favourable outcome was confirmed by clinical (a significant decrease of SCORAD and DLQI indices) and immunological criteria (a significant decrease of IL-4 and an increase of TGF-β).

Interpretation of the results of this study is based on data of a small number of patients, which requires further study of this problem with the involvement of more patients.

References

1. Pineiro M, Asp NG, Reid G, Macfarlane S, Morelli L, Brunser O, et al. FAO Technical meeting on prebiotics. J Clin Gastroenterol. 2008; 42(Suppl 3, Pt 2):S156-9. https://doi.org/10.1097/MCG.0b013e31817f184e PMid:18685504

2. Sánchez B, Delgado S, Blanco-Míguez A, Lourenço A, Gueimonde M, Margolles A. Probiotics, gut microbiota, and their influence on host health and disease. Mol Nutr Food Res. 2017; 61(1). https://doi.org/10.1002/mnfr.201600240

3. Baldini M, Lohman IC, Halonen M, Erickson RP, Holt PG, Martinez FD. A Polymorphism* in the 5' flanking region of the CD14 gene is associated with circulating solubleCD14 levels and with total serum immunoglobulin E. Am J Respir Cell Mol Biol. 1999; 20(5):976-83. https://doi.org/10.1165/ajrcmb.20.5.3494 PMid:10226067

4. Zhao L, Bracken MB. Association of CD14 -260 (-159) C>T and asthma: a systematic review and meta-analysis. BMC Med Genet. 2011; 12:93. https://doi.org/10.1186/1471-2350-12-93 PMid:21745379 PMCid:PMC3148550

5. Leynaert B, Guilloud-Bataille M, Soussan D, Benessiano J, Guénégou A, Pin I, et al. Association between farm exposure and atopy, according to the CD14 C-159T polymorphism. J Allergy Clin Immunol. 2006; 118(3):658-65. https://doi.org/10.1016/j.jaci.2006.06.015 PMid:16950285

6. Rennie DC, Karunanayake CP, Chen Y, Nakagawa K, Pahwa P, Senthilselvan A, et al. CD14 gene variants and their importance for childhood croup, atopy, and asthma. Dis Markers. 2013; 35(6):765-71. https://doi.org/10.1155/2013/434920 PMid:24347797 PMCid:PMC3856132

7. Koppelman GH, Reijmerink NE, Colin Stine O, Howard TD, Whittaker PA, Meyers DA, et al. Association of a promoter polymorphism of the CD14 gene and atopy. Am J Respir Crit Care Med. 2001; 163(4):965-9. https://doi.org/10.1164/ajrccm.163.4.2004164 PMid:11282774

8. O'Donnell AR, Toelle BG, Marks GB, Hayden CM, Laing IA, Peat JK, et al. Age-specific relationship between CD14 and atopy in a cohort assessed from age 8 to 25 years. Am J Respir Crit Care Med. 2004; 169(5):615-22. https://doi.org/10.1164/rccm.200302-278OC PMid:14617510

9. Leung TF, Tang NL, Sung YM, Li AM, Wong GW, Chan IH, et al. The C-159T polymorphism in the CD14 promoter is associated with serum total IgE concentration in atopic Chinese children. Pediatr Allergy Immunol. 2003; 14(4):255-60. https://doi.org/10.1034/j.1399-3038.2003.00048.x PMid:12911501

10. Han D, She W, Zhang L. Association of the CD14 gene polymorphism C-159T with allergic rhinitis. Am J Rhinol Allergy. 2010; 24(1):e1-3. https://doi.org/10.2500/ajra.2010.24.3411 PMid:20109306

11. Kabesch M, Hasemann K, Schickinger V, Tzotcheva I, Bohnert A, Carr D, et al. A promoter polymorphism in the CD14 gene is associated with elevated levels of soluble CD14 but not with IgE or atopic diseases. Allergy. 2004; 59(5):520-5. https://doi.org/10.1111/j.1398-9995.2004.00439.x PMid:15080833

12. Zare Marzouni H, Farid-Hosseini R, Jabari-Azad F, Tavakkol-Afshari J, Tehranian F, Khoshkhui M, et al. CD14 as A Serum Immune Biomarker and Genetic Predisposition Factor for Allergic Rhinitis. Iran J Otorhinolaryngol. 2019; 31(102):1-9. PMid:30783593 PMCid:PMC6368989

13. Wang IJ, Wang JY. Children with atopic dermatitis show clinical improvement after Lactobacillus exposure. Clin Exp Allergy. 2015; 45(4):779-87. https://doi.org/10.1111/cea.12489 PMid:25600169

14. Bieber T, Vieths S, Broich K. New opportunities and challenges in the assessment of drugs for atopic diseases. Allergy. 2016; 71(12):1662-1665. https://doi.org/10.1111/all.13063 PMid:27716946

15. Wollenberg A, Barbarot S, Bieber T, Christen-Zaech S, Deleuran M, Fink-Wagner A, et al. Consensus-based European guidelines for treatment of atopic eczema (atopic dermatitis) in adults and children: part I. J Eur Acad Dermatol Venereol. 2018; 32(5):657-682. https://doi.org/10.1111/jdv.14891 PMid:29676534

16. Gerbens LA, Chalmers JR, Rogers NK, Nankervis H, Spuls PI;Harmonising Outcome Measures for Eczema (HOME) initiative. Reporting of symptoms in randomized controlled trials of atopic eczema treatments: a systematic review. Br J Dermatol. 2016; 175(4):678-86. https://doi.org/10.1111/bjd.14588 PMid:27012805

17. Hongbo Y, Thomas CL, Harrison MA, Salek MS, Finlay AY. Translating the science of quality of life into practice: What do dermatology life quality indexscores mean? J Invest Dermatol. 2005; 125(4):659-64. https://doi.org/10.1111/j.0022-202X.2005.23621.x PMid:16185263

18. Ballini A, Santacroce L, Cantore S, Bottalico L, Dipalma G, Topi S, et al. Probiotics Efficacy on Oxidative Stress Values in Inflammatory Bowel Disease: A Randomized Double-Blinded Placebo-Controlled Pilot Study. Endocr Metab Immune Disord Drug Targets, 2018. https://doi.org/10.2174/1871530319666181221150352

19. Campanella V, Syed J, Santacroce L, Saini R, Ballini A, Inchingolo F. Oral probiotics influence oral and respiratory tract infections in pediatric population: a randomized double-blinded placebo-controlled pilot study. Eur Rev Med Pharmacol Sci. 2018; 22(22):8034-8041. PMid:30536353

20. Baquerizo Nole KL, Yim E, Keri JE. Probiotics and prebiotics in dermatology. J Am Acad Dermatol. 2014; 71(4):814-21. https://doi.org/10.1016/j.jaad.2014.04.050 PMid:24906613

21. Winkler P, Ghadimi D, Schrezenmeir J, Kraehenbuhl JP. Molecular and cellular basis of microflora-host interactions. Nutr. 2007; 137(3 Suppl 2):756S-72S.

https://doi.org/10.1093/jn/137.3.756S PMid:17311973

22. Feleszko W, Jaworska J, Rha RD, Steinhausen S, Avagyan A, Jaudszus A, et al. Probiotic-induced suppression of allergic sensitization and airway inflammation is associated with an increase of T regulatory-dependent mechanisms in a murine model of asthma. Clin Exp Allergy. 2007; 37(4):498-505. https://doi.org/10.1111/j.1365-2222.2006.02629.x PMid:17430345

23. Karimi K, Inman MD, Bienenstock J, Forsythe P. Lactobacillus reuteri-induced regulatory T cells protect against an allergic airway response in mice. Am J Respir Crit Care Med. 2009; 179(3):186-93. https://doi.org/10.1164/rccm.200806-951OC PMid:19029003

24. Kwon HK, Lee CG, So JS, Chae CS, Hwang JS, Sahoo A, et al. Generation of regulatory dendritic cells and CD4+Foxp3+ T cells by probiotics administrationsuppresses immune disorders. Proc Natl Acad Sci U S A. 2010; 107(5):2159-64. https://doi.org/10.1073/pnas.0904055107 PMid:20080669 PMCid:PMC2836639

25. Drago L, Iemoli E, Rodighiero V, Nicola L, De Vecchi E, Piconi S. Effects of Lactobacillus salivarius LS01 (DSM 22775) treatment on adult atopic dermatitis: a randomized placebo-controlled study. Int J Immunopathol Pharmacol. 2011; 24(4):1037-48. https://doi.org/10.1177/039463201102400421 PMid:22230409

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Apr 15; 7(7):1059-1066.
https://doi.org/10.3889/oamjms.2019.247
eISSN: 1857-9655
Basic Science

Neuroprotective Activity of *Evolvulus alsinoides* & *Centella asiatica* Ethanolic Extracts in Scopolamine-Induced Amnesia in Swiss Albino Mice

Mukesh Kumar Yadav[1*], Santosh Kumar Singh[2], Manish Singh[3], Shashank Shekhar Mishra[4], Anurag Kumar Singh[2], Jyoti Shankar Tripathi[1], Yamini Bhusan Tripathi[5]

[1]Department of Kayachikitsa, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India; [2]Centre of Experimental Medicine & Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India; [3]Department of Pharmacology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India; [4]Department of Vikriti Vigyan, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India; [5]Department of Medicinal Chemistry, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India

Citation: Yadav MK, Singh SK, Singh M, Mishra SS, Singh AK, Tripathi JS, Tripathi YB. Neuroprotective Activity of *Evolvulus alsinoides* & *Centella asiatica* Ethanolic Extracts in Scopolamine-Induced Amnesia in Swiss Albino Mice. Open Access Maced J Med Sci. 2019 Apr 15; 7(7):1059-1066. https://doi.org/10.3889/oamjms.2019.247

Keywords: *Centella asiatica; Evolvulus alsinoides L.*; Ethanolic extracts; In vivo antioxidant; Neuroprotective; Acetylcholine esterase

***Correspondence:** Prof. Jyoti Shankar Tripathi and Mukesh Kumar Yadav. Department of Kayachikitsa, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. E-mail: mukesh.yadav@bhu.ac.in

Received: 07-Feb-2019; **Revised:** 25-Mar-2019; **Accepted:** 26-Mar-2019; **Online first:** 12-Apr-2019

Funding: This research did not receive any financial support

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

Abstract

AIM: To carry out the comparative nootropic, neuroprotective potentials of two medicinal plant species.

MATERIAL AND METHODS: For neuroprotective activity; behavior models (elevated plus maze & morris water maze), in vivo antioxidant (superoxide dismutase, catalase, lipid peroxidation & reduced glutathione), inflammatory markers (IL-1β, IL-6 & TNF-α) and acetylcholine esterase (AChE) assessment procedures followed at different dosages i.e. 250 & 500 mg/kg of Evolvulus alsinoides and Centella asiatica ethanolic extracts. At the end of the study, it was performed histopathological analysis of the following organs: brain, heart, liver, and kidney.

RESULTS: In oral administration of different doses of ethanolic extracts of both medicinal plants i.e. Sco + EEA 250 = 2.49 ± 0.29 , Sco + EEA 500 = 2.67 ± 0.36, Sco + ECA 250 = 2.33 ± 0.17, Sco + ECA 500 = 2.77 ± 0.21, Sco + EEA + ECA 250 = 2.61 ± 0.32 and Sco + EEA + ECA 500 = 2.79 ± 0.16 U/mg of protein respectively against the scopolamine induced group Sco (control) = 5.51 ± 0.35 U/mg of protein extracts shows neuroprotective and nootropic activity with reducing AChE level in the brain homogenate of swiss albino mice.

CONCLUSION: Since the *E. alsinoides* & *C. asiatica* are already used in traditional Indian medicine as the neuroprotective agent and also found promising effects over inflammatory diseases, wound healing, and immunomodulatory activity. The neuroprotective effect of both plants extracts attributed to inhibition of AChE activity and improve the spatial memory formation.

Introduction

Alzheimer's disease is a progressive neurodegenerative disease which is characterised by the loss of learning and memory abilities with ageing [1]. This impairment of memory is correlated with the loss of cholinergic neurons [2], [3]. Learning and memory are complex processes and thought not to be regulated by only one muscarinic receptor subtype; scopolamine a non-selective muscarinic receptor antagonist has been reported to impair both acquisition and long-term memory formation. Scopolamine-induced amnesic animal models are used to screen for drugs that potentially have anti-dementia activities by stimulating the cholinergic system, make them a candidate for the treatment of Alzheimer's disease [4]. Ayurveda, the Indian system of medicine describe a group of medicinal plants under the category of 'Medhya Rasayana', which possess the memory-enhancing effect and facilitate learning acquisition. Medicinal plants are rich sources of important metabolites, which are potential sources of antioxidant, antimicrobial, anti-inflammatory, and anticancer activities [5], [6], [7]. The utilisation of herbal medicine in treating infectious diseases have

been practised for 1000s of years and will continue to provide humanity with new remedies [8].

Evolvulus alsinoides L.

Evolvulus alsinoides L. an important medicinal plant is employed for different ailments in India traditionally and grows in the open and grassy places almost throughout India and mostly in the region from India to west Cameroon and widely dispersed elsewhere in tropical Africa and worldwide. Some vernacular names of the plant in India are vishnukranta, vishnugandhiy (sanskrit), shankapushpi (hindi). *Evolvulus alsinoides* L. is used in Ayurveda as a brain tonic in the treatment of neurodegenerative diseases, asthma and amnesia [9].

Centella asiatica

Centella asiatica is a very important medicinal herb also known as Mandukparni, which has been used as a medicine in the Ayurvedic tradition of India for thousands of years and mentioned in many classical texts of Ayurveda. *Centella asiatica* (CA) is a rejuvenative nervine recommended for nervous disorders, epilepsy, senility and premature ageing and several other medical conditions. As a brain tonic, it is said to aid intelligence and memory [10]. The extract of *Centella asiatica* especially from roots and leaves contain a high anti-oxidative activity, which was as good as tocopherol, a natural anti-oxidant, have been reported to play a significant role in wound healing [11]. *Centella asiatica* extract contains four principle bioactive compounds: asiatic acids, made classic acid, asiaticoside and madecassoside [12], [13], in which asiaticoside was identified as the main active constituent responsible for wound healing.

We are working on the development of anti-dementia natural drug using an *in vivo* screening of herbal materials due to their safety and cost-effectiveness [14].

Material and Methods

Plant material

Whole plant material of *Centella asiatica* and *Evolvulus alsinoides* were collected from village Ramnapur, Varanasi, Uttar Pradesh, India in October 2015 and authentication was done by Department of Botany, Banaras Hindu University, India and also herbarium of *Centella asiatica* (voucher specimen no. Apia/02/2015) and *Evolvulus alsinoides* (voucher specimen no. Convolvul./03/2015) of plant was deposited in the Department of Botany, Banaras Hindu University, India.

Preparation of extracts

The extraction of whole plants was done with Soxhlet method in ethanolic solvents at 72-82°C for 72 hours. The Soxhlet extraction has widely been used for extracting valuable bioactive compounds from various natural sources. It is used as a model for the comparison of new extraction alternatives. A small amount of dry sample is placed in a thimble. The thimble is then placed in distillation flask which contains the solvent of particular interest. After reaching an overflow level, the solution of the thimble-holder is aspirated by a siphon. Siphon unloads the solution back into the distillation flask. This solution carries extracted solutes into the bulk liquid. The solute remains in the distillation flask, and solvent passes back to the solid bed of plant. The processrepeatedly runs until the extraction is completed.

Preparation of extract samples

Ethanolic extracts of *C. asiatica* (ECA) and *E. alsinoides* (EEA) were solubilized in distilled water to obtain solutions of 250 and 500 mg/ml [15].

Animals

The experimental Swiss albino mice were issued by Animal house of Institute of Medical Sciences, Banaras Hindu University Varanasi, Uttar Pradesh. Animals were divided into experimental groups, housed in plastic cages and maintained on a 12-hour light and 12-hour dark cycle. They were given standard food and water ad libitum. The Central Animal Ethical Committee of Banaras Hindu University approved all experimental procedures (CAEC/196).

Experimental design and Drug administration

Scopolamine (1.0 mg/kg; dissolved in distilled water) was administered intraperitoneal (i.p.) to the experimental animals. The ethanolic extracts of *Evolvulus alsinoides* (EEA) and *Centella asiatica* (ECA) in the following doses: EEA 250 mg/kg/day & 500 mg/kg/day, ECA 250 mg/kg/day & 500 mg/kg/day and combination of EEA + ECA 250 mg/kg/day & 500 mg/kg/day, dissolved in 0.3% CMC and was administered orally (p.o.). Nine groups, each consisting of 6 animals, were included in the study.

Group I (normal) was treated with vehicle daily (0.3% CMC; p.o).

Group II (Sco control) was treated with scopolamine (1.0 mg/kg/day; i.p.).

Group III (Sco + Doz) was treated with a donepezil (1.5 mg/kg/day; i.p.) and scopolamine (1.0 mg/kg/day; i.p.).

Group IV (Sco + EEA 250) was treated with low dose EEA (250 mg/kg/day; p.o.) and scopolamine (1.0 mg/kg/day; i.p.).

Group V (Sco + EEA 500) was treated with high dose of EEA (500 mg/kg/day; p.o.) and scopolamine (1.0 mg/kg/day; i.p.).

Group VI (Sco + ECA 250) was treated with low dose ECA (250 mg/kg/day; p.o.) and scopolamine (1.0 mg/kg/day; i.p.).

Group VII (Sco + ECA 500) was treated with high dose of ECA (500 mg/kg/day; p.o.) and scopolamine (1.0 mg/kg/day; i.p.).

Group VIII (Sco + EEA + ECA 250) was treated with low dose of EEA (250 mg/kg/day; PO) & ECA (250 mg/kg/day; p.o.) and scopolamine (1.0 mg/kg/day; i.p.).

Group IX (Sco + EEA + ECA 500) was treated with high dose of EEA (500 mg/kg/day; p.o.) & ECA (500 mg/kg/day; p.o.) and scopolamine (1.0 mg/kg/day; i.p.).

Behavioural study

Elevated plus maze

The elevated plus maze (EPM) is designed to study the behavioural pattern of experimental animals such as sensitivity to external stimuli (exteroceptive behaviour), anxiety, exploration as well as learning and memory [16], [17]. The EPM consists of four arms (two open and two closed), each 49 cm x 10 cm, with 40 cm high walls in closed arms and the open roof. The whole structure is elevated 50 cm above the ground. On the 10th day, 60 min after the drug treatment, each mice was placed at the end of an open arm, facing away from the central platform. The time is taken by the mice to enter any of the closed arms was recorded and considered as the transfer latency (TL) and served as a parameter for acquisition/ learning. If the mice did not enter into any one of the closed arms within 180 sec, it was gently pushed into one of the two closed arms, and the TL was assigned as 180 sec. For the next 15 sec, the mice were allowed to explore the maze before returning it to its home cage. On the 14th day, TL was recorded again, which served as a parameter for retention of memory. Between each session, the maze was carefully cleaned with 30% ethanol tissue to remove any olfactory cues.

Morris water maze

The Morris water maze (MWM) [18], [19] test was performed to assess the learning and spatial memory of the experimental animals. The advantage of MWM over other models of learning and memory lies in the fact that no motivational stimuli such as food and water deprivation, electrical stimulation and buzzer sounds are applied. The apparatus consists of a circular pool (45 cm in height and 100 cm in diameter) with a featureless inner surface. The pool was filled with opaque water, maintained at the temperature of 22 ± 2°C, to a height of 30 cm, and was divided into four quadrants of equal area, marked by different visual cues. A platform (29 cm X 6 cm) was placed one centimetre below the level of water at the centre of one of the four quadrants, which was considered as the target quadrant. The position of the platform was kept unaltered throughout the experiment. The MWM test was performed on the 10th day after drug administration was started. On the first experimental day, the mice were allowed to acclimatise in the pool and swim for 120 sec without the platform. During the next four consecutive days, each animal received four learning trials of 120 sec with an inter-trial interval of 60 sec.

For each learning trial, the mice were placed in the water facing the pool wall diagonally opposite to the quadrant in which the platform was kept. The time taken by the animal to locate the submerged platform was recorded as Escape latency time (ELT) for each trial. If the animal were unable to locate the platform within 120 sec, it was directed to the platform and allowed to rest there for 60 sec, and in this case, the ELT was recorded as 120 sec. These sessions were recorded as hidden platform trials for acquisition test. On the 14th day after the learning trial session, the platform was removed from the pool, and the mice were subjected to a Probe trial session to assess memory retention. Each mouse was placed into the water diagonally opposite to the target quadrant, and for 60 sec was allowed to swim and find the quadrant in which the platform was previously placed. The swimming time of the animal to reach the target quadrant was recorded as probe trial memory retention test.

Preparation of Brain homogenate

On the terminal experimental day, animals were euthanized, and the whole brains of all mice were isolated after performing cardiac perfusion with normal saline for biochemical estimations. Then further rinsed in ice-cold isotonic saline and were homogenized with ice-cold 0.1 M phosphate buffer saline (pH 7.4) to form 10% w/v homogenates. These homogenates were then further centrifuged at -4°C (10,000 rpm; C-24 cooling centrifuge instrument, Model no. C-24 Remi, India) for 15 min and the supernatant was used for estimation of biochemical parameters [20].

In vivo antioxidants assessment

Assessment of Superoxide dismutase activity

Every 3 ml of reaction mixture contained 2.8

ml of potassium phosphate buffer (0.1 M, pH 7.4), 0.1 ml of brain homogenate and 0.1 ml of pyrogallol solution (2.6 mM in 10 mM HCl). The change in absorbance was recorded at 325 nm for 5 min with 30 sec. Interval. One unit of SOD is equivalent to the amount of enzyme required to cause 50% inhibition of pyrogallol autoxidation per 3 ml of the assay mixture [21].

Assessment of Catalase activity

The reaction mixture contained 2.0 ml of diluted homogenate in 0.1 M phosphate buffer (enzyme extract). The reaction was started by adding 1.0 ml of 200 mM H_2O_2. The decrease in OD per min was recorded against the blank (all the reagents except enzyme extract) for 3 min at 240 nm at intervals of 15 sec. CAT activity was expressed as U/mg protein [22].

Assessment of Lipid peroxidation activity

To 100 µl of tissue homogenate, 1.5 ml of 10% TCA solution was added. After 10 min, centrifuge at 5000 rpm for 10 min. The supernatant was separated and mixed with 1.5 ml of TBA. The tubes were kept in boiling water bath for 30 min to complete reaction and were cooled under tap water. The absorbance of the sample at 535 nm against distilled water [23].

Assessment of Reduced glutathione (GSH) activity

The GSH assays were performed as described by Smith I K et al., [24]. In GSH assay, 3 ml of reaction mixture consisted of 2.9 ml of 5, 5-dithiobis (2-nitrobenzoate) (DTNB) prepared in potassium phosphate buffer (0.1 M, pH 7.4) and 0.1 ml of tissue homogenate. The reaction mixture was incubated at 37°C for 15 min, and the absorbance was recorded at 412 nm, and the results were expressed as GSH/mg protein.

Analysis of IL-1β, IL-6 and TNF-α in the brain

The levels of IL-1β, IL-6, and TNF-α in the brain homogenates were determined using commercial (Elabscience) ELISA kits according to the manufacturer's instructions. The levels of these cytokines in the brain tissues were normalized to the protein content.

AChE estimation

AChE activity was estimated in the whole brain homogenates according to Ellman's method [25]. Briefly, the brain homogenate was incubated for 5 min with 2.7 ml of phosphate buffer and 0.1 ml of 5, 5-dithiobis (2-nitrobenzoate) (DTNB). Further, 0.1 ml of freshly prepared acetylcholine iodide (pH 8) was added, and the change in absorbance was recorded at 412 nm.

Statistical analysis

The relative haematological and biochemical data were expressed as the mean ± standard error of the mean (SEM). Data were submitted to analysis of variance (one-way ANOVA) followed by Dunnett multiple comparison tests. The results were expressed as mean ± SEM. The software GraphPad Prism 6.0 (GraphPad Software, USA) was used for statistical analysis. $P < 0.05$ were considered statistically significant.

Results

Behavioral study

Elevated plus maze

The elevated plus maze (EPM) was used to study the behavioral pattern of mice such as anxiety, exploration as well as learning and memory and results are shown in Figure 1. At the days 1st, 6th, 10th and 14th with transfer latency time (sec) for Sco + ECA 250 = 23 ± 0.7, 19 ± 2.27, 18 ± 0.67, 16 ± 0.73; Sco + ECA 500 = 27 ± 2.39, 26 ± 0.79, 20 ± 0.97, 17 ± 0.97; Sco + EEA + ECA 250 = 20 ± 1.73, 18 ± 1.32, 18 ± 0.51, 16 ± 0.72 groups have shown good activity in respect to Sco (control) = 45 ± 2.1, 48 ± 2.1, 50 ± 2.3, 52 ± 2.3 group and Sco + Doz (standard)= 22 ± 1.28, 20 ± 2.86, 18 ± 2.3, 14 ± 2.52 group.

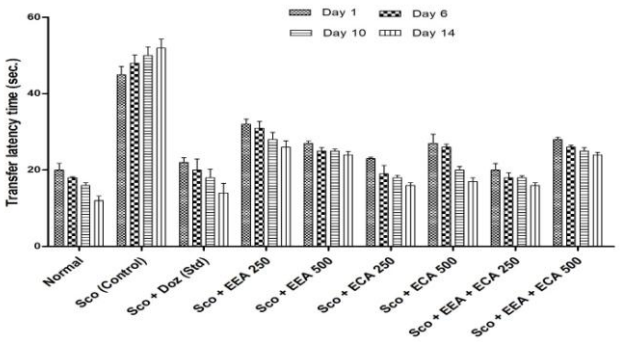


Figure 1: Transfer latency time for all groups in elevated plus maze behavioral study. Data expressed as mean ± sem, n = 6. P < 0.01, compared with the Sco group and P < 0.01 compared to the normal group using one-way ANOVA followed by Dunnett's test as a post – ANOVA test

Morris water maze

The Escape Latency Time (ELT) was measured to assess spatial memory in mice, and the

results are shown in Figure 2. On 1st and 6th day and we found that there was no significant difference in ELT in all mice groups. On the 10th and 14th day all plant extract treated groups were found to improve the spatial memory in a dose-dependent manner with a significant ($P < 0.01$).

On 14th day plant extracts treatment groups significantly reduced the ELT at Sco + EEA 250 = 56 ± 2.88, 54 ± 2.3, 43 ± 1.85, 36 ± 1.72; Sco + ECA 250 = 49 ± 2.33, 46 ± 2.5, 46 ± 2.88, 36 ± 2.98; Sco + ECA 500 = 48 ± 1.95, 47 ± 1.45, 45 ± 1.73, 24 ± 1.5; Sco + EEA + ECA 500 = 44 ± 3.2, 43 ± 2.66, 33 ± 1.85, 24 ± 1.76 & Sco + Doz (standard) = 39 ± 2.66, 38 ± 1.62, 35 ± 1.73, 33 ± 2.88 and showed significantly improvement in spatial memory on both the days during treatment regimen ($P < 0.01$) in comparison with Scopolamine treated mice group Sco (control) = 92 ± 2.8, 95 ± 3.4, 112 ± 1.76, 116 ± 1.45.

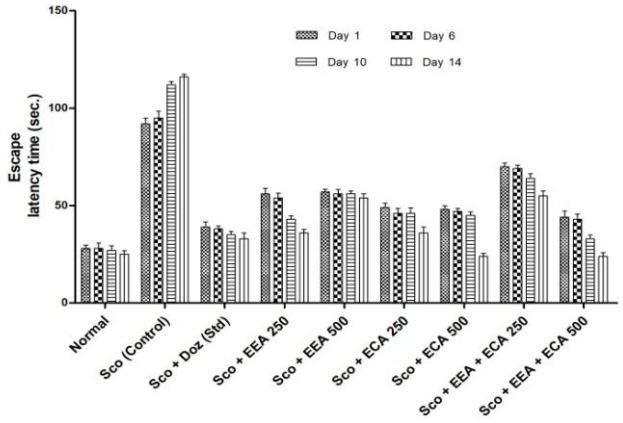


Figure 2: Escape Latency Time (ELT) for all groups in morris water maze behavioral study. Data expressed as mean ± sem, n = 6. P < 0.01, compared with the Sco group and P < 0.01 compared to the normal group using one-way ANOVA followed by Dunnett's test as a post-ANOVA test

In vivo antioxidant activity

Super oxide Dismutase (SOD)

SOD is the major antioxidant enzyme scavenging the free radicals generated during oxidative stress were estimated and results are showed in Figure 3. The Sco + EEA 250 = 146 ± 6.01, Sco + EEA 500 = 148.66 ± 5.23 & Sco + ECA 250 = 148.89 ± 6.63 treatment groups significantly increased the SOD activity at all doses levels ($P < 0.01$) except Sco + ECA 500 = 141.13 ± 5.18, Sco + EEA + ECA 250 = 141.63 ± 6.11 and Sco + EEA + ECA 500 = 131.55 ± 4.25 found not significant in comparison with Sco (Control) = 119.78 ± 4.57 treated mice group.

Sco + Doz (standard) = 150.52 ± 4.61 administration also showed protection from oxidative stress by elevating the SOD activity as compared to Sco (control) mice ($P < 0.01$).

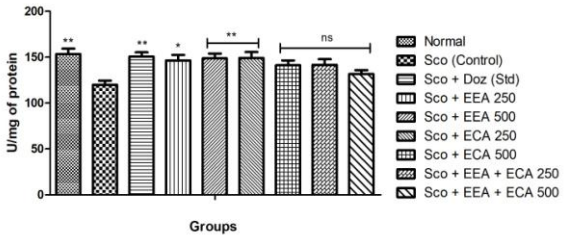


*Figure 3: Super oxide dismutase activity in all mice groups show significant increase. Data expressed as mean ± sem, n = 6. P < 0.01, compared with the Sco (control) group and P<0.01 compared to the normal group using one-way ANOVA followed by Dunnett's test as a post-ANOVA test. (*P < 0.05, **P < 0.01, ***P < 0.001, ns = not significant)*

Catalase

In the all treatment groups; Sco + EEA 500 = 126.8 ± 5.7, Sco + ECA 500 = 126.0 ± 5.2 & Sco + EEA + ECA 500 = 126.7 ± 5.8 & Sco + Doz (standard) = 131.1 ± 4.7 significantly increased the catalase activity ($P < 0.01$) except Sco + ECA 250 = 103.1 ± 6.6 which found not significant in comparison with Sco (control) = 98.32 ± 5.3 treated mice group (Figure 4).

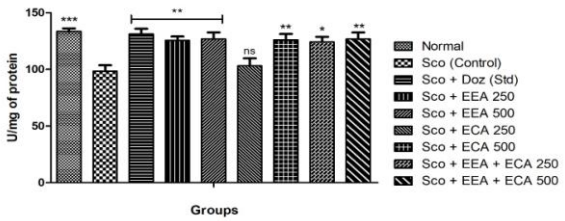


*Figure 4: Catalase activity in all mice groups and Data expressed as mean ± sem, n = 6. P < 0.01, compared with the Sco group and P < 0.01 compared to the normal group using one-way ANOVA followed by Dunnett's test as a post-ANOVA test. (*P < 0.05, **P < 0.01, ***P < 0.001, ns = not significant)*

Lipid Peroxidation

In the all treatment groups; Sco + EEA 250 = 0.48 ± 0.05, Sco + EEA 500 = 0.37 ± 0.03 & Sco + ECA 500 = 0.45 ± 0.02 and Sco + EEA + ECA 250 = 0.6 ± 0.03 significantly increased the LPO activity ($P < 0.05$) except Sco + ECA 250 = 0.66 ± 0.03 and Sco + EEA + ECA 500 = 0.7 ± 0.03 in comparison with Sco (control) = 0.81 ± 0.06 treated mice group as well as Sco + Doz (standard) = 0.45 ± 0.02 also decreased the elevated MDA levels.

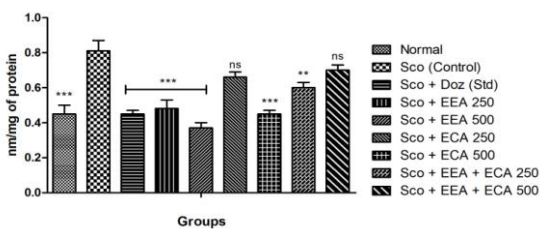


*Figure 5: Lipid peroxidation assay in all mice groups showed significant decrease in comparison to Sco group. Data expressed as mean ± sem, n = 6. P < 0.01, compared with the Sco (control) group and P < 0.01 compared to the normal group using one-way ANOVA followed by Dunnett's test as a post-ANOVA test. (*P < 0.05, **P < 0.01, ***P < 0.001, ns = not significant)*

Reduced glutathione (GSH)

Reduced glutathione (GSH) level were increased after treatment with all the experimental animal groups; Sco + EEA 250 = 4.75 ± 0.47, Sco + EEA 500 = 4.61 ± 0.18, Sco + ECA 500 = 4.26 ± 0.06 & Sco + EEA+ ECA 250 = 4.18 ± 0.09 which found to be significant ($P < 0.05$) except Sco + ECA 250 = 4.08 ± 0.16 and Sco + EEA + ECA 500 = 4.02 ± 0.11 which found not significant in comparison to Sco (control) = 3.2 ± 0.26 treated group and Sco + Doz (standard) = 4.8 ± 0.15 significantly elevated GSH levels ($P < 0.05$).

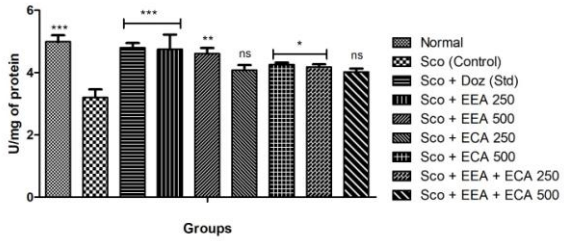


*Figure 6: Reduced glutathione activity in all mice groups showed significant increase in comparison to Sco group. Data expressed as mean ± sem, n = 6. P < 0.05, compared with the Sco group and P < 0.05 compared to the normal group using one-way ANOVA followed by Dunnett's test as a post-ANOVA test. (*P < 0.05, **P < 0.01, ***P < 0.001, ns = not significant)*

Inflammatory markers

IL-1β

The effect of plant extracts treated at different concentrations Sco + EEA 250 = 394.1 ± 23.03, Sco + EEA 500 = 355.5 ± 14.64, Sco + ECA 500 = 387.26 ± 25.3 & Sco + EEA + ECA 250 = 386.21 ± 14.38 & Sco + Doz (standard) = 396.38 ± 15.28 on whole brain markedly decrease the levels of IL-1β when compared with Sco (control) = 485.85 ± 14.76 group ($P < 0.01$) although Sco + ECA 250 = 405.5 ± 23.16 and Sco + EEA + ECA 500 = 406.38 ± 24.65 groups founds to be not significant.

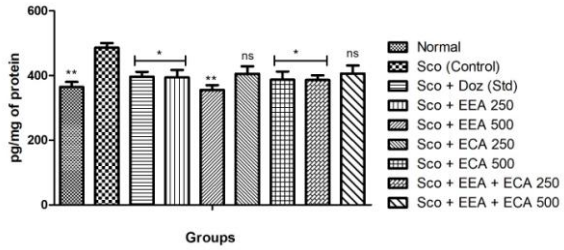


*Figure 7: Effect of plant extracts on brain IL-1β levels in Sco induced mice model. Data expressed as mean ± sem, n = 6. P < 0.01, compared with the Sco group and P < 0.01 compared to the normal group using one-way ANOVA followed by Dunnett's test as a post-ANOVA test. (*P < 0.05, **P < 0.01, ***P < 0.001, ns= not significant)*

IL-6

Figure 8 shows the effect of plant extracts on the IL-6 levels in the whole brain and we found that Sco + EEA 500 = 395.54 ± 14.17, Sco + ECA 250 = 398.87 ± 16.25 Sco + ECA 500 = 395.12 ± 23.68 & Sco + EEA + ECA 250 = 413.66 ± 20.26 & Sco + Doz (standard) = 360.54 ± 20.86 except Sco + EEA 250 = 465.75 ± 13.62 all treatment groups markedly decrease the levels of IL-6 when compared with Sco (control) = 541.58 ± 19.82 group.

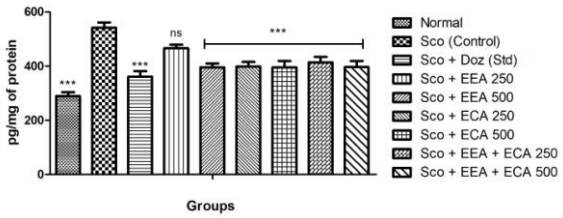


*Figure 8: Effect of plant extracts on brain IL-6 levels in Sco induced mice model. Data expressed as mean ± sem, n = 6. P < 0.01, compared with the Sco group and P < 0.01 compared to the normal group using one-way ANOVA followed by Dunnett's test as a post-ANOVA test. (*P < 0.05, **P < 0.01, ***P < 0.001, ns = not significant)*

TNF-α

Figure 9 shows the effect of plant extracts on the TNF-α level in the whole brain on treatment with Sco + EEA 250 = 112.17 ± 11.52; Sco + EEA 500 = 108.97 ± 12.32; Sco + ECA 250 = 130.12 ± 11.50; Sco + ECA 500 = 176.92 ± 14.72; Sco + EEA + ECA 250 = 109.61 ± 15.86; Sco + EEA + ECA 500 = 107.69 ± 14.63 & Sco + Doz (standard) = 83.97 ± 14.42 subsequently decrease the levels of TNF-α activity when compared with Sco (control) = 233.97 ± 12.4 group.

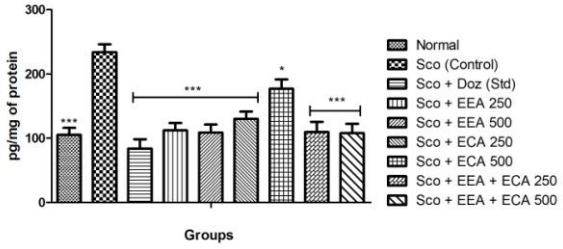


*Figure 9: Effect of plant extracts on brain TNF-α levels in Sco induced mice model. Data expressed as mean ± sem, n = 6. P < 0.01, compared with the Sco group and P < 0.01 compared to the normal group using one-way ANOVA followed by Dunnett's test as a post-ANOVA test. (*P < 0.05, **P < 0.01, ***P < 0.001, ns = not significant)*

Acetylcholine esterase (AChE) activity

Sco administration resulted in significant increase in the brain AChE activity. All the treatment groups Sco + EEA 250 = 2.49 ± 0.29; Sco + EEA 500 = 2.67 ± 0.36; Sco + ECA 250 = 2.33 ± 0.17; Sco + ECA 500 = 2.77 ± 0.21; Sco + EEA + ECA 250 = 2.61 ± 0.32; Sco + EEA + ECA 500 = 2.79 ± 0.16 & Sco + Doz (standard) = 2.74 ± 0.3 significantly inhibit the AChE activity in comparison with Sco (control) = 5.51

± 0.35 treated mice group.

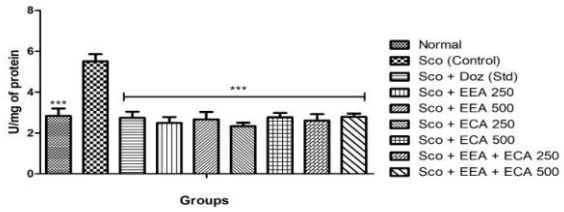


*Figure 10: Acetylcholine esterase activity in all mice groups found significant in comparison to Sco group. Data expressed as mean ± sem, n = 6. P < 0.01, compared with the Sco group and P < 0.01 compared to the normal group using one-way ANOVA followed by Dunnett's test as a post-ANOVA test. (*P < 0.05, **P < 0.01, ***P < 0.001, ns = not significant)*

Discussion

In neurodegeneration, oxidative stress plays an important role in the ageing process, and the brain is highly susceptible to the oxidative imbalance due to its high energy demand, high oxygen consumption [26]. Basal forebrain and amygdala are involved in learning and memory formation and are more vulnerable areas susceptible to oxidative stress [27]. Mitochondrial dysfunction caused by increased reactive oxygen species generation has been involved in ageing and neurodegenerative disorders [28]. In this study, Scopolamine treatment significantly decreased the reactive oxygen species scavenging enzymes activities like superoxide dismutase, catalase and also reduced glutathione levels in brain tissue. Scopolamine treatment also significantly increased lipid peroxidation as compared to the normal group. Both plants extract me. e. EEA, ECA, EEA + ECA treatments were found to significantly elevate the SOD, catalase activity, lipid peroxidation activity and reduced glutathione levels in comparison with Scopolamine group. The expression of cytokine receptors is temporally and spatially regulated in the central nervous system, and they are closely involved in cell proliferation, gliogenesis, neurogenesis, cell migration, apoptosis, and synaptic release of neurotransmitters [29], [30]. The level of cytokines IL-1β, IL-6 and TNF-α are significantly decreased on treatment with plant extracts which increased by Scopolamine administration. The elevated activity of AChE leads to increased degradation of acetylcholine (Ach) neurotransmitter and which in turn declines the ACh pool in the brain which is essential in learning and memory [31]. Scopolamine administration amplifies the AChE activity which is one of the major causes for the cholinergic deficit occurrence after its administration [20]. In this study we found that treatment with both plants ethanolic extracts significantly reduced the AChE activity as compared to the Scopolamine treated mice. It reveals that inhibition of AChE activity by plant extracts have a protective role in acetylcholine degradation and improved the cholinergic neurotransmission. Thus, plant extracts reduced the cholinergic deficits produced by Scopolamine administration resulting in an enhanced neuroprotective effect.

Since the *E. alsinoides* & *C. asiatica* are already used in traditional Indian medicine as the neuroprotective agent and also found promising effects over inflammatory diseases, wound healing, and immunomodulatory activity. The neuroprotective effect of both plants extracts attributed to inhibition of AChE activity and improve the spatial memory formation. The neuroprotective activity could be ascribed to extracts strong antioxidant potential, inhibitory role on AChE activity. The research above findings of this study prospect ethanolic extracts of both plants, i.e. EEA, ECA and EEA + ECA extracts as the promising therapeutic candidate for neurodegenerative diseases.

Acknowledgement

This research work was done in collaboration with the Department of Kayachikitsa & Centre of Experimental Medicine & Surgery (CEMS), Institute of medical sciences, Banaras Hindu University, Varanasi. All authors contributed equally.

References

1. Terry Jr AV, Callahan PM, Hall B, Webster SJ. Alzheimer's disease and age-related memory decline (preclinical). Pharmacology Biochemistry and Behavior. 2011; 99(2):190-210. https://doi.org/10.1016/j.pbb.2011.02.002 PMid:21315756 PMCid:PMC3113643

2. Auld DS, Kornecook TJ, Bastianetto S, Quirion R. Alzheimer's disease and the basal forebrain cholinergic system: relations to β-amyloid peptides, cognition, and treatment strategies. Progress in neurobiology. 2002; 68(3):209-45. https://doi.org/10.1016/S0301-0082(02)00079-5

3. Mufson EJ, Counts SE, Perez SE, Ginsberg SD. Cholinergic system during the progression of Alzheimer's disease: therapeutic implications. Expert review of neurotherapeutics. 2008; 8(11):1703-18. https://doi.org/10.1586/14737175.8.11.1703 PMid:18986241 PMCid:PMC2631573

4. Herrera-Morales W, Mar I, Serrano B, Bermúdez-Rattoni F. Activation of hippocampal postsynaptic muscarinic receptors are involved in long-term spatial memory formation. European Journal of Neuroscience. 2007; 25(5):1581-8. https://doi.org/10.1111/j.1460-9568.2007.05391.x PMid:17355252

5. Hernandez NE, Tereschuk ML, Abdala LR. Antimicrobial activity of flavonoids in medicinal plants from Tafí del Valle (Tucumain, Argentina). J Ethnopharmacol. 2000; 73:317-322. https://doi.org/10.1016/S0378-8741(00)00295-6

6. Polya G. Biochemical targets of plant bioactive compounds: a

pharmacological reference guide to sites of action and biological effects. CRC press, 2003. https://doi.org/10.1201/9780203013717 PMid:12901082

7. Maruthanila VL, Poornima J, Mirunalini S. Attenuation of carcinogenesis and the mechanism underlying by the influence of indole-3-carbinol and its metabolite 3, 3′-diindolylmethane: a therapeutic marvel. Advances in pharmacological sciences. 2014; 2014:1-7. https://doi.org/10.1155/2014/832161 PMid:24982671 PMCid:PMC4060499

8. Cragg GM, Newman DJ. Drug from nature: past achievement, future prospect. Adv Phytomed. 2007; 1:23-37. https://doi.org/10.1016/S1572-557X(02)80010-1

9. Goyal PR, Singh KP. Shankhpuspi (Evolvulus alsinoides Linn.): a medicinal herb. Int J Mendel. 2005; 2:124.

10. Kartning T. Clinical Application of Centella asiatica (L.) Urb. in Craker L.E. and Simon J.E (eds.), Herbs Spices and Medicinal Plants. Vol. II. Oryx Press, Phoemix, AZ, 1988: 145.

11. Zainol MK, Abd-Hamid A, Yusof S, Muse R. Antioxidative activity and total phenolic compounds of leaf, root and petiole of four accessions of Centella asiatica (L.) Urban. Food Chemistry. 2003; 81(4):575-81. https://doi.org/10.1016/S0308-8146(02)00498-3

12. Inamdar PK, Yeole RD, Ghogare AB, De Souza NJ. Determination of biologically active constituents in Centella asiatica. Journal of Chromatography A. 1996; 742(1-2):127-30. https://doi.org/10.1016/0021-9673(96)00237-3

13. Zheng CJ, Qin LP. Chemical components of Centella asiatica and their bioactivities. Journal of Chinese Integrative Medicine. 2007; 5:348-351. https://doi.org/10.3736/jcim20070324

14. Myers SP, Cheras, PA. The other side of the coin: safety of complementary and alternative medicine. Med. J. Aust. 2004; 181:222-225. PMid:15310261

15. Derelanko M J, Hollinger M A. Handbook of toxicology, 2nd edition, CRC press, 2002. PMid:12454789

16. Walf AA, Frye CA. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. Nature protocols. 2007; 2(2):322-328. https://doi.org/10.1038/nprot.2007.44 PMid:17406592 PMCid:PMC3623971

17. Broadbent NJ, Squire LR, Clark RE. Spatial memory, recognition memory, and the hippocampus. Proc Natl Acad Sci USA. 2004; 101:14515-14520. https://doi.org/10.1073/pnas.0406344101 PMid:15452348 PMCid:PMC521976

18. Gacar N, Mutlu O, Utkan T, Komsuoglu Celikyurt I, Gocmez SS, Ulak G. Beneficial effects of resveratrol on scopolamine but not mecamylamine induced memory impairment in the passive avoidance and morris water maze tests in rats. Pharmacol Biochem Behav. 2011; 99(3):316-323. https://doi.org/10.1016/j.pbb.2011.05.017 PMid:21624386

19. Harrison FE, Hosseini AH, Dawes SM, Weaver S, May JM. Ascorbic acid attenuates scopolamine-induced spatial learning deficits in the water maze. Behavioural brain research. 2009; 205(2):550-8. https://doi.org/10.1016/j.bbr.2009.08.017 PMid:19703495 PMCid:PMC2759855

20. Goverdhan P, Sravanthi A, Mamatha T. Neuroprotective effects of meloxicam and selegiline in scopolamine-induced cognitive impairment and oxidative stress. International Journal of Alzheimer's Disease. 2012; 2012.

21. Li X. Improved pyrogallol autoxidation method: a reliable and cheap superoxide scavenging assay suitable for all antioxidants. J Agric Food Chem. 2012; 60:6418-6424. https://doi.org/10.1021/jf204970r PMid:22656066

22. Aebi H. Catalase In: Bergmeyer U, ed. Methods of enzymatic analysis. New York and London: Academic Press, 1974:673-680. https://doi.org/10.1016/B978-0-12-091302-2.50032-3

23. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical biochemistry. 1979; 95(2):351-8. https://doi.org/10.1016/0003-2697(79)90738-3

24. Smith IK, Vierheller TL, Thorne CA. Assay of glutathione reductase in crude tissue homogenates using 5, 5′-dithiobis (2-nitrobenzoic acid). Analytical biochemistry. 1988; 175(2):408-13. https://doi.org/10.1016/0003-2697(88)90564-7

25. Ellman GL, Courtney KD, Andres Jr V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical pharmacology. 1961; 7(2):88-95. https://doi.org/10.1016/0006-2952(61)90145-9

26. Nunomura A, Perry G, Aliev G, Hirai K, Takeda A, Balraj EK, Jones PK, Ghanbari H, Wataya T, Shimohama S, Chiba S. Oxidative damage is the earliest event in Alzheimer disease. Journal of Neuropathology & Experimental Neurology. 2001; 60(8):759-67. https://doi.org/10.1093/jnen/60.8.759

27. Mattson MP, Pedersen WA, Duan W, Culmsee C, Camandola S. Cellular and molecular mechanisms underlying perturbed energy metabolism and neuronal degeneration in Alzheimer's and Parkinson's diseases. Ann NY Acad Sci. 1999; 839:154-175. https://doi.org/10.1111/j.1749-6632.1999.tb07824.x

28. Swerdlow RH. Brain aging, Alzheimer's disease, and mitochondria. Biochem Biophys Acta. 2011; 1812(12):130-1639. https://doi.org/10.1016/j.bbadis.2011.08.012

29. Borsini A, Zunszain PA, Thuret S, Pariante CM. The role of inflammatory cytokines as key modulators of neurogenesis. Trends Neurosci. 2015; 38 (3):145-57. https://doi.org/10.1016/j.tins.2014.12.006 PMid:25579391

30. Boulanger LM. Immune proteins in brain development and synaptic plasticity. Neuron. 2009; 64 (1):93-109. https://doi.org/10.1016/j.neuron.2009.09.001 PMid:19840552

31. Singh M, Kaur M, Kukreja H, Chugh R, Silakari O, Singh D. Acetylcholinesterase inhibitors as Alzheimer therapy: from nerve toxins to neuroprotection. European Journal of Medicinal Chemistry. 2013; 70:165-88. https://doi.org/10.1016/j.ejmech.2013.09.050 PMid:24148993

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Apr 15; 7(7):1067-1070.
https://doi.org/10.3889/oamjms.2019.245
eISSN: 1857-9655
Basic Science

The Effects of Cinnamaldehyde (Cinnamon Derivatives) and Nystatin on *Candida Albicans* and *Candida Glabrata*

Sedigheh Bakhtiari[1], Soudeh Jafari[1], Jamileh Bigom Taheri[1], Tahereh Sadat Jafarzadeh Kashi[2], Zahra Namazi[2*], Maryam Iman[3], Mohammad Poorberafeyi[1]

[1]Department of Oral Medicine, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran; [2]Department of Dental Biomaterial, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran; [3]Chemical Injuries Research Center, Systems Biology and Poisonings Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran

Citation: Bakhtiari S, Jafari S, Taheri JB, Kashi TSJ, Namazi Z, Iman M, Poorberafeyi M. The Effects of Cinnamaldehyde (Cinnamon Derivatives) and Nystatin on *Candida Albicans* and *Candida Glabrata*. Open Access Maced J Med Sci. 2019 Apr 15; 7(7):1067-1070. https://doi.org/10.3889/oamjms.2019.245

Keywords: Cinnamon; Cinnamaldehyde; Nystatin; Candida albicans; Candida glabrata

***Correspondence:** Zahra Namazi. Department of Dental Biomaterials, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran. E-mail: zahra.t.namazi@gmail.com

Received: 19-Feb-2019; **Revised:** 22-Mar-2019; **Accepted:** 23-Mar-2019; **Online first:** 10-Apr-2019

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Candida species are the most common opportunistic fungal infections. Today, cinnamon plants have been considered for anti-Candida properties.

AIM: This study aimed to investigate the effectiveness of cinnamaldehyde extract (from cinnamon derivatives) on *Candida albicans* and *Candida glabrata* species and comparison with nystatin.

MATERIAL AND METHODS: In this study, cinnamaldehyde and nystatin were used. The specimens included *Candida albicans* and *Candida glabrata*. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were measured for each one by the microdilution method. This experiment was repeated three times.

RESULTS: Cinnamaldehyde extract at a concentration of 62.5 µl/ml was able to prevent the growth of *Candida albicans*, at a concentration of 93.7 µl/ml, causing *Candida albicans* to disappear, at 48.8 µl/ml, to prevent the growth of *Candida glabrata*, and in the concentration of 62.5 µl/ml, causes the loss of *Candida glabrata*. In comparison, nystatin at 0.5 µg/ml concentration prevented the growth of *Candida albicans*, at concentrations of 1 µg/ml causing *Candida albicans* to be destroyed, at 4 µg/ml concentration to prevent the growth of *Candida glabrata*, and at a concentration of 8 µg/ml causes the loss of *Candida glabrata*. The results were the same every three times.

CONCLUSIONS: Although cinnamaldehyde extract had an effect on fungal growth in both *Candida albicans* and *Candida glabrata* with a fatal effect; the effect on these two species was lower than nystatin.

Introduction

Candidiasis is an important and common disease of the oral mucosa, caused by various *Candida* species [1], [2]. *Candida albicans* is a major species of pathogens that exist in the form of commensal in the human mouth. The importance of non-albicans such as *Candida glabrata* has increased in recent years [3]. *Candida glabrata* species is known as the most common cause of infection after *Candida albicans* which is seen in about 31-55% of healthy people. These microorganisms can become pathogens with weakening the immune system [4], [5].

Cinnamon zeylanicum blume has been used as an aromatic herb for a long time in the Asian and European herbal medicine for food and medicine application. This herb has been used in traditional medicine for treatment of respiratory, digestive and genital tract defects [6]. The majority of cinnamon essential oil is cinnamaldehyde [7]. Various studies have been done on cinnamon properties [8], [9], [10]. They considered cinnamon as an antioxidant, anti-inflammatory, anti-bacterial, anti-diabetes, anticancer and lipid-lowering agent. The standard topical drug to resolve *candida* infection is nystatin mouthwash. However the inappropriate taste of nystatin mouthwash and repeated preparation and use of it during the day leads to dissatisfaction of the patients. Hence the presence of mouthwashes with proper flavour, easier use, and proper effect seems necessary [11].

Few reports showed the positive effects of cinnamon on the treatment of *Candida albicans* [8], [9], [10] and *Candida glabrata* [9], and limited studies have investigated its comparative effects with nystatin therapy [8], [12]. Providing such herbal medicines that have antimicrobial effects can be useful in reducing side effects and toxic effects on the tissue, as well as economically viable [13], [14], [15].

The purpose of the present study was to evaluate the effects of Cinnamaldehyde (cinnamon derivatives) and Nystatin on *Candida albicans* and *Candida glabrata*. Furthermore, we compared the antifungal effects of Cinnamaldehyde and Nystatin relative to each other using an experimental laboratory intervention method.

Material and Methods

An experimental laboratory intervention was designed at the Institute of Biology and Poisoning, School of Medicine, Baqiyatallah University of Medical Sciences. The studied samples included standard species (ATCC*) Candida albicans* (ATCC 90028) and *Candida glabrata*, both of which were obtained from the Mycology Laboratory of the School of Veterinary Medicine, University of Tehran. The present study was carried out using pure cinnamaldehyde (purchased from Merck Germany) and nystatin (purchased from Sigma).

To determine the lowest inhibitory concentration of cinnamaldehyde extract on two strains *Candida albicans* and *Candida glabrata*, we used eight different concentrations. For each microorganism, a plate was used to evaluate the effect of cinnamaldehyde extract, and overall two plates for two types of *candida* were considered. To prepare the required dilutions for nystatin, according to the protocol of the Sigma factory, 1.024 mg of nystatin powder were added to 1 ml of 5% Dimethyl sulfoxide (DMSO) and a concentration of 1.024 mg/ml of nystatin obtained. The culture media were prepared according to the manufacturer's instructions. Then, the *candidates* prepared on the culture medium with sterile inoculating loops and the plate was incubated for 24 hours in a 37°C incubator, and then candida colonies appeared on the culture medium. Fungal suspensions were prepared according to the turbidity McFarland criterion 0.5. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of cinnamaldehyde extract were determined by dilution in the tube. To determine the MIC for a series of eight tubes, we tested the dilutions of each extract. Also, a tube as a positive control containing diluted extract and a negative control medium containing microbial suspension and culture medium was considered. A total of eight dilution series of cinnamaldehyde extract was prepared as 187.5, 125, 93.75, 62.5, 46.8, 31.2, 23.4, 15.6 µl/ml. According to the microdilution method contained in CLSI, in each of the eight microplates, these concentrations, plus 10 µl of fungal suspension were placed. They were incubated 24 hours at 37°C and eventually the first transparent well that indicated the lack of microorganism growth was considered as MIC. The contents of wells without growth were cultured in a solid culture medium, and after 24 hours of incubation, the lowest concentration in which growth was not observed was considered as MFC. To control the test, positive control and negative control were considered, so that we used the culture medium and *candida* for positive control and the culture medium and cinnamaldehyde for negative control. Ethics committee approval was not required for this study.

Regarding the fact that in this type of microbiological experiment, the trials are repeated three times, in the present study, for each *candida* in each concentration, three replications were repeated. The results of the study were reported descriptively in different groups of intervention and control.

Results

To detect the antifungal effects of cinnamaldehyde extract and nystatin on the growth of two species of *Candida albicans* and *Candida glabrata*, we used the microdilution method to determine MIC and MFC with three replications.

Table 1: Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of Cinnamaldehyde Extract on Candida albicans and Candida glabrata growth

Row	Concentrations of cinnamaldehyde extract(µg/ml)	Candida albicans		Candida glabrata	
		MIC	MFC	MIC	MFC
1	187/5	-	-	-	-
2	125	-	-	-	-
3	93/75	+	-	-	-
4	62/5	+	+	+	-
5	46/8	+	+	+	+
6	31/2	+	+	+	+
7	23/4	+	+	+	+
8	15/6	+	+	+	+

This method was also used to determine the MIC and MFC of nystatin. The results of MIC and MFC for cinnamaldehyde and nystatin are presented in Table 1, 2, and 3.

Table 2: Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of Nystatin on Candida albicans

	Sample (oil)		Nystatin	
Fungi	MIC (µl/ml)	MFC (µl/ml	MIC (µg/ml)	MFC (µg/ml)
Candida albicans ATCC 90028	62.5	93.7	0.5	1

This experiment was repeated three times. We also compared the MIC and MFC of cinnamaldehyde and nystatin on the growth of

Candida albicans and *Candida glabrata* (Figure 1).

Table 3: Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of Nystatin on Candida glabrata

Fungi	Sample (oil)		Nystatin	
	MIC (µl/ml)	MFC(µl/ml)	MIC (µg/ml)	MFC (µg/ml)
Candida glabrata ATCC	48.8	62.5	4	8

The results indicated that nystatin, with a much lower concentration than cinnamaldehyde extract, exerts a lethal effect on *Candida albicans* and *Candida glabrata*.

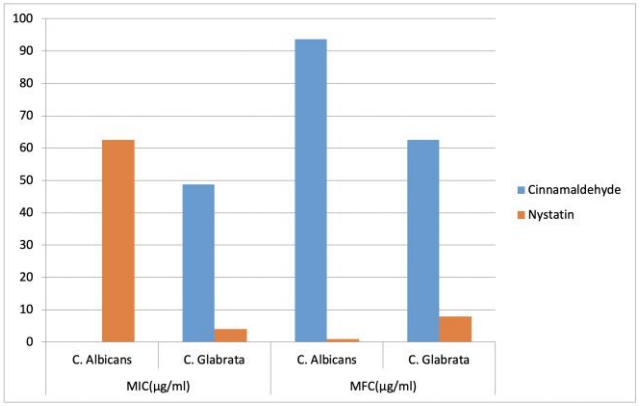


Figure 1: Comparison of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of Cinnamaldehyde extract and Nystatin on Candida albicans and Candida glabrata

Discussion

Topical treatment for oral candidiasis is nystatin mouthwash, which its requirement to frequent use and unpleasant taste, often leads to patients' dissatisfaction and sometimes lack of continuity of treatment. For this reason, offering a proper flavour mouthwash with the ease of use and proper efficacy seems necessary and practical [16]. Studies conducted in the last decade clearly show the unpleasant effects of chemical drugs alongside their beneficial effects. Recent studies have paid more attention to the use of medicinal plants due to increased drug resistance to new chemical drugs. This study aimed to evaluate the antifungal effects of cinnamaldehyde extract against *Candida albicans* and *Candida glabrata*, and compare it with the antifungal effect of nystatin. Most previous studies have been conducted on specific pathogens that affect the skin, respiratory, digestive and urogenital system, and few studies have been done on oral mucosal pathogens [17], [18], [19]. For many years, *Candida glabrata* was considered a relatively non-pathogenic saprophytic flora of normal people, unconnected with a serious infection in humans. However, following the large and widespread use of immunosuppressive agents, along with treatment with broad-spectrum antibiotics, the number of systemic and mucosal infections caused by *Candida glabrata* has increased dramatically [20]. In Fani and Kohanteb (2011) [9], the antifungal effects of cinnamon on *Candida albicans* are mentioned. Also, Casrto and Lima (2013) pointed to the antifungal effects of essential oil of cinnamon, which the main component of it is eugenol, on *Candida albicans*, *Candida tropicalis*, and *Candida krusei*. They found the direct effect of cinnamon essential oil on the cell wall synthesis of the yeast [21]. Many researchers have indicated that there is a correlation between the chemical composition and the antifungal activity. They also showed that the strong antifungal activity of the oils derived from the skin and the cinnamon leaf was due to the high levels of cinnamaldehyde (44.2%) and eugenol (90.2%) [22]. However, other compounds may also contribute to antifungal activity. The study of Arbabi et al., (2011) aimed at comparing the effect of thyme, cloves and cinnamon extract with nystatin on the inhibition of *Candida albicans*. The diameter of the inhibition zone of each plant extract was compared with the diameter of the positive control growth zone with ANOVA. Candida inhibition in the nystatin group was 32.6 ± 0.84, and in cinnamon, extract group was 31.3 ± 0.82. In addition, *candida* inhibition in clove extract group was 27.4 ± 0.82 and finally in thyme evaluated 13 ± 0.82. As a result, the highest effect was first on nystatin, cinnamon, cloves and ultimately thyme ($P < 0.000$). The final result was that thyme, cloves, and cinnamon had antifungal effects on the *Candida albicans* [12]. However, in these two studies, the method of measuring the inhibitory effect of cinnamon extract is not the same, but both studies have confirmed that cinnamon has antifungal effects. The aim of the study of Ataei et al., (2007) was an experimental evaluation on the antifungal effects of absinthium Artemisia, eucalyptus, onion, cinnamon, turmeric, sage, mint, and Calendula officinalis on a standard strain of *Candida albicans* compared to nystatin mouthwash. The results showed that the extract of each of the six herbs had an antifungal effect. Cinnamon has been shown to be more potent and more effective than the onion, mint, Calendula officinalis and sage, with the same effect as turmeric, absinthium Artemisia and eucalyptus. Also, cinnamon, absinthium Artemisia, eucalyptus exhibited significant antifungal effects in comparison with nystatin [8]. Carvalhinho et al., (2012) and Condò et al., (2018) also showed a significant activity of cinnamon against *Candida albicans* [23], [24]. Gucwa et al., (2018) exhibited both fungistatic and fungicidal activity of some plant oils toward *Candida albicans* and *Candida glabrata* isolates and the highest activity was demonstrated for cinnamon oil [25]. The results of these studies confirm the result of the present study and the antifungal properties of cinnamon.

In conclusion, although the cinnamaldehyde extract in comparison with nystatin can prevent the

growth and loss of *Candida albicans* and *Candida glabrata* with higher concentrations; given the antifungal effects of this plant, it is expected that it could be considered as an effective medicinal plant. Since limited studies have been conducted on cinnamon plants, it is suggested to perform extraction of other active substances and its compounds, molecular studies to understand the mechanisms of action of the ingredients, Invivo tests in the animal model following Invitro tests and cell culture experiments.

Acknowledgements

We thank our colleagues from Shahid Beheshti University of Medical Sciences and Baqiyatallah University of Medical Sciences who provided insight and expertise that greatly assisted the research, although they may not agree with all of the conclusions of this paper.

References

1. Lalla RV, Patton LL, Dongari-Bagtzoglou A. Oral candidiasis: pathogenesis, clinical presentation, diagnosis and treatment strategies. Journal of the California Dental Association. 2013; 41(4):263-8. PMid:23705242

2. Millsop JW, Fazel N. Oral candidiasis. Clinics in dermatology. 2016; 34(4):487-94. https://doi.org/10.1016/j.clindermatol.2016.02.022 PMid:27343964

3. Mortazavi H, Safi Y, Baharvand M, Jafari S, Anbari F, Rahmani S. Oral White Lesions: An Updated Clinical Diagnostic Decision Tree. Dentistry journal. 2019; 7(1):15. https://doi.org/10.3390/dj7010015 PMid:30736423

4. Suvirya S, Gandhi R, Agarwal J, Patil R. Erythematous candidiasis leading to systemic manifestations of human immunodeficiency virus co-infection with secondary syphilis: a diagnostic and therapeutic dilemma. European journal of dentistry. 2015; 9(3):449. https://doi.org/10.4103/1305-7456.163219 PMid:26430379 PMCid:PMC4570002

5. Gürgan CA, Zaim E, Bakirsoy I, Soykan E. Short-term side effects of 0.2% alcohol-free chlorhexidine mouthrinse used as an adjunct to non-surgical periodontal treatment: a double-blind clinical study. Journal of periodontology. 2006; 77(3):370-84. https://doi.org/10.1902/jop.2006.050141 PMid:16512751

6. Gruenwald J, Freder J, Armbruester N. Cinnamon and health. Critical reviews in food science and nutrition. 2010; 50(9):822-34. https://doi.org/10.1080/10408390902773052 PMid:20924865

7. Rao PV, Gan SH. Cinnamon: a multifaceted medicinal plant. Evidence-Based Complementary and Alternative Medicine. 2014; 2014:642942.

8. Ataei Z, Ansari M, AYAT EM, Mirzaei A. In-vitro study of antifungal effects of selected herbal extracts on standard and wild strains of Candida albicans. The Journal of Islamic Dental Association of IRAN. 2007; 19:91-97

9. Fani MM, Kohanteb J. Inhibitory activity of Cinnamomum zeylanicum and eucalyptus globulus oils on Streptococcus mutans, Staphylococcus aureus, and Candida species isolated from patients with oral infections. Journal of Dentistry, Shiraz University of Medical Sciences. 2011; 11:14-22.

10. Almeida LD, Paula JF, Almeida RV, Williams DW, Hebling J, Cavalcanti YW. Efficacy of citronella and cinnamon essential oils on Candida albicans biofilms. Acta Odontologica Scandinavica. 2016; 74(5):393-8. https://doi.org/10.3109/00016357.2016.1166261 PMid:27098375

11. Lyu X, Zhao C, Yan ZM, Hua H. Efficacy of nystatin for the treatment of oral candidiasis: a systematic review and meta-analysis. Drug design, development and therapy. 2016; 10:1161–1171. https://doi.org/10.2147/DDDT.S100795 PMid:27042008 PMCid:PMC4801147

12. SHerzaee M, Poorzamani M. Inhibitory Effects of Plant Extracts Containing Thyme, Clore and Cinnamon Compared to Nystatin On Candida Albicans.(Invitro). Res Dent Sci. 2012; 8(4):175-9.

13. Baharvand M, Jafari S, Mortazavi H. Herbs in oral mucositis. Journal of clinical and diagnostic research. 2017; 11(3):ZE05–ZE11. https://doi.org/10.7860/JCDR/2017/21703.9467

14. Shekar BR, Nagarajappa R, Suma S, Thakur R. Herbal extracts in oral health care-A review of the current scenario and its future needs. Pharmacognosy reviews. 2015; 9(18):87–92. https://doi.org/10.4103/0973-7847.162101 PMid:26392704 PMCid:PMC4557240

15. Li CL, Huang HL, Wang WC, Hua H. Efficacy and safety of topical herbal medicine treatment on recurrent aphthous stomatitis: a systemic review. Drug design, development and therapy. 2016; 10:107–115. PMid:26770058

16. Ahmad I, Beg AZ. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. Journal of ethnopharmacology. 2001; 74(2):113-23. https://doi.org/10.1016/S0378-8741(00)00335-4

17. Herrera-Arellano A, López-Villegas EO, Rodríguez-Tovar AV, Zamilpa A, Jiménez-Ferrer E, Tortoriello J, Martínez-Rivera MA. Use of antifungal saponin SC-2 of Solanum chrysotrichum for the treatment of vulvovaginal candidiasis: in vitro studies and clinical experiences. African Journal of Traditional, Complementary and Alternative Medicines. 2013; 10(3):410-7. https://doi.org/10.4314/ajtcam.v10i3.4

18. Taguchi Y, Ishibashi H, Takizawa T, Inoue S, Yamaguchi H, Abe S. Protection of oral or intestinal candidiasis in mice by oral or intragastric administration of herbal food, clove (Syzygium aromaticum). Nippon Ishinkin Gakkai Zasshi. 2005; 46(1):27-33. https://doi.org/10.3314/jjmm.46.27

19. Sheidaei S, Sadeghi T, Jafarnejad F, Rajabi O, Najafzadeh M. Herbal medicine and vaginal candidiasis in Iran: a review. Evidence Based Care. 2017; 7(2):71-7.

20. Silva S, Negri M, Henriques M, Oliveira R, Williams DW, Azeredo J. Candida glabrata, Candida parapsilosis and Candida tropicalis: biology, epidemiology, pathogenicity and antifungal resistance. FEMS microbiology reviews. 2012 Mar 1; 36(2):288-305. https://doi.org/10.1111/j.1574-6976.2011.00278.x PMid:21569057

21. Castro RD, Lima EO. Anti-candida activity and chemical composition of Cinnamomum zeylanicum blume essential oil. Brazilian Archives of Biology and Technology. 2013; 56(5):749-55. https://doi.org/10.1590/S1516-89132013000500005

22. Tabassum N, Vidyasagar G. Antifungal investigations on plant essential oils. A review . Int J Pharm Sci. 2013; 5:19-28

23. Carvalhinho S, Costa AM, Coelho AC, Martins E, Sampaio A. Susceptibilities of Candida albicans mouth isolates to antifungal agents, essentials oils and mouth rinses. Mycopathologia. 2012; 174(1):69-76. https://doi.org/10.1007/s11046-012-9520-4 PMid:22246961

24. Condò C, Anacarso I, Sabia C, Iseppi R, Anfelli I, Forti L, de Niederhäusern S, Bondi M, Messi P. Antimicrobial activity of spices essential oils and its effectiveness on mature biofilms of human pathogens. Natural product research. 2018:1-8. https://doi.org/10.1080/14786419.2018.1490904 PMid:30317865

25. Gucwa K, Milewski S, Dymerski T, Szweda P. Investigation of the antifungal activity and mode of action of Thymus vulgaris, Citrus limonum, Pelargonium graveolens, Cinnamomum cassia, Ocimum basilicum, and Eugenia caryophyllus essential oils. Molecules. 2018; 23(5):1116. https://doi.org/10.3390/molecules23051116 PMid:29738503 PMCid:PMC6099571

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Apr 15; 7(7):1071-1076.
https://doi.org/10.3889/oamjms.2019.209
eISSN: 1857-9655
Basic Science

In Vivo Toxicity Study of Ethanolic Extracts of *Evolvulus alsinoides* & *Centella asiatica* in Swiss Albino Mice

Mukesh Kumar Yadav[1*], Santosh Kumar Singh[2], Manish Singh[3], Shashank Shekhar Mishra[4], Anurag Kumar Singh[2], Jyoti Shankar Tripathi[1], Yamini Bhusan Tripathi[5]

[1]Department of Kayachikitsa, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India; [2]Centre of Experimental Medicine & Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India; [3]Department of Pharmacology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India; [4]Department of Vikriti Vigyan, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India; [5]Department of Medicinal Chemistry, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India

Citation: Yadav MK, Singh SK, Singh M, Mishra SS, Singh AK, Tripathi JS, Tripathi YB. In Vivo Toxicity Study of Ethanolic Extracts of *Evolvulus alsinoides* & *Centella asiatica* in Swiss Albino Mice. Open Access Maced J Med Sci. 2019 Apr 15; 7(7):1071-1076. https://doi.org/10.3889/oamjms.2019.209

Keywords: Centella asiatica; Evolvulus alsinoides L.; Ethanolic extracts; Sub-acute; Toxicity

***Correspondence:** Prof. Jyoti Shankar Tripathi and Mukesh Kumar Yadav. Department of Kayachikitsa, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. E-mail: mukesh.yadav@bhu.ac.in

Received: 09-Jan-2019; **Revised:** 04-Mar-2019; **Accepted:** 05-Mar-2019; **Online first:** 11-Apr-2019

Funding: This research did not receive any financial support

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

Abstract

AIM: We aimed to investigate several parameters after the *in vivo* acute and sub-acute administration of ethanolic extracts from *E. alsinoides* & *C. asiatica.*

METHODS: Malignant Ovarian Germ Cell Tumors for *in vivo* toxicity study guidelines 423 and 407 of Organization for Economic Co-operation and Development (OECD) were followed for acute and sub-acute toxicity assays respectively. For LD50 evaluation, a single dose of ethanolic extracts of Evolvulus alsinoides L. (EEA) and ethanolic extracts of Centella asiatica (ECA) was orally administered to mice at doses of 200, 400, 800, 1600 and 2000 mg/kg. Then the animals were observed for 72 hours. For acute toxicity evaluation, a single dose of both extracts was orally administered to mice at doses of 300, 600, 1200 and 2000 mg/kg and the animals were observed for 14 days. In the sub-acute study, the extracts were orally administered to mice for 28 days at doses of 300, 600, 1200 and 2000 mg/kg. To assess the toxicological effects, animals were closely observed on general behaviour, clinical signs of toxicity, body weight, food and water intake. At the end of the study, it was performed biochemical and hematological evaluations, as well as histopathological analysis from the following organs: brain, heart, liver, and kidney.

RESULTS: The oral administration of E. alsinoides and C. asiatica ethanolic extracts, i.e. EEA 300, EEA 600, EEA 1200, EEA 2000, ECA 300, ECA 600, ECA 1200 & ECA 2000 mg/kg doses showed no moral toxicity effect in LD50, acute and sub-acute toxicity parameters.

CONCLUSION: In this study, we had found that *E. alsinoides* & *C. asiatica* extract at different doses cause no mortality in acute and sub-acute toxicity study. Also, histopathology of kidney, liver, heart, and brain showed no alterations in tissues morphology.

Introduction

Centella asiatica belonging to the family: Apiaceae is native to most of the Asian countries. Being herbaceous, it contains stems which are long, filiform and prostrate with long internodes containing roots, 1-5 leaves per node which are 50-350 cm in radius, uninformed, deeply cordate, long-petioled and oval or orbicular in shape, 3-6 small flowers which are purple to white-green in color and are arranged in umbels arising from the axis of the leaves. *Centella asiatica* grows well in both tropical and sub-tropical countries. The pharmacological activity of *Centella asiatica* is due to several active constituents including kaempferol-3-o-β-d-glucuronide, quercetin-3-o-β-d-glucuronide, castillicetin, apigenin, rutin, luteolin, naringin [1], [2] rosmarinic acid, chlorogenic acid, 3,4-di-o-caffeoyl quinic acid, 1,5-di-o-caffeoyl quinic acid, 3,5-di-o-caffeoyl quinic acid, 4,5-di-o-caffeoyl quinic acid, isochlorogenic acid, asiaticoside, centelloside, madecassoside, brahmoside, brahminoside (saponin glycosides), asiaticentoic acid, centellic acid, madecassic acid, terminolic acid and betulic acid [3].

The plant *Centella asiatica* has been reported

as traditionally used for various ailments including wound healing, bronchitis, asthma, diabetes, allergy, cancer, diuretic, and hypertension and to improve mental ability [4], [5], [6], [7].

Evolvulus alsinoides L. (Family: *Convolvulaceae*) is a small genus composed of about 10–15 species widely distributed in Asian and American countries, with some of its species used medicinally. *Evolvulus alsinoides* is one of the several well-known Ayurvedic crude drugs that have a significant place in the traditional medicinal system of India due to its memory enhancing properties. In *Evolvulus alsinoides* some active chemical constituents present like triacontane, pentatriacontane, evolving, β-sitosterol, two alkaloids betaine and shankpushpin, caffeic acid, 6-methoxy-7-O-b-glucopyranoside coumarin, kaempferol-7-O-b-glucopyranoside, kaempferol-3-O-b-glucopyranoside and kaempferol-3-O-b-glucopyranoside and quercetine- 3-O-b-glucopyra-noside in this species [8].

This plant has some traditional pharmacological activities such as gastro protective [9], antibacterial [10], antiulcer [11], immunomodulatory [12], cytoprotective [13], adaptogenic and antiamnesic [14], anxiolytic [15], diabetes [16], syphilis [17], tonic to brain strength & memory enhancer [18], analgesic and anti-inflammatory activity [19].

We aimed to investigate several parameters after the *in vivo* acute and sub-acute administration of ethanolic extracts from *E. alsinoides* & *C. asiatica.*

Material and Methods

Plant material

Whole plant material of *Centella asiatica* and *Evolvulus alsinoides* were collected from village Ramnapur, Varanasi, Uttar Pradesh, India in October 2015 and authentication was done by Department of Botany, Banaras Hindu University, India and also herbarium of *Evolvulus alsinoides* (voucher specimen no. Convolvul./03/2015) and *Centella asiatica* (voucher specimen no. Apia/02/2015) plants were deposited in the Department of Botany, Banaras Hindu University, India.

Preparation of extracts

The extraction of both plants was done with soxhlet method in ethanolic solvents at 72-82°C for 72 hours. The Soxhlet extraction has widely been used for extracting valuable bioactive compounds from various natural sources. It is used as a model for the comparison of new extraction alternatives. Generally, a small amount of dry sample is placed in a thimble. The thimble is then placed in distillation flask which contains the solvent of particular interest. After reaching an overflow level, the solution of the thimble-holder is aspirated by a syphon.

Syphon unloads the solution back into the distillation flask. This solution carries extracted solutes into the bulk liquid. The solute has remained in the distillation flask, and solvent passes back to the solid bed of plant. The process repeatedly runs until the extraction is completed. Per cent yield for *Centella Asiatica* and *Evolvulus alsinoides* were 16.7% w/w and 15.3% w/w respectively.

Preparation of extract samples

Ethanolic extracts of *E. alsinoides* (EEA) and *C. asiatica* (ECA) were solubilized in distilled water to obtain solutions of 30, 60, 120 and 200 mg/ml. The doses were evaluated as 300, 600, 1200 and 2000 mg/kg.

Toxicity assays

The safety parameters assessed by conducting the acute and sub-acute toxicity study according to the OECD guidelines [20] 423 and 407 respectively.

Animals

The experimental Swiss albino mice (male and female) 7-8-week-old of 25-30 gm weight were issued by Animal house of Institute of Medical Sciences, Banaras Hindu University Varanasi, Uttar Pradesh. Animals were divided into experimental groups, housed in plastic cages and maintained on a 12-hour light and 12-hour dark cycle. They were given standard food and water ad libitum. The Central Animal Ethical Committee of Banaras Hindu University approved all experimental procedures (CAEC/196).

LD 50 assay

LD50 (Lethal Dose) is the amount of a drug or extracts given at once, which causes the death of 50% population of test animals. This is one way to measure the short-term toxicity of the drug or extract. For LD50 a single dose of ethanolic extracts of both plants *Centella asiatica* and *Evolvulus alsinoides* L. was orally administered to mice at doses of 200, 400, 800, 1600 and 2000 mg/kg. Then the animals were observed for 72 hours.

Acute toxicity assay

The animals were divided into nine experimental groups of 6 animals each (3 male and 3 female). Group 1 received 10 µl/g of distilled water and served as control.

Groups 2 to 5 treated with ethanolic extract of *E. alsinoides* (EEA) at the doses of 300, 600, 1200 and 2000 mg/kg.

Groups 6 to 9 were treated with ethanolic extract of *C. asiatica* (ECA) at doses of 300, 600, 1200 and 2000 mg/kg respectively.

All treatments were administered once by oral gavage. Animals were closely observed for 4 hours following administration and once a day for 14 days on general behaviour, clinical signs of toxicity, mortality, food and water intake. Body weight was measured before and after administration on days 4, 7, 10 and 14. At the end of the experiment, animals were anaesthetized with ketamine (20 mg/kg i.p.). After the anaesthesia has reached depth, the cardiac puncture was performed to collect blood for biochemical and haematological evaluations.

Sub-acute toxicity assay

The animals were divided into nine experimental groups of 6 animals each (3 male and 3 female). Group 1 received 10 µl/g of distilled water and served as control.

Groups 2 to 5 treated with ethanolic extract of *E. alsinoides* (EEA) at the doses of 300, 600, 1200 and 2000 mg/kg.

Groups 6 to 9 were treated with ethanolic extract of *C. asiatica* (ECA) at doses of 300, 600, 1200 and 2000 mg/kg respectively.

All treatments were administered once by oral gavage daily 7 days each week for 28 days. Animals were closely observed for 28 days on general behaviour, clinical signs of toxicity, mortality, food and water intake. Body weight was measured before and after administration on days 7, 14, 21 and 28. At the end of the experiment, animals were anaesthetised with ketamine (20 mg/kg i.p.). After the anaesthesia has reached depth, the cardiac puncture was performed to collect blood for biochemical and haematological evaluations.

Haematological analysis

The haematological evaluation was performed in all surviving animals at the end of the experiment. The complete blood count was performed using an automated haematology analyser. Haematological evaluations included haemoglobin concentration (HGB), red blood cell count (RBC), platelet count (PLT), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and white blood cell count (WBC).

Blood serum biochemistry analysis

The biochemical evaluation was performed in all surviving animals at the end of the experiment. The collected blood was transferred to tubes without anticoagulant and allowed to stand for 60 min at room temperature and centrifuged at 4000 rpm for 10 min. The serum from each blood sample was recovered and stored in cryogenic tubes at -80°C deep freezer. Urea, Creatinine, Serum glutamic oxaloacetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT) and alkaline phosphatase were evaluated.

Histopathology

The organs were collected from all surviving animals, washed with saline solution 0.9% (w/v), weight and fixed in 40% formaldehyde solution. Then organs were processed for paraffin embedding. Five µm thick sections were prepared and stained with hematoxylin and eosin (H&E). The tissues were analysed in an optical microscope for their general structure, signs of inflammation, degenerative changes and necrosis evidence. The images were captured with the microscope Motic B1 series, scanned through micro camera Moticam 480 using the Motic Images Plus 2.0ML Application Suite software.

Statistical analysis

The relative haematological and biochemical data were expressed as mean ± standard error of the mean (SEM). Data were submitted to analysis of variance (one-way ANOVA) followed by Dunnett's multiple comparison tests. The results were expressed as mean ± SEM. The software GraphPad Prism 6.0 (GraphPad Software, USA) was used for statistical analysis. $P < 0.05$ were considered statistically significant.

Results

Acute toxicity & Sub-acute toxicity

General signs and mortality

No deaths were recorded within 72 hours in LD50 assay after administration of the extracts. No signs of toxicity were observed in animal groups after the treatment with EEA 300, EEA 600, EEA 1200, EEA 2000, ECA 300, ECA 600, ECA 1200 and ECA 2000 mg/kg.

Body weight, relative organ weight, food and water intake

Animal treated with EEA and ECA at the three evaluated doses showed weight gain throughout the entire experiment duration. The increase was the same in treated and control group animals, and the treatment did not affect relative organs weights, food, and water intake (Table 1).

Table: 1 List of different parameters and general signs assessed during toxicity study

Parameters	Dose (mg/kg)							
	EEA 300	EEA 600	EEA 1200	EEA 2000	ECA 300	ECA 600	ECA 1200	ECA 2000
Body weight	N	N	N	N	N	N	N	N
Feed Intake	N	N	N	N	N	N	N	N
Water Intake	N	N	N	N	N	N	N	N
Fur Condition	N	N	N	N	N	N	N	N
Nails Colour	N	N	N	N	N	N	N	N
Eye Colour	N	N	N	N	N	N	N	N
Convulsion	N	N	N	N	N	N	N	N
Locomotion	N	N	N	N	N	N	N	N
Dysponea	N	N	N	N	N	N	N	N
Sedation	N	N	N	N	N	N	N	N
Aggressive Behavior	N	N	N	N	N	N	N	N

(Normal = N, Abnormal = Ab).

Table: 2 Hematological parameters of Swiss mice treated for 28 days with different doses (300, 600, 1200 and 2000 mg/kg) of ethanolic extracts of *E. alsinoides* (EEA) and *C. asiatica* (ECA) n = 6 swiss albino mice.

	Haematological parameters							
Groups	HGB (g/dL)	RBC (10^6 /µL)	PLT (10^3/µL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	WBC (10^3/µL)
Control	12.3 ± 0.28	6.30 ± 0.30	901.2 ± 17.2	41.2 ± 1.09	57.0 ± 0.99	19.5 ± 0.50	32.1 ± 1.01	5.94 ± 0.20
EEA 300	13.0 ± 0.46	6.90 ± 0.17	771.3 ± 39.8	39.6 ± 1.07	54.0 ± 0.36	17.2 ± 0.72	31.3 ± 1.65	5.85 ± 0.55
EEA 600	12.7 ± 0.28	6.24 ± 0.24	936.4 ± 41.0	41.7 ± 1.06	57.4 ± 1.56	20.4 ± 1.06	33.7 ± 1.61	5.89 ± 0.42
EEA 1200	12.93 ± 0.73	6.46 ± 0.44	928.8 ± 23.8	43.66 ± 1.45	57.1 ± 0.96	19.26 ± 0.76	32.56 ± 0.63	5.48 ± 0.63
EEA 2000	13.33 ± 0.63	7.06 ± 0.69	795.9 ± 26.1	43.16 ± 2.00	57.20 ± 1.83	20.33 ± 1.80	31.86 ± 1.68	5.51 ± 0.39
ECA 300	13.63 ± 0.52	7.22 ± 0.31	816.1 ± 10.2	41.52 ± 1.29	55.09 ± 1.09	18.75 ± 1.10	30.77 ± 1.21	5.46 ± 0.29
ECA 600	12.6 ± 0.3	6.66 ± 0.24	795.7 ± 9.1	39.2 ± 0.46	53.43 ± 0.66	16.86 ± 0.17	28.36 ± 1.53	4.89 ± 0.16
ECA 1200	13.5 ± 0.4	7.25 ± 0.5	862.5 ±14.7	45.5 ± 1.9	55.8 ± 0.8	18.8 ± 0.7	32.9 ± 0.7	5.27 ± 0.5
ECA 2000	13.6 ± 0.57	7.46 ± 0.78	784.3 ± 25.5	46.2 ± 1.12	57.5 ± 1.47	20.8 ± 1.28	33.5 ± 0.40	5.79 ± 0.21

One-way ANOVA followed by Dunett's multiple comparison tests. *p < 0.05, **p < 0.01, ***p < 0.001. Abbreviations: Hemoglobin concentration (HGB), Red blood cell count (RBC), platelet count (PLT), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and white blood cell count (WBC).

Haematological parameters

Treatment with EEA and ECA at all doses did not produce any changes on animal haematological parameters.

Biochemical parameter

Treatment with EEA and ECA at all doses did not produce any statistically significant changes on Urea, Creatinine, SGOT, SGPT and alkaline phosphatase (Table 3).

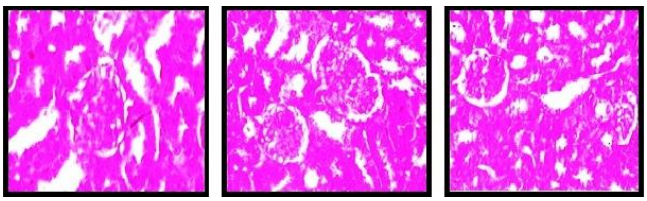
Table 3: Blood serum biochemical parameters of Swiss albino mice treated with dosage (300, 600, 1200 & 2000 mg/kg) of ethanolic extracts of *E. alsinoides* & *C. asiatica.* N = 6 swiss albino mice.

Groups	Urea Normal value = 25-30 mg/dl	Creatinine Normal value = 0.2-0.9 mg/dl	SGOT Normal value = 54-298 mg/dl	SGPT Normal value = 17-77 mg/dl	Alkaline phosphatase Normal value = 35-96 mg/dl
Normal	26.80 ± 1.12	0.3 ± 0.06	57.92 ± 1.62	58.18 ± 3.22	39.41 ± 3.22
EEA 300	26.54 ± 0.82	0.4 ± 0.08	58.59 ± 4.19	52.69 ± 1.11	55.45 ± 4.76
EEA 600	27.45 ± 0.62	0.6 ± 0.1	55.19 ± 2.06	62.67 ± 6.26	46.51 ± 5.73
EEA 1200	27.68 ± 0.68	0.4 ± 0.08	55.26 ± 1.30	59.10 ± 2.8	48.87 ± 2.05
EEA 2000	27.88 ± 0.82	0.4 ± 0.12	61.82 ± 4.28	51.45 ± 8.93	54.38 ± 3.33
ECA 300	27.38 ± 1.02	0.5 ± 0.13	55.08 ± 0.57	63.82 ± 5.79	51.54 ± 4.08
EAC 600	26.33 ± 0.68	0.4 ± 0.12	56.24 ± 1.24	52.80 ± 1.77	55.55 ± 5.08
ECA 1200	27.36 ± 1.80	0.5 ± 0.05	58.24 ± 2.00	59.36 ± 3.56	41.32 ± 3.69
ECA 2000	26.24 ± 0.83	0.2 ± 0.03	57.94 ± 4.51	57.49 ± 2.53	50.82 ± 2.08

One-way AVOVA followed by Dunett's multiple comparison test. *p < 0.05, **p < 0.01, ***p < 0.001.

Histopathological analysis

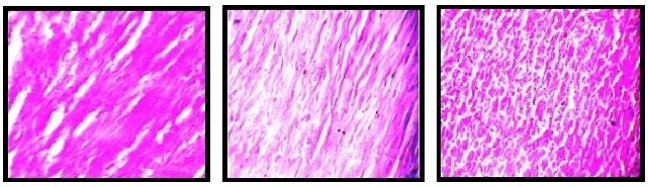
The oral administration of ECA and EEA did not produce significant dose-dependent Histopathological alterations. At the four evaluated doses, it was not observed any tissue damage on the kidney, heart, liver and brain of mice.



(a) Normal (b) EEA (c) ECA

Figure 1: Histopathological slides of kidney organ shown no changes in Swiss albino mice treated with (b) EEA & (c) ECA extracts

Microscopic histological slides from different organs of Swiss albino mice treated with ethanolic extracts of *E. alsinoides* & *C. asiatica,* i.e. EEA & ECA respectively are shown in Figures 1 to 4.



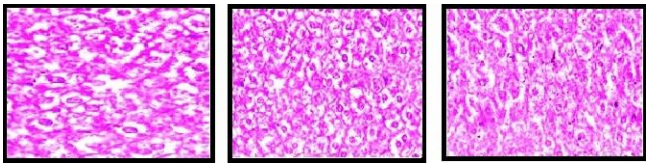
(a) Normal (b) EEA (c) ECA

Figure 2: Histopathological slides of heart organ shown no changes in Swiss albino mice treated with (b) EEA & (c) ECA extracts

Discussion

To assess preliminary toxicity study animal models are widely used because the early

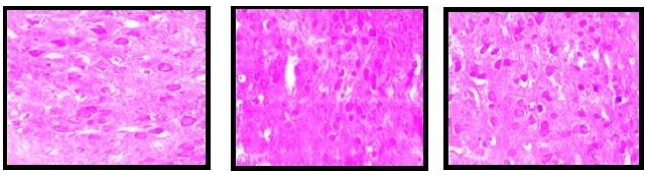
identification of side effects is usually predictive of the toxicity in humans and can save time, resources and efforts [21]. In this study, several parameters evaluated after the *in vivo* acute and sub-acute administration of ethanolic extracts from *E. alsinoides* & *C. asiatica* were investigated. In toxicological evaluation mortality is an important criterion [22] and there was no mortality seen in both acute and sub-acute evaluation of extracts. For LD50 no death was recorded in 72 hours of administration of extracts.



(a) Normal (b) EEA (c) ECA

Figure 3: Histopathological slides of LIVER organ shown no changes in Swiss albino mice treated with (b) EEA & (c) ECA extracts

In acute toxicity, no death was recorded in 14 days extracts administration and in sub-acute toxicity study also no death recorded for 28 days extract administration. Clinical signs of toxicity were observed after the acute administration and during the sub-acute evaluation for all extract dosage. Liver damage is usually assessed by the determination of SGOT, SGPT, and alkaline phosphatase.



(a) Normal (b) EEA (c) ECA

Figure 4: Histopathological slides of brain organ shown no changes in Swiss albino mice treated with (b) EEA & (c) ECA extracts

It was not observed any significant alterations in serum levels of these three markers of liver function after acute and sub-acute administration of extracts and histopathological analysis did not show liver damage. Renal function was evaluated by serum levels of urea, creatinine and by histological analysis.

The histopathological evaluation did not reveal alterations in this organ of any treated groups of sub-acute toxicity. Also, no tissue alterations found in the heart and brain of animals treated with 28 days *E. alsinoides* & *C. asiatica* extracts.

In this study, we had found that *E. alsinoides* & *C. asiatica* extracts at different doses cause no mortality in acute and sub-acute toxicity study. In addition, histopathology of kidney, liver, heart, and brain showed no alterations in tissues morphology.

Acknowledgement

The authors would like to thank the staff of Centre of Experimental Medicine & Surgery (CEMS), Institute of medical sciences, Banaras Hindu University, Varanasi for providing the well-equipped laboratory & animal house to conduct this study.

References

1. Bhandari P, Kumar N, Gupta AP, Singh B, Kaul VK. A rapid RP-HPTLC densitometry method for simultaneous determination of major flavonoids in important medicinal plants. Journal of separation science. 2007; 30(13):2092-6. https://doi.org/10.1002/jssc.200700066 PMid:17654615

2. Zheng C, Qin L. Chemical components of Centella asiatica and their bioactivities. Journal of Chinese Integrative Medicine. 2007; 5(3):348-51. https://doi.org/10.3736/jcim20070324 PMid:17498500

3. Barnes J, Anderson LA, Phillipson JD. Herbal medicines. Pharmaceutical Press, 2007.

4. Kumar MV, Gupta YK. Effect of different extracts of Centella asiatica on cognition and markers of oxidative stress in rats. Journal of ethnopharmacology. 2002; 79(2):253-60. https://doi.org/10.1016/S0378-8741(01)00394-4

5. Pittella F, Dutra R, Junior D, Lopes MT, Barbosa N. Antioxidant and cytotoxic activities of Centella asiatica (L) Urb. International journal of molecular sciences. 2009;10(9):3713-21. https://doi.org/10.3390/ijms10093713 PMid:19865514 PMCid:PMC2769141

6. Park BC, Bosire KO, Lee ES, Lee YS, Kim JA. Asiatic acid induces apoptosis in SK-MEL-2 human melanoma cells. Cancer letters. 2005; 218(1):81-90. https://doi.org/10.1016/j.canlet.2004.06.039 PMid:15639343

7. Tang B, Zhu B, Liang Y, Bi L, Hu Z, Chen B, Zhang K, Zhu J. Asiaticoside suppresses collagen expression and TGF-β/Smad signaling through inducing Smad7 and inhibiting TGF-βRI and TGF-βRII in keloid fibroblasts. Archives of dermatological research. 2011; 303(8):563-72. https://doi.org/10.1007/s00403-010-1114-8 PMid:21240513

8. Gupta P, Siripurapu KB, Ahmad A, Palit G, Arora A, Maurya R. Anti-stress Constituents of Evolvulus alsinoides: An Ayurvedic Crude Drug. Chem Pharma Bull. 2007; 55:771. https://doi.org/10.1248/cpb.55.771

9. Ratnasooriya WD, Hewageegana HGSP, Jayakody JRAC, Ariyawansa HAS, Kulatunga RDH. Gastroprotective activity of Evolvulus alsinoides L. powder. Aust J Med Herbalism. 2005; 17:55-60.

10. Tharan NT, Vadivu R, Palanisamy M, Justin V. Antibacterial Activity of Evolvulus alsinoides. Indian Drugs. 2003; 40:585-586.

11. Purohit MG, Shanthaveerappa BK, Badami S, Swamy HKS, Shrishailappa B, Antiulcer and anticatatonic activity of alcoholic extract of Evolvulus alsinoides (Convolvulaceae). Ind J Pharma Sci. 1996; 58:110-112.

12. Ganju L, Karan D, Chanda S, Srivastava KK, Sawhney RC, Selvamurthy W. Immunomodulatory effects of agents of plant origin. Biomed-Pharmacother. 2003; 57:296-300. https://doi.org/10.1016/S0753-3322(03)00095-7

13. Bhatnagar M, Shukla SD, Jain S, Mundra A. Cytoprotective effects of Shankhpushpi - an E. alsinoides preparation on Hippocampal cells in mice. Indian Drugs. 2000; 37:280-285.

14. Siripurapu KB, Gupta P, Bhatia G, Maurya R, Nath C, Palit G. Adaptogenic and anti-amnesic properties of Evolvulus alsinoides in

rodents. Pharmacol Biochem Behav. 2005; 81:424-432. https://doi.org/10.1016/j.pbb.2005.03.003 PMid:15899513

15. Alok Nahata, U.K. Patil, and V.K. Dixit. Anxiolytic activity of Evolvulus alsinoides and Convulvulus pluricaulis in rodents. Pharmaceutical Biology. 2009; 47(5):444-451. https://doi.org/10.1080/13880200902822596

16. Alam MM, Siddiqui MB, Hussain W. Treatment of diabetes through herbal drugs in rural India. Fitoterpia. 1990; 61:240-242.

17. Goyal PR et al. Shankhpushpi (Evolvulus alsinoides Linn): a medicinal herb. Int J Mendel. 2005; 22:124.

18. Auddy B, Ferreira M, Blasina F et al. Screening of Antioxidant activity of some three Indian medicinal plants traditionally used for the management of neurodegenerative diseases. Journal of Ethnopharmacology. 2003; 84:131-138. https://doi.org/10.1016/S0378-8741(02)00322-7

19. Kankariya RD, Shetty SC, Shete RV, Ingale SD. Deccan J Pharmacology. 2011; 2(4).

20. Organization for Economic Co-operation and Development guideline 407 & 423.

21. Kramer JA, O'Neill E, Phillips ME, Bruce D, Smith T, Albright MM, Bellum S, Gopinatan S, Heydorn WE, Liu X, Nouraldeen A, Payne BJ, Read R, Vogel P, Yu XQ, Wilson AGE. Early toxicology signal generation in the mouse. Toxicol. Pathol. 2010; 38:452-471. https://doi.org/10.1177/0192623310364025 PMid:20305093

22. Asare GA, Gyan B, Bugyei K, Adjei S, Mahama R, Addo P, Otu-Nyarko L, Wiredu EK, Nyarko A. Toxicity potentials of the nutraceutical Moringa oleifera at supra-supplementation levels. Journal of ethnopharmacology. 2012; 139(1):265-72. https://doi.org/10.1016/j.jep.2011.11.009 PMid:22101359

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Apr 15; 7(7):1077-1081.
https://doi.org/10.3889/oamjms.2019.279
eISSN: 1857-9655
Basic Science

Chemical Characterization of Hydrogels Crosslinked with Polyethylene Glycol for Soft Tissue Augmentation

Damiano Monticelli[1], Virginia Martina[2], Roberto Mocchi[3], Raffaele Rauso[4], Umberto Zerbinati[5], Giovanna Cipolla[5], Nicola Zerbinati[6]*

[1]University of Insubria, Department of Science and High Technology, Como, Italy; [2]MatexLab S.p.a., Department of Research and Development, Brindisi, Italy; [3]UB-CARE S.r.l. Spin-off University of Pavia, Pavia, Italy; [4]University of Foggia, Department of Plastic Reconstructive Surgery, Foggia, Italy; [5]Centro Medico Polispecialistico, Dermatology Department, Pavia, Italy; [6]University of Insubria, Department of Medicine and Surgery, School of Medicine, Varese, Italy

Citation: Monticelli D, Martina V, Mocchi R, Rauso R, Zerbinati U, Cipolla G, Zerbinati N. Chemical Characterization of Hydrogels Crosslinked with Polyethylene Glycol for Soft Tissue Augmentation. Open Access Maced J Med Sci. 2019 Apr 15; 7(7):1077-1081. https://doi.org/10.3889/oamjms.2019.279

Keywords: Hydrogels; Polyethylene glycol; Soft tissue augmentation

***Correspondence:** Zerbinati, University of Insubria, Department of Medicine and Surgery, School of Medicine, Varese, Italy. E-mail: nicola.zerbinati@uninsubria.it

Received: 27-Feb-2019; **Revised:** 04-Apr-2019; **Accepted:** 05-Apr-2019; **Online first:** 14-Apr-2019

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Hyaluronic acid (HA) based hydrogels for esthetic applications found widespread use. HA should be crosslinked for this application to achieve the correct viscoelastic properties and avoid fast degradation by the hyaluronidase enzyme naturally present in the skin: these properties are controlled by the amount of crosslinker and the fraction that is effectively crosslinked (i.e. that binds two HA chains).

AIM: Crosslinking by polyethylene glycol diglycidyl ether (PEGDE) has been more recently introduced and showed attractive features in terms of viscoelastic properties and reduced biodegradation. Aim of this paper is to define a method for the determination of the crosslinking properties of these recently introduced fillers, method that is lacking at the moment.

MATERIAL AND METHOD: The percentage of crosslinker and the fraction that is effectively crosslinked were determined by proton Nuclear Magnetic Resonance (1H NMR) and by 13C NMR, respectively. The filler were preliminarily washed with acetonitrile to remove residual PEG and then digested by hyaluronidase to obtain a sample that can be analysed by NMR.

RESULTS: The crosslinking parameters were determined in four samples of NEAUVIA PEG-crosslinked dermal fillers (produced by MatexLab S.p.A., Italy). The percentage of crosslinker was between 2.8% and 6.2% of HA, whereas the effective crosslinker ratios were between 0.07 and 0.16 (ratio between the moles of effectively crosslinked PEG and total moles of PEG). Moreover, a digestion procedure alternative to enzymatic digestion, based on acidic hydrolysis, was successfully tested for the determination of crosslinker percentage.

CONCLUSIONS: The proposed method successfully determined the two crosslinking parameters in PEG-crosslinked dermal fillers. The estimated percentage of crosslinker is similar to previously reported data for other crosslinkers, whereas the effective crosslinker ratio is lower for PEG crosslinked hydrogels.

Introduction

Hyaluronic acid (HA) based hydrogels attracted much attention since the first years of the new millennium [1], [2]. Interest in this material and its chemical modifications stems from its high biocompatibility and flexibility, encompassing several very interesting and promising fundamental study and clinical applications. Tissue engineering [3], [4], drug delivery [5], cell scaffolding [4], [6], wound healing [7], dermal filler [8] are all extremely active field of fundamental research, product development and clinical activity. As far as dermal fillers are involved, the introduction of HA-based filler was a big change in paradigm in this field, moving from permanent or semipermanent to biodegradable fillers like the ones based on hyaluronic acid [8], [9]. The latter property prompted for the chemical modification of HA to reduce the biodegradation rate and give the possibility to modulate viscoelastic properties to achieve compatibility with different tissues [10]. The most successful chemical modification was achieved by crosslinking agents, i.e. chemical species connecting two sections of the HA chain in a bridge-like fashion. Several crosslinking species were introduced [11]:

1,4-Butanediol diglycidyl ether (BDDE), 1, 2, 7, 8-diepoxyoctane (DEO), divinyl sulfone (DVS), hexamethylenediamine (HMDA) and polyethylene glycol diglycidyl ether (PEGDE [12]). Crosslinked HA hydrogel is an adaptable material whose properties may be tailored for the intended purpose. Chemical and biochemical characterisation of hydrogels were accordingly performed with several different aims, ranging from safety assessment, quality assurance to the understanding of hydrogel properties (rheology, degradation, fitness for purpose). In particular, the crosslinking parameters play a major role in determining the rheological and swelling properties of the hydrogel [9], [10], [11], features that are of the utmost importance for clinical applications [10]. Two parameters are typically investigated: the degree of modification and the effective crosslinker ratio [13]. The first figure states the percentage of disaccharide units bound to a crosslinker molecule: as an example, a 50% modification means that half of the HA is modified by the crosslinker. The effective crosslinker ratio is esteem of the fraction of crosslinker that connects two disaccharide units, i.e. that acts as a crosslinker, bridge-like a binder.

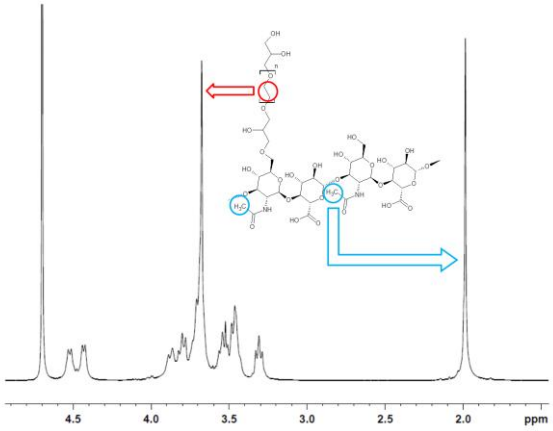


Figure 1: 1H NMR spectrum of PEGDE crosslinked hydrogel disaccharide unit after enzymatic digestion (sample #1). Signals due to the protons in the repetitive unit –C2H4O– in PEG (red) and to the methyl group on the HA chain (blue) are evidenced. The signals from the remaining 10 PEG protons overlap the HA ones (3.2-4.0 ppm) completely

Hyaluronic acid hydrogel crosslinked with polyethene glycol (PEG) has been recently introduced [12], [14], [15]. Several features of these materials have already been investigated, namely the properties directly involved in the safe use as dermal fillers (degradation by hyaluronidase [16] and biosafety [17]).

Nevertheless, the possibility to investigate crosslinking feature in these materials have not yet been investigated. Here we introduce a method for estimating the crosslinking parameters for PEG crosslinked hyaluronic acid-based hydrogels. Using as a starting point published general methodologies, several issues specific to this material were efficiently tackled, leading to the definition of a general approach for the characterisation of crosslinked hydrogels: critical steps were pinpointed, and an alternative procedure for sample treatment is proposed.

Instrumentation

Nuclear magnetic resonance (NMR) spectra were acquired on a Brucker 400 MHz Advance NMR spectrometer equipped with a 5 mm PABBO probe. The zg30 sequence from the TopSpin library was used to acquire 1H spectra, whereas 13C spectra were acquired by the zgig30 sequence. 1H spectra were acquired accumulating 64 scans with a recycle delay (D1) time of 5 seconds, whereas 13C spectra acquisition required a D1 of 10 seconds and the accumulation of 7000 scans. These delays ensured full relaxation of the signals achieving quantitative signals.

A VWR pHenomenal MU6100L pHmeter was used for pH measurements. A refrigerated incubator (model FOC225i) from VELP Scientifica was used for sample incubation at 37°C.

Reagents and solutions

Polyethylene glycol diglycidyl ether (PEGDE, mean MW 500 g/mol) was purchased from Fluka. The general formula of the product is shown below (see PEGDE characterization for a discussion on the structure of this species).

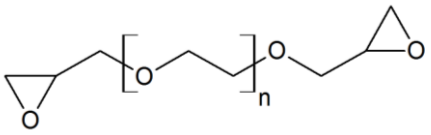


Figure 2: General formula polyethylene glycol diglycidyl ether (PEGDE)

Hyaluronidase from sheep tested was purchased from Sigma Aldrich (type V, lyophilized powder, > 1500 U/mg). Sodium hyaluronate from *Bacillus Subtilis* was used for the synthesis of the crosslinked hydrogel.

Acetonitrile (> 99.5%, Sigma Aldrich), concentrated sodium hydroxide (32%, Carlo Erba), concentrated hydrochloric acid (37%, Sigma Aldrich), deuterium oxide (D_2O, 99.97%D, Eurisotop), sodium azide (99%, Aldrich), sodium dihydrogenphosphate monohydrate (> 99.0%, Fluka), disodiumhydrogen-phosphate dihydrate (99.5%, Merck) and sodium chloride (> 99%, Carlo Erba) were used as received from the producers.

A phosphate buffer saline (PBS) solution was prepared dissolving 34.4 mg of NaH_2PO_4, 44.4 mg of

Na_2HPO_4, 400 mg of NaCl and 20 mg of NaN_3 in 50 mL of ultrapure water (final pH adjusted to 7.4).

Finally, crosslinked hydrogel samples were obtained from MatexLab S.p.A. (Brindisi, Italy) and consisted of PEG crosslinked hyaluronic acid hydrogels with HA concentrations ranging from 22 to 28 mg/L.

Hydrogel sample preparation

Five, 1 mL syringes of hydrogel samples were transferred into a 10 mL round-bottomed flask and washed three times with a 95:5 acetonitrile: water mixture. The resulting solid was dried under vacuum (diaphragm pump, KNF LABOPORT) at 60°C in a water bath for 30 minutes to remove any trace of the solvent. This procedure was used to remove the unreacted, hydrolysed PEGDE from the hydrogel.

The classic procedure (enzymatic digestion[13]) involves enzymatic digestion: the dried hydrogel was rehydrated in 5 mL of PBS to which 100 µL of the enzyme solution was added. The hydrogel was then incubated at 37°C for 18 hours. The simplified procedure (acidic digestion) involves the digestion for 1 hour at 60°C in 2 mL of 0.2 M HCl in D_2O: the solution was buffered to pH 7 by neutralisation with 32% NaOH and the addition of 10 mM phosphate buffer.

1H NMR spectra were collected directly on the abovementioned solutions obtained after digestion, whereas solutions for 13C NMR were concentrated under vacuum to around 1 mL to increase hydrogel concentration and reduce acquisition time.

Results

PEGDE characterisation

Commercially available polyethylene glycol diglycidyl ether shows an average molar mass of 500 g/mol. It is a mixture of several compounds of the general structure depicted in the Reagent and solution section with n (number of $–CH_2–CH_2–O–$ groups) ranging from 2 to 15 as shown by preliminary mass spectrometry measurements. This is the first feature specific to PEG crosslinked hydrogels: opposing to BDDE, DEO, DVS and HMDA, the crosslinker is not a single molecule but a mixture of oligomers with different chain length. Measuring the typical crosslinking parameters, i.e. degree of modification and effective crosslinker ratio, requires a preliminary investigation on the crosslinker to establish average parameters. In particular, as NMR spectroscopy was used for the hydrogel characterisation, the composition of the PEGDE crosslinker should be known in term of average hydrogen and carbon atoms. In other words, the term “n” in the formula reported in the Reagent and solution section should be experimentally determined and represents the average number of repetitive $–CH_2–CH_2–O–$ groups. The 13C NMR spectrum of PEGDE dissolved in D_2O obtained with a 20 s recycle time yielded a value of n of 7.63, which is close to the theoretical value of 8.4 expected for the commercially reported average molecular weight of 500 g/mol. This figure (n = 7.63) was used for all of the following calculations.

Hydrogel washing

The removal of unreacted polyethylene glycol, i.e. the fraction that did not react with hyaluronic acid, is of the utmost importance if reliable results on linked PEG is to be achieved. If any unreacted PEG is left in the sample, the following NMR analysis would not be able to distinguish between bound and unbounded PEG, leading to unreliable results. Unbounded crosslinker was typically removed by washing with water and saline aqueous solutions [18]: this procedure could not be followed for PEG crosslinked hydrogels as their swelling rate is much higher than other crosslinkers’, or at least than BDDE crosslinked hydrogels. Washing with water is completely inefficient in removing unreacted PEG as the only result is the swelling of the hydrogel. Two alternatives were accordingly tested: ethanol and acetonitrile. Both proved efficient, leading to an efficient washing accompanied by the removal of water from the hydrogel. A further complication is due to the possible interference in the NMR spectrum of the residual solvent (acetonitrile or ethanol) after drying (see Experimental): acetonitrile washing was preferred as its 1H signal (2.06 ppm in D_2O) overlaps HA methyl signal (1.98 ppm) if acetonitrile is present in significant amounts, whereas traces do not cause any issue. On the other hand, ethanol $–CH_2–$ signal (3.66 ppm) falls in the region of both HA and PEG proton signals.

Calculation of crosslinking parameters

The calculation of crosslinking parameters by NMR for 1, 4-butanediol diglycidyl ether (BDDE) crosslinked HA has been recently introduced [13], [18]. The BDDE crosslinker enables a relatively easy determination of these figures as it is a single molecule and shows 1H and 13C signals well separated from HA signals [18]. Conversely, crosslinked PEG 1H signals overlap completely hyaluronic acid signals when a 400 MHz NMR spectrometer is used (see Figure 1). 13C signals of bound PEG, used to calculate the effective crosslinker ratio, do not overlap HA carbon signals but still fall in the region where the HA signals are (see Figure 3).

The degree of modification, i.e. the moles of crosslinker bound per disaccharide unit (usually expressed as a percentage), was calculated as

follows. The peaks with a chemical shift in the range 3.1-4.0 include both the hyaluronic acid protons and the PEG protons: the latter ones can be assigned to the peak at ~3.6 ppm, but cannot be integrated separately (see Figure 1). The integral of the region 3.1-4.0 ppm ($I^{\delta 3.1-4.0}$) is subtracted by 10 protons, which are the protons of hyaluronic acid in this range of chemical shifts (the chemical shift of the other two protons, the anomeric protons, is centered at 4.5 ppm). The subtracted integral ($I^{\delta 3.1-4.0}_{corrected}$) is accordingly the integral of the PEG protons: based on the previously reported analysis (see PEGDE characterisation), 40.52 protons on average are present on the PEG residue. The degree of modification is consequently calculated as follows:

$$\text{The degree of modification}\% = \frac{\frac{I^{\delta 3.1-4.0}_{corrected}}{40.52}}{\frac{I^{\delta 2.0}}{3}} \cdot 100$$

where $I^{\delta 2.0}$ Is the integral of the peak at 2.0 ppm of the three protons of the methyl group on the N-acetylglucosamine residue. This value is an average degree of modification as crosslinked PEG shows a distribution of molar masses as already mentioned.

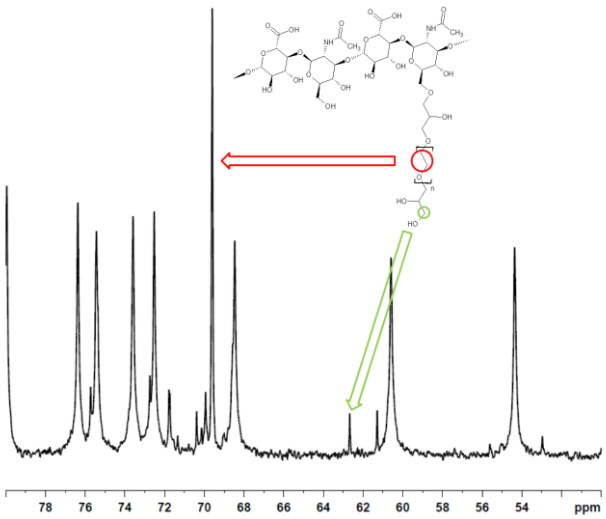


Figure 3: 13C spectrum of PEGDE crosslinked hydrogel after enzymatic digestion limited to the 50-80 ppm range (sample #1)

The effective crosslinker ratio, i.e. the fraction of PEG molecules that cross-link between two HA chains, was calculated using 13C NMR data as follows. The signal at 69.58 ppm are attributed to the 15.26, on average, carbon atoms in the repetitive -C_2H_4O- unit of PEG, whereas the peak at 62.60 ppm was attributed to the carbon atom in the terminal -CH_2OH group of the monolinked PEG molecule (see[18] for the attribution of these signals and Figure 2 for the actual 13C NMR spectrum). The effective crosslinker ratio is determined according to the following equation:

$$\text{Effective crosslinker ratio} = 1 - \frac{I^{\delta 62.60}}{\frac{I^{\delta 69.58}}{15.26}}$$

Accordingly, the effective crosslinker ratio is 1 when all of the PEG is crosslinked among two HA chains, and there is no -CH_2OH group ($I^{\delta 62.60}$ = 0).

A grade of modification and effective crosslinker ratio for four samples of hydrogel crosslinked with PEG are reported in Table 1.

Table 1: Grade of modification and effective crosslinker ratio for four PEG crosslinked HA hydrogels

Sample	HA grade of modification	Effective crosslinker ratio
Neauvia Stimulate Lot HA1161101	6.2%	0.07
Neauvia Intense Lot HA2170204	5.6%	0.14
Neauvia Intense Flux Lot HA2170303	2.8%	0.16
Neauvia Rheology Lot HA2170207	5.0%	0.09

Acidic digestion

Acidic digestion of the hydrogel was tested as an alternative, simplified procedure for hydrogel preparation before NMR measurements of crosslinking parameters. Acidic digestion is a known method to cleavage hyaluronic acid, leading to the hydrolysis of the glycosidic bond without inducing any further chemical modification [19]: accordingly, this treatment is expected to lead to a reduction in the length of HA chains without altering the HA structure and, possibly, the bound crosslinker. The reliability of this treatment was checked by applying the enzymatic and acidic digestion to six aliquots of the same sample and checking the results for comparability: three aliquots were treated by the enzymatic digestion and three by 0.2 M HCl. The 1H NMR spectra obtained by the two procedures are virtually indistinguishable, showing the same signal pattern. Signals were integrated and the crosslinking parameters calculated for the six sample aliquots: the results are reported in Table 2.

Table 2: Comparison of the crosslinking properties obtained by enzymatic and acidic digestions. Data from the digestion of three different sample aliquots reported as average ± standard deviation for the calculation of the modification degree

	Modification degree %	
	Enzymatic	Acidic
Lot#1	6.3 ± 0.63	5.8 ± 0.85
Lot#2	3.4 ± 0.52	3.6 ± 0.70
Lot#3	5.9 ± 0.78	6.4 ± 0.33

Data on the three samples clearly show that the acidic treatment leads to figures not significantly different from enzymatic digestion, as far as the calculation of the degree of modification is involved, leading to a simplification in the digestion procedure in terms of required time, manipulation and involved reagents.

In conclusion, the crosslinking properties of

hydrogel based on hyaluronic acid crosslinked with polyethylene glycol were for the first time determined: the modification degree ranges from 2.8% to 6.2% and is similar to previously reported data (3.4%-8.2%, with one outlier at 32%, see [18]), whereas the effective crosslinker ratio seems on average lower than the reported ones (average in this work 0.11 against 0.27 in [18]). The latter difference is worth further investigations: at the present stage of investigation its significance should be assessed as the determination of the effective crosslinker ratio is affected by a relatively high error. Nevertheless, this difference may be one of the factors determining the contrast in the macroscopic behaviour of the two hydrogels like viscoelastic properties and swelling rate. The latter is an interesting property to be investigated: the evidence collected during this study point to a significant difference in swelling rate *in vitro* (speed of water inclusion into the hydrogel network) between different crosslinkers, with PEGDE showing a much higher rate than BDDE based formulations. In case this difference also occurred *in vivo*, it would clearly have a strong clinical significance. As a more general conclusion, this study puts forward a general method for the determination of crosslinking parameters in HA hydrogel with complex crosslinkers. Two critical steps were highlighted: hydrogel washing and data extraction from NMR spectra. The present study highlighted that, even for a complex crosslinker as PEGDE, the crosslinking parameters could be measured: generally, it is to be expected that this approach may be useful for any kind of crosslinker, under the unique, easily fulfilled condition, that it shows 1H or 13C NMR signals. Furthermore, a simplified sample treatment, i.e. acidic digestion, was successfully tested: although preliminary, results are indistinguishable from the ones obtained by enzymatic digestion.

A deeper understanding of the chemical factors controlling clinically relevant properties of the hydrogel, like viscoelastic behaviour and swelling, would be beneficial, finally leading to a rational approach to hydrogel design. In this regard, PEGDE shows unique features in that it is the longest crosslinker employed and it introduces unequal spacers among the HA chains as opposed to simpler molecules like BDDE, DVS and HMDA. An understanding of the role of these factors, i.e. crosslinker length and length distribution, on hydrogel properties should be accordingly achieved. More generally, establishing quantitative structure-activity relationship (QSAR) is surely a further goal for the research in this field. This achievement would allow a quantitative description of the effect of the formulation (crosslinker identity, HA/crosslinker ratio, reaction time, etc.) on hydrogel properties, which would translate into a deep understanding of the linkage between chemical structure and hydrogel characteristics. Nevertheless, a QSAR approach is at the moment a challenging task, mainly due to the lack of sufficient data.

References

1. Hoffman AS. Hydrogels for biomedical applications. Adv Drug Deliv Rev. 2012; 64(suppl.):18-23. https://doi.org/10.1016/j.addr.2012.09.010

2. Huerta-Ángeles G, Nešporová K, Ambrožová G, Kubala L, Velebný V. An Effective Translation: The Development of Hyaluronan-Based Medical Products From the Physicochemical, and Preclinical Aspects. Front Bioeng Biotechnol. 2018; 6. https://doi.org/10.3389/fbioe.2018.00062

3. Hemshekhar M, Thushara RM, Chandranayaka S, Sherman LS, Kemparaju K, Girish KS. Emerging roles of hyaluronic acid bioscaffolds in tissue engineering and regenerative medicine. Int J Biol Macromol. 2016; 86:917-928. https://doi.org/10.1016/j.ijbiomac.2016.02.032 PMid:26893053

4. Drury JL, Mooney DJ. Hydrogels for tissue engineering: scaffold design variables and applications. Biomaterials. 2003; 24(24):4337-4351. https://doi.org/10.1016/S0142-9612(03)00340-5

5. Santos LF, Correia IJ, Silva AS, Mano JF. Biomaterials for drug delivery patches. Eur J Pharm Sci. 2018; 118:49-66. https://doi.org/10.1016/j.ejps.2018.03.020 PMid:29572160

6. Diekjürgen D, Grainger DW. Polysaccharide matrices used in 3D in vitro cell culture systems. Biomaterials. 2017; 141:96-115. https://doi.org/10.1016/j.biomaterials.2017.06.020 PMid:28672214

7. Boateng JS, Matthews KH, Stevens HNE, Eccleston GM. Wound Healing Dressings and Drug Delivery Systems: A Review. J Pharm Sci. 2008; 97(8):2892-2923. https://doi.org/10.1002/jps.21210 PMid:17963217

8. Jones DH. Semipermanent and Permanent Injectable Fillers. Dermatol Clin. 2009; 27(4):433-444. https://doi.org/10.1016/j.det.2009.08.003 PMid:19850193

9. Tezel A, Fredrickson GH. The science of hyaluronic acid dermal fillers. J Cosmet Laser Ther. 2008; 10(1):35-42. https://doi.org/10.1080/14764170701774901 PMid:18330796

10. Santoro S, Russo L, Argenzio V, Borzacchiello A. Rheological properties of cross-linked hyaluronic acid dermal fillers. J Appl Biomater Biomech. 2011; 9(2):127-136. https://doi.org/10.5301/JABB.2011.8566

11. Yeom J, Bhang SH, Kim B-S, et al. Effect of cross-linking reagents for hyaluronic acid hydrogel dermal fillers on tissue augmentation and regeneration. Bioconjug Chem. 2010; 21(2):240-247. https://doi.org/10.1021/bc9002647 PMid:20078098

12. Zerbinati N, D'Este E, Farina A, Rauso R, Cherubino M, Calligaro A. Morphological evidence following pegylated filler treatment in human skin. J Biol Regul Homeost Agents. 2017; 31(2 Suppl. 2):79-85. PMid:28702967

13. Kenne L, Gohil S, Nilsson EM, et al. Modification and cross-linking parameters in hyaluronic acid hydrogels - Definitions and analytical methods. Carbohydr Polym. 2013; 91(1):410-418. https://doi.org/10.1016/j.carbpol.2012.08.066 PMid:23044151

14. Zerbinati N, Rauso R, Gonzalez P, et al. In vitro evaluation of collagen production on human fibroblasts treated with hyaluronic acid peg cross-linked with micromolecules of calcium hydroxyapatite in low concentration. J Biol Regul Homeost Agents. 2017; 31(Suppl 2):87-90. PMid:28702968

15. Zerbinati N, Haddad RG, Bader A, et al. A new hyaluronic acid polymer in the augmentation & restoration of labia majora. J Biol Regul Homeost Agents. 2017; 31(2 Suppl 2):153-161. PMid:28702976

16. Zerbinati N, Lotti T, Monticelli D, et al. In vitro evaluation of the sensitivity of a hyaluronic acid PEG cross-linked to bovine testes hyaluronidase. Open Access Maced J Med Sci. 2018; 6(1).

17. Zerbinati N, Lotti T, Monticelli D, et al. In vitro evaluation of the biosafety of hyaluronic acid PEG cross-linked with micromolecules of calcium hydroxyapatite in low concentration. Open Access Maced J Med Sci. 2018; 6(1).

18. Wende FJ, Gohil S, Nord LI, Helander Kenne A, Sandström C. 1D NMR methods for determination of degree of cross-linking and BDDE substitution positions in HA hydrogels. Carbohydr Polym. 2017; 157:1525-1530. https://doi.org/10.1016/j.carbpol.2016.11.029 PMid:27987864

19. Tømmeraas K, Melander C. Kinetics of hyaluronan hydrolysis in acidic solution at various pH values. Biomacromolecules. 2008; 9(6):1535-1540. https://doi.org/10.1021/bm701341y PMid:18452332

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Apr 15; 7(7):1082-1086.
https://doi.org/10.3889/oamjms.2019.243
eISSN: 1857-9655
Clinical Science

Efficacy and Safety of Intravenous Ketorolac versus Nalbuphine in Relieving Postoperative Pain after Tonsillectomy in Children

Islam Adel Eladi, Karim Hussein Mourad, Ahmed Nabih Youssef*, Abdelrazek Ahmed Abdelrazek, Mohammad Ahmed Ramadan

Department of Anesthesia, Surgical Intensive Care Unit and Pain Management, Faculty of Medicine, Cairo University, Cairo, Egypt

Citation: Eladi IA, Mourad KH, Youssef AN, Abdelrazek AA, Ramadan MA. Efficacy and Safety of Intravenous Ketorolac versus Nalbuphine in Relieving Postoperative Pain after Tonsillectomy in Children. Open Access Maced J Med Sci. 2019 Apr 15; 7(7):1082-1086. https://doi.org/10.3889/oamjms.2019.243

Keywords: Ketorolac; Analgesia; Nalbuphine; Postoperative pain; Adenotonsillectomy

***Correspondence:** Ahmed Nabih Youssef. Department of Anesthesia, Surgical Intensive Care Unit and Pain Management, Faculty of Medicine, Cairo University, Cairo, Egypt. E-mail: nabihomar100@yahoo.com

Received: 14-Feb-2019; **Revised:** 22-Mar-2019; **Accepted:** 23-Mar-2019; **Online first:** 29-Mar-2019

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Pain is a major postoperative complication worldwide, which in turn impairs normal body performance and increases postoperative morbidity, hospitalisation, and the susceptibility to infections which also lead to chronic pain development.

AIM: The purpose of this study was to evaluate the efficacy of intravenous ketorolac versus nalbuphine as analgesia after adenotonsillectomy surgery to determine the optimal procedure for pain control and postoperative reduction of analgesic use.

METHODS: A group of 100 pediatric patients undergoing tonsillectomy or adenotonsillectomy were assigned as follows to two equal groups: Group A: 50 patients received intravenous ketorolac 0.9 mg/Kg. Group B: 50 patients received intravenous nalbuphine 0.25 mg/Kg.

RESULTS: FLACC (Face, Legs, Activity, Cry, Consolability) pain score was measured after recovery from anaesthesia (postoperative). There was a statistically significant difference concerning pain score between group 'A' and group 'B' as pain score in 'A' (ranging from 3.18 ± 0.87 to 4.68 ± 0.74) is lower compared to 'B' (ranging from 3.90 ± 0.76 to 5.54 ± 0.73) and probability value < 0.05 except at 90 & 120 min which was observed statistically insignificant. There was no serious postoperative complication detected in either group.

CONCLUSION: It is concluded that intravenous ketorolac is more effective than intravenous nalbuphine in reducing pain intensity and postoperative analgesic requirements after adenotonsillectomy in children.

Introduction

Adenotonsillectomy is one of the most frequent ENT surgeries performed in children. Postoperative pain can influence the ability of the child to tolerate medication for oral pain and fluid intake, resulting in nausea and dehydration in a considerable number of children postoperatively [1], [2].

The operation involves pain in more than 80 per cent of children on the first day of surgery. It is assumed that pain is not treated adequately in half of all surgical procedures [3].

Although opioids are widely used in postoperative pain management, their side effects, especially respiratory depression, bradycardia, nausea and vomiting, have reduced the use of these analgesics, especially in children. Non-steroidal anti-inflammatory drugs (NSAIDs) have been widely used with very good results in pain reduction and postoperative opioid requirements after adenotonsillectomy in children and adults [4], [5], [6], [7], [8].

Nalbuphine is an opioid agonist-antagonist of the phenanthrene series which was synthesised in an attempt to provide analgesia without the undesirable side effects of the pure agonists. Its

analgesic and possibly certain anti-pruritic effects are mediated via actions on the μ and κ-receptors, and nalbuphine has been indicated for mild to moderate pain [9].

NSAIDs act on prostaglandin synthesis for pain reduction with adverse effects such as bleeding problems in both gastrointestinal tracts and from the surgical site and potential renal dysfunction that have caused some concerns in their widespread application [5], [6], [8], [10].

Ketorolac is a non-steroidal anti-inflammatory drug (NSAID) that has an analgesic efficacy similar to commonly used opioids, and that recently has found wide acceptance in the treatment of postoperative pain in a variety of surgical procedures. Ketorolac is used for moderate pain relief; it may be used to treat severe pain when associated with opioids, reducing the opioid dose. The advantage of this association is the reduction of opioid side effects such as respiratory depression, pruritus, urinary retention, sedation and nausea [11], [12], [13].

This study aimed to evaluate the efficacy of intravenous ketorolac versus nalbuphine as analgesia after adenotonsillectomy surgery to determine the optimal procedure for pain control and postoperative reduction of analgesic use.

Patients and Methods

This is a prospective randomised clinical study designed to assess the effect of intravenous ketorolac versus intravenous nalbuphine on postoperative pain after tonsillectomy in children.

The study was carried out in Children Hospital of Cairo University (Abu El Reish) on 100 pediatric patients following the approval of the Ethical committee and obtaining informed consent from parents.

Inclusion criteria:

- ASA physical status I & II.
- Age between 2 years & 10 years old.
- Body weight below 30 Kg.

Exclusion criteria:

- ASA physical status ≥ III.
- Age below 2 years & above 10 years old.
- Body weight above 30 Kg.
- Any contraindications to any drug used.

Methodology in details

One hundred patients meeting the inclusion criteria were randomly assigned into two equal groups:

Group A: 50 patients received intravenous ketorolac 0.9 mg/Kg**.**

Group B: 50 patients received intravenous nalbuphine 0.25 mg/Kg.

The investigators did not know the details of the series and the group assignment was kept in the assets of sealed envelopes with only the case number on the outside.

Anesthetic Management

All patients were visited preoperatively on the day before surgery. The entire procedure has been explained to the patient, and informed consent has been taken. Full history has been taken including diseases, bleeding, drug intake, allergy or drug sensitivity and previous anaesthetic experience. Complete general examination including airway evaluation, chest and cardiac auscultation was conducted. All patients fasted for an appropriate period (6 hours for food, 4 hours for water). Laboratory evaluation: complete blood count (CBC), prothrombin time and concentration (PT&PC), INR, liver function tests, renal function tests. Upon arrival at the operating theatre, IV access was established by 22 G cannula insertion.

Five lead ECG pulse oximeter and non-invasive ABP were monitored. Premedication injection: atropine sulfate intravenously 0.01 mg/kg and dexamethasone intravenously 0.2 mg/kg. Induction of anaesthesia by inhalation sevoflurane and oral endotracheal intubation of appropriate size was done. After induction of anaesthesia, children were randomly allocated to receive 0.9 mg/Kg ketorolac or 0.25 mg/Kg nalbuphine administered as an intravenous injection. Anaesthesia was maintained by isoflurane inhalation. IV fluids according to body weight “Hartmann's solution”, 1st 10 kg 4 ml/kg, 2nd 10 kg 2 ml/kg and for each kg above 20 kg 1 ml/kg fluids.

Data collected

1-patients characteristics and demographic data were collected; age, gender, weight.

2-Postoperative pain, using “FLACC score “(face, legs, activity, cry,consolability) this scale (Table 1) is scored between a range of 0-10 with zero representing no pain. The scale has 5 criteria each assigned a score of 0, 1, 2. Pain was recorded at 15, 30, 45, 60, 90, 120, 180, 240, 300 and 360 minutes postoperatively. Postoperatively intramuscular ketoprofen (1 mg/kg) was used as ‘‘rescue analgesia” if pain score > 5.

Table: 1 FLACC pain score [14]

Criteria	Score 0	Score 1	Score 2
Face	No particular expression or smile	Occasional grimace or frown, withdrawn, uninterested	Frequent to constant quivering chin, clenched jaw
Legs	Normal position or relaxed	Uneasy, restless, tense	Kicking or legs were drawn up
Activity	Lying quietly, normal position moves easily	Squirming, shifting, back and forth, tense	Arched, rigid or jerking
Cry	No cry (awake or asleep)	Moans or whimpers; occasional complaint	Crying steadily, screams or sobs, frequent complaints
Consolability	Content, relaxed	Reassured by occasional touching, hugging or being talked to, distractible	Difficult to console or comfort

3-Postoperative sedation, using Ramsay sedation score (Table 2).

Table 2: Ramsay sedation score [15]

Score	Observation
1	Anxious, agitated or restless.
2	Cooperative, oriented and tranquil
3	Responsive to commands.
4	Asleep, but with brisk response to a light glabellar tap or loud auditory stimulus.
5	Asleep, sluggish response to a glabellar tap or auditory Stimulus.
6	Asleep, no response.

4-Postoperative vomiting occurrence.

5-Postoperative side effects: respiratory depression, oedema, rash or itching were also assessed.

Statistical analysis

Data were statistically described, when appropriate, in terms of mean ± standard deviation (± SD), frequencies (number of cases) and relative frequencies (percentages). The student t-test was used to compare quantitative variables among the study groups. For comparing categorical data, Chi-square ($\chi 2$) test was performed. When the expected frequency is less than 5, an exact test was used instead. A probability value (*p*-value) less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs Microsoft Excel 2010 (Microsoft Corporation, NY, and USA) and SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 21 for Microsoft Windows.

Results

One hundred (100) patients were included in this study and randomly allocated into 2 groups: group A (ketorolac) and group B (nalbuphine).

Table 3: Demographic data (age & weight)

Demographic data/Groups	A	B
Age	5.18 ± 1.17	5.04 ± 1.26
Weight	17.06 ± 4.2	16.28 ± 4.63

Data expressed as mean ± (SD); P value > 0.05.

ASA status is the same in all patients of both groups “ASA 1”.

There was no statistical difference in age, weight, and sex between the groups (Table 3 and 4).

Table 4: Demographic data (sex)

Sex/Groups	A	B
Male	39/50	36/50
Female	11/50	14/50

Data expressed as count.; P value > 0.05.

FLACC pain score was measured after recovery from anaesthesia (postoperative). There was a statistically significant difference concerning pain score between group ‘A’ and group ‘B’ as pain score in 'A' (ranging from 3.18 ± 0.87 to 4.68 ± 0.74) is lower compared to 'B' (ranging from 3.90 ± 0.76 to 5.54 ± 0.73) and P-value < 0.05 except at 90 & 120 min which was observed statistically insignificant (Table 5) (Figure 1).

Table 5: FLACC score at (6 hours) postoperatively

	Group A	Group B
15 min	3.18 ± 0.87	3.96 ± 0.83*
30 min	3.36 ± 0.80	3.90 ± 0.76*
45 min	3.40 ± 0.67	4.34 ± 0.48*
60 min	3.48 ± 0.65	4.34 ± 0.48*
90 min	3.96 ± 0.61	4.04 ± 0.19
120 min	3.96 ± 0.61	4.04 ± 0.19
180 min	4.28 ± 0.53	4.52 ± 0.51*
240 min	4.48 ± 0.65	5.34 ± 1.00*
300 min	4.68 ± 0.74	5.54 ± 0.73*
360 min	4.52 ± 0.58	5.00 ± 0.49*

Data expressed as mean ± (SD); *P value < 0.05.

A number of patients who needed rescue analgesia (ketoprofen 1 mg/kg I.M.) were recorded. None of the patients required analgesia in the first two hours (as pain score was < 5) postoperatively in both groups.

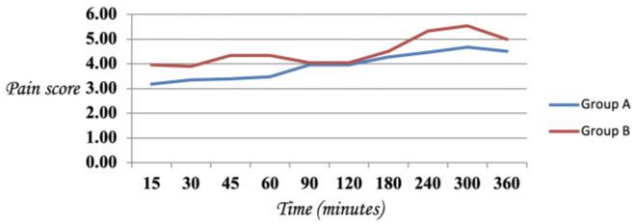


Figure 1: FLACC pain scale 6 hours postoperative

There was a significant difference concerning the analgesic need between the two groups being lower in ‘A’ group compared to ‘B’ group which was statistically significant in 2-4 hours after surgery (group A 8% of cases while group B 32% of cases producing p-value < 0.05) and statistically insignificant in 4-6 hours after end of the surgery, however lower number of cases in group A compared to B (12% and 18% of cases respectively) producing p-value > 0.05 (Table 6).

Ramsay sedation score was significantly higher in group ‘B’ when compared to group ‘A’ at 0 and 30 minutes after recovery. But both groups have the same score (which is 2) from 30 minutes to 6 hours postoperatively (Table 7).

Table 6: Need for rescue analgesia (Ketoprofen) at 6 hours postoperative

		Group A	Group B
1st 2 hours postoperatively	Count	0/50	0/50
	% within gruop	0%	0%
2nd 2 hours postoperatively	Count	4/50	16/50*
	% within gruop	8%	32%*
3rd 2 hours postoperatively	Count	6/50	9/50
	% within gruop	12%	18%
Total	Count	10/50	25/50*
	% within gruop	20%	50%*

* P value < 0.05.

The incidence of vomiting was monitored after surgery and was statistically different and significant between both groups being high in 'B' group compared to 'A' group which required intervention (Table 8).

Table 7: Ramsay sedation score

	Group A	Group B
15 min	1 ± 0	2.08 ± 0.9*
30 min	1 ± 0	2.56 ± 0.541*
30-360 min	2 ± 0	2±0

* P value < 0.05.

Although 6% of cases (3 cases out of 50) in group B developed respiratory depression "which was statistically insignificant as p-value <0.05", it is clinically significant and vitally important required immediate intervention (Table 9).

Table 8: Incidence of vomiting

		Group A	Group B
Vomiting	Count	2/50	8/50*
	% within gruop	4%	16%*

* P value < 0.05.

There was no serious postoperative complication detected in either group.

Table 9: Respiratory depression

		Group A	Group B
Respiratory depression	Count	0/50	3/50
	% within gruop	0%	6%

P value > 0.05.

Discussion

Many studies were concerned with the post-tonsillectomy pain management. These studies studied different routes, drugs and mechanisms for that pain management, e.g. I.V, I.M, rectal, local infiltration, etc. [16]. In this study, we found that patients had less pain in 'group A' with FLACC pain score (ranging from 3.18 ± 0.87 to 4.68 ± 0.741) compared to 'group B' with FLACC pain score (ranging from 3.90 ± 0.763 to 5.54 ± 0.734) six hours postoperatively and rescue analgesia was needed in less number of patients at 2-4 and 4-6 hours in 'group A' (20%) compared to 'group B' (50%) which was statistically significant. So intravenous ketorolac 'group A' reduces pain intensity and postoperative analgesic requirements compared to nalbuphine 'group B' group after adenotonsillectomy in children.

In consistency with this study, Forrest et al. reported that ketorolac was more advantageous compared to opioid analgesics in the control of post-tonsillectomy pain. This was due to lower rates of sedation, nausea, vomiting, and respiratory depression and a similar level of analgesia when using ketorolac, compared to commonly used opioids [17]. In agreement of the study, Tarkkila and Saarnivaara compared ketorolac, ketoprofen, and diclofenac postoperatively following elective tonsillectomy, and reported lower use of opioids, improved pain control, and a similar complication rate compared to placebo. These findings are similar to those we found in our trial [18]. According to Carney et al., ketorolac reduced the use of opioids and lowered the morbidity during the first 48 hours after pediatric surgery. Furthermore, patients receiving ketorolac did not present increased bleeding or kidney toxicity compared to the group that received morphine only. Shende and Das reported a lower rate of vomiting and pain when using ketorolac postoperatively after strabismus surgery in children compared to the placebo group [19], [20].

In this study, we found that patients in 'group B' were significantly more sedated compared to patients in 'group A' using the Ramsay sedation score at 15 and 30 minutes after recovery. Sedation score was (2.08 ± 0.9 at 15 min. and 2.56 ± 0.541 at 30 min.) in 'group B' and was (1 ± 0 at 15 & 30 min.) in 'group A' which was statistically significant. Sedation score was the same in the remaining observational hours after recovery in both groups and was statistically insignificant. So nalbuphine produces more sedation and less agitation during the early postoperative period which not preferred in ENT operations in general and pediatric surgeries in specific.

In this study, we found that the incidence of postoperative vomiting was significantly higher in 'group B' (16%) compared to that of 'group A' (4%). This is explained as opioids alter lower oesophagal sphincter activity, resulting in sphincter relaxation. Gastric emptying is delayed by opioids via supraspinal (vagus nerve-mediated), spinal and peripheral mechanisms [21]. Also, the study revealed that incidence of postoperative respiratory depression occurred in 'group B' (6%) compared to that of 'group A' in which no patient developed respiratory depression (0%) which was statistically insignificant but clinically vital as it required immediate intervention [22].

In agreement with that Hamza et al., 2012 who compared the efficacy of ketorolac and pethidine for postoperative pain relief in the first 24 hours after tonsillectomy? One hundred patients age 5-12 years under going tonsillectomy were divided into group A and B randomly, who received either inj. ketorolac 0.5

mg/kg or inj. Pethidine 1 mg/kg I.M respectively postoperatively on 6 hourly bases. Patients were assessed in the recovery room and ENT ward for pain according to faces pain scale and for any side effects. Amount of rescue analgesia required by both groups was also recorded. They concluded that ketorolac provides similar analgesic effects as pethidine in the doses mentioned above with much less incidence of nausea, vomiting and drowsiness in the first 24 hours after adenotonsillectomy [23].

Limitation to the study was that patients were not followed up more than six “6” hours, as adequate acute pain management should be controlled and followed up in the first three “3” months as regarding the definition of acute pain of IASP “International Association for the Study of Pain” for better health care service and reducing a lot of morbidities which is an important issue for future studies. This was because tonsillectomy was performed on an ambulatory basis and most of the patients came from rural areas, where it would have been very difficult to track them after they had left the hospital.

It was concluded that intravenous ketorolac is effective in reducing pain intensity and postoperative analgesic requirements after adenotonsillectomy in children. Also, it is generally safe as absent respiratory depression and a very low incidence of postoperative complications. It is recommended that the use of multimodal analgesia is the best way for acute postoperative pain management after adenotonsillectomy surgery.

References

1. Owezarzak V, Haddad J. Comparison of oral versus rectal administration of acetaminophen with codeine in postoperative pediatric adenotonsillectomy patients. Laryngoscope. 2006, 116:1485-88. https://doi.org/10.1097/01.mlg.0000227530.64179.1f PMid:16885758

2. Pickering AE, Dridge HS, Nolan J, Stoddart PA. Double-blind placebo-controlled study of ibuprofen or rofecoxib in combination with paracetamol for tonsillectomy in children. Br J Anaesth. 2002; 88:72-77. https://doi.org/10.1093/bja/88.1.72 PMid:11881888

3. Lee WC, Sharp JF. Complications of pediatric tonsillectomy postdischarge.J Laryngol Otol. 1996; 110:136-140. https://doi.org/10.1017/S0022215100132979 PMid:8729496

4. Mc Callum PL, MacRae FL, Sukerman S, MacRae E. Ambulatory adenotonsillectomy in children less than 5 years of age. J Otolaryngol. 2001; 30:75-78. https://doi.org/10.2310/7070.2001.19826

5. Kokki H, Ahonen R. Pain and activity disturbance after pediatric day care adenotonsillectomy. Pediatr Anest. 1997; 7:227-31. https://doi.org/10.1046/j.1460-9592.1997.d01-76.x PMid:9189969

6. Carr DB, Jacox AK, Chapman CR, Ferrell B, Fields HL, Heidrich G. Clinical practice guidelines for acute pain management: operative or medical procedures and trauma. Washington, DC: Agency for Health Care Policy and Research. 1992:95-0034.

7. Rusy LM, Houck CS, Sullivan LJ, Ohlms CB, et al. A double blind evaluation of ketorolac tromethamine versus acetaminophen in pediatric tonsillectomy: analgesia and bleeding. Anesth Analg. 1995; 80:226-9. PMid:7818104

8. Hiller A, Silvanto M, Savolainen S, Tarkkila P. Propacetamol and diclofenac alone and in combination for analgesia after elective tonsillectomy. Acta Anaesthesiology Scand; 2004, 48:1185-89. https://doi.org/10.1111/j.1399-6576.2004.00473.x PMid:15352967

9. Zacny JP, Conley K, Marks S. Comparing the subjective, psychomotor and physiological effects of intravenous nalbuphine and morphine in healthy volunteers. Journal of Pharmacology and Experimental Therapeutics. 1997; 280(3):1159-69. PMid:9067299

10. Issioui T, Klein KW, White PF, Watcha MF, et al. The efficacy of premedication with celecoxib and acetaminophen in preventing pain after otolaryngologic surgery. Anesth Analg. 2002; 94:1188-93. https://doi.org/10.1097/00000539-200205000-00025 PMid:11973187

11. Strom BL, Berlin JA, Kinman JL, Spitz PW, Hennessy S, Feldman H et al. Parenteral ketorolac and risk of gastrointestinal and operative site bleeding. A postmarketing surveillance study. JAMA. 1996; 275(5):376- 82. https://doi.org/10.1001/jama.1996.03530290046036 PMid:8569017

12. Carney DE, Nicolette LA, Ratner MH, Minerd A, Baesl T. Ketorolac reduces postoperative narcotic requirements. J Ped Surg. 2001; 36(1):76- 9. https://doi.org/10.1053/jpsu.2001.20011 PMid:11150441

13. Dsida RM, Wheeler M, Birmingham PK, Wang Z, Heffner CL, Coté CJ, Avram MJ. Age-stratified phermacokinetics of ketorolac tromethamine in pediatric surgical patients. Anesth Analg. 2002; 94:266-70. https://doi.org/10.1213/00000539-200202000-00007 PMid:11812682

14. Merkel S, et al. The FLACC: A behavioral scale for scoring postoperative pain in young children. Pediatr Nurse. 1997; 23:293–97. PMid:9220806

15. Ramsay MAE, Savege TM, Simpson BRJ, and Goodwin R.Controlled sedation with alpaxalone-alphadolone. British Medical Journal. 1974; 2:656–659. https://doi.org/10.1136/bmj.2.5920.656 PMid:4835444 PMCid:PMC1613102

16. Alhashemi JA, Daghistani MF. Effects of intraoperative i.v. acetaminophen vs i.m. meperidine on post-tonsillectomy pain in children. Br J Anaesth. 2006; 96:790-5. https://doi.org/10.1093/bja/ael084 PMid:16613928

17. Forrest JB, Heitlinger EL, Revell S. Ketorolac for postoperative pain management in children. Drug Safety. 1997; 16(5):309-29. https://doi.org/10.2165/00002018-199716050-00003 PMid:9187531

18. Tarkkila P, Saarnivaara L. Ketoprofen, doclofenac or ketorolac for pain after tonsillectomy in adults? Br J Anaesth. 1999; 82(1):56-60. https://doi.org/10.1093/bja/82.1.56 PMid:10325837

19. Carney DE, Nicolette LA, Ratner MH, Minerd A, Baesl T. Ketorolac reduces postoperative narcotic requirements. J Ped Surg. 2001; 36(1):76-9. https://doi.org/10.1053/jpsu.2001.20011 PMid:11150441

20. Shende D, Das K. Comparative effects of intravenous ketorolac and pethidine on perioperative analgesia and postoperative nausea and vomiting (PONV) for paediatric strabismus surgery. Acta Anaesthesiol Scand. 1999; 43:265-9. https://doi.org/10.1034/j.1399-6576.1999.430305.x PMid:10081531

21. Schurizek BA, Willacy LH, Kraglund K. Antroduodenal motility, pH and gastric emptying during balanced anaesthesia: Comparison of pethidine and fentanyl. Br J Anaesth. 1989; 62:674–82. https://doi.org/10.1093/bja/62.6.674 PMid:2751923

22. Tarkkila P, Tuominen M, Lindgren L. Comparison of respiratory effects of tramadol and pethidine. Eur J Anaesthesiol. 1998; 15:64-8. https://doi.org/10.1097/00003643-199801000-00013 PMid:9522144

23. AMIR H, UMER H, QAISER K, MUHAMMAD K. Compare the Efficacy of Ketorolac and Pethidine for Postoperative Pain Relief in First 24 Hours after Tonsillectomy. PJMHS. 2012; 6:326-328.

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Apr 15; 7(7):1088-1092.
https://doi.org/10.3889/oamjms.2019.269
eISSN: 1857-9655
Clinical Science

The Effectiveness of Early Mobilization Time on Balance and Functional Ability after Ischemic Stroke

Umi Budi Rahayu[1*], Samekto Wibowo[2], Ismail Setyopranoto[2]

[1]Department of Physiotherapy, Faculty of Health Science, Universitas Muhammadiyah Surakarta, Indonesia; [2]Department of Neurology, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia

Citation: Rahayu UB, Wibowo S, Setyopranoto I. The Effectiveness of Early Mobilization Time on Balance and Functional Ability after Ischemic Stroke. Open Access Maced J Med Sci. 2019 Apr 15; 7(7):1088-1092. https://doi.org/10.3889/oamjms.2019.269

Keywords: Early mobilisation; Ischemic post-stroke; Balance; Functional ability

***Correspondence:** Umi Budi Rahayu. Department of Physiotherapy Faculty of Medicine, Universitas Muhammadiyah, Surakarta, Indonesia. E-mail: ubr155@ums.ac.id

Received: 23-Nov-2018; **Revised:** 03-Apr-2019; **Accepted:** 04-Apr-2019; **Online first:** 13-Apr-2019

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Early mobilisation (EM) after-ischemic stroke is a motor learning intervention aimed to restore nerve cells and to improve balance and functional ability. Unfortunately, the study of when this intervention began has not been widely studied.

AIM: On this study was compared the effect of EM started at 24 hours and 48 hours after an ischemic stroke on balance and functional ability.

MATERIAL AND METHODS: Randomized controlled trial involving 40 patients on 2 groups meeting predefined inclusion criteria. The levels of balance were measured using the Berg Balance Scale, and the functional ability was measured using the Barthel Index, at 5th and 7th day.

RESULTS: A significant difference was observed in both balance ($p = 0.038$) and functional ability ($p = 0.021$) obtained on the 7th day of assessment between both groups. A significant difference on the 5th day was observed only in the functional ability ($p = 0.002$) and not in the balance ($p = 0.147$), between the groups.

CONCLUSION: EM started at 24 hours after the ischemic stroke has been found to have a better impact on balance and functional ability compared to that at 48 hours.

Introduction

Early mobilisation (EM) is the following step in few days after stroke manifestation. In general, some physiotherapists apply mobilisation after 48 hours or even afterwards due to hospital bureaucracy. EM started at 24 hours after ischemic stroke which has been recommended by the AVERT Trial Collaboration Group [1], is part of neurorestoration to help restore nerve lesions. The recovery is probably mediated through basic mechanisms of nervous system restoration improving its function [2]. The reason for EM started 24 hours after stroke is related to the increase of certain proteins at the molecular level that plays a role in neuroplasticity, i.e. decreased caspase-3, increased expression of Bcl-2, MidKine (MK), Brain-derived Neurotropic Factor (BDNF), anti-platelet endothelial cell (PECAM-1) which will inhibit the apoptosis of nerve cells and increase the strength of nerve synaptic transmission, and subsequently enhance its motion and functional ability [3], [4], [5], [6]. However, most studies on EM gave 24 hours after a stroke are still tested in experimental animals (rats).

The expression of several proteins that act as a nerve growth factor (NGF) will be released several hours after ischemia. So, it is hypothesised that EM started at 24 hours after stroke will give better results than those that started at 48 after stroke.

The application of the EM started 24 hours after stroke in this study was included in The TIDIeR (Template for Intervention Description and Replication) guides based on content validity testing of earlier research [7]. The TIDIeR guides have been claimed as a good model for reporting an intervention [8]. EM can be expected to impact on the balance and functional ability in patients with ischemic stroke [9], [10]. The balance could be measured using the Berg Balance Scale (BBS) [9], [11], while the functional ability could be measured using the Barthel Index (BI) and has been reported as a good tool for measuring functional ability up to 3 months after stroke [10].

The aim of this study was compared to the effect of EM started at 24 hours and 48 hours after an ischemic stroke on balance and functional ability.

Material and Methods

Forty (40) patients were recruited based on a predefined inclusion criterion, admitted from three hospitals in Indonesia (Dr Moewardi Hospital, Dr Oen Surakarta Hospital, Government Surakarta Hospital) and had signed informed consent by the requirements of the local ethics committee. The study was conducted from April until October 2018. The inclusion criteria included, (1) patients diagnosed as ischemic stroke with decreased motor control, (2) patients having sensory and proprioceptive deficits with a muscle strength score of at least 2+, (3) patients with first-stage stroke scan, one side of the first attacked stroke and second stroke hemiparesis which had been confirmed without any deterioration of neurological complications (e.g. decreased awareness, sepsis, shock due to embolism), (4) patients without aphasia, (5) patients without severe cognitive impairment (MMSE score greater than 19).

Patients meeting inclusion criteria were randomly divided into 2 groups, i.e. treatment group was 20 subjects received EM at 24 hours and control group was 20 subjects received EM at 48 hours after diagnosis of ischemic stroke with CTScan. The EM of learning stages is a cognitive stage, associative stage and autonomous stage. The cognitive stage is sensory, visual, verbal, proprioceptive stimuli to the upper and lower limb, starting from the proximal part with clear commands. The associative stage: active assisted and free active exercise stimuli in the upper limbs and lower limbs. The associative stage to the autonomous stage using functional training.

The group receiving mobilisation at 24 hours groups underwent an assessment using the validated TIDIeR Guidelines (CVI and CVR) [7] and the group receiving mobilisation at 48 hours underwent routine hospital procedures (Table 1).

Table 1. Early mobilisation an application for groups

Provisions	Treatment group activity	Control group activity
Time	30 – 60 minutes	30 – 60 minutes
Focus of mobilisation	Exercise gradation: cognitive stage – associative stage – automatic stage	Nothing
Day 1	Stimulation on the skin, visual, verbal and join by active assisted exercise on extremities (focusing on patients cognitive)	-
Day 2	Stimulation on the skin, visual, verbal and join by active assisted exercise on extremities (Based Bobath and PNF Method)	Passive exercise and assisted exercise on extremities
Day 3	Active assisted and active exercise on extremities to functional pattern (focusing on patients' association) (Based Bobath and PNF Method)	Passive exercise and assisted exercise on extremities (Based Bobath and PNF Method)
Day 4	Control posture exercise Preparing to roll and sitting process	Rolling and sitting exercise
Day 5	Sitting stabilisation and posture control on sitting (focusing on doing association to get automatic movement)	Sitting stabilisation exercise
Day 6	Sitting and standing stabilisation Standing Functional ability exercise (like as feeding and dressing) (based CIMT Method)	Standing stabilisation exercise
Day 7	Functional ability exercise (like as feeding and dressing) (based CIMT Method) Gait exercise	Gait exercise

The balance was measured using BBS which has a maximum score of 56 [9], while the functional ability measured using BI with a score of 100 in 5 rating points [6]. Measurements BBS and BI conducted on the 1st, 5th, and 7th days with the reason, (1) consideration by the protocol that the time of observation during a hospital stay is 7 days, (2) to see the initial improvement that occurred.

The BBS score can predict the ability to activities because it determines the value to functional ability. BBS can be used to support hospitalised stroke patients who have a BBS score of 20 indicate the availability of speed [9]. It's a bit different from the other balance measurement like a Romberg test, Single-leg stance test, Step test; they are functional performance test. In terms of reliability and validity that BBS is one of the most popular and recognised balance measurement.

The evaluate tool of functional ability post-stroke was commonly done using the National Institute of Health Stroke Scale (NIHSS) and IB. The NIHSS is a method for assessing the severity of a stroke while IB is a better parameter for functional ability post-stroke. The reliability and consistency of internal IB for stroke patients is excellent, as well as of the contents are also excellent. The average change in IB score reaches 1.85 points which are considered very important by the time [12].

The differences in balance and functional ability scores respectively obtained at day 5th and day 7th against the baseline scores at 1st day with a reason to observe improvement from day to day at the beginning of the hospital treatment were statistically compared using Mann Whitney Test. Statistical significance was set at 5 %. Statistical analysis was performed using SPSS software version 20.

Results

Forty (40) patients with ischemic stroke consisting of 20 patients receiving early mobilisation at 24 hours and another 20 patients receiving mobilisation at 48 hours, were included in the study. There were no differences in the ages and sex between the two groups (Table 2).

Table 2. Demographic characteristic of the patients

The characteristic	Treatment group		Control group		p-value
	N = 20	%	N = 20	%	
Age					0.645
< 60 years	14	70%	12	60%	
> 60 years	6	30%	8	40%	
Mean (minimum: maximum)	57.95 (47:80)		58.75 (44:86)		
Median	56.5		58		
Sex					0.739
Male	14	70%	13	65%	
Female	6	30%	7	35%	
Location of lesion					0.209
Right	9	45%	13	65%	
Left	11	55%	7	35%	
Severity of Stroke					
ASPECTS > 7	20	100%	20	100%	-
Complicating factors					0.08
None/1 complicating factor	12	60%	8	40%	
Two (2)/more complicating	8	40%	12	60%	
factors	1	5%	-	0%	
None	11	55%	7	35%	
Hypertension	-	0%	1	5%	
Cor disease	1	5%	9	45%	
DM and Dyslipidemia	-	0%	1	5%	
DM and Cor disease	6	30%	1	5%	
Hypertension and DM	1	5%	1	5%	
Hypertension, DM, and Cor disease					

Most ischemic stroke patients were at the ages of less than 60 years and predominantly male gender. Patients receiving EM at 24 hours mostly had a left lesion the patients receiving EM at 48 hours mostly had right-side lesion was in the control group. Almost all patients have complication factors such as hypertension, diabetes mellitus and dyslipidemia. There were no significant differences between the 24 hours EM and 48 hours EM in the complication profiles. Meanwhile, the level of severity of stroke for subjects using CTScan by ASPECTS score was higher than 7.

The balance and functional ability showed significant differences between 24 hours EM and 48 hours EM on the 7th day, and in functional abilities on the 5th day (Table 3).

Table 3. An overview of the balance and functional abilities of patients after ischemic stroke after early mobilisation

Balance	Treatment Group, n = 20			Control Group, n = 20			p-value
	Median	Mean	SD	Median	Mean	SD	
1st day	0	0.50		0	0.50		
5th day	12.50	23.18	12.70	8.00	17.83	7.31	0.147
7th day	38.50	33.75	16.55	20.50	22.1	13.75	0.038
Functional Ability							
1st day	20.00	22.90		22.00	23.40		
5th day	56.50	55.05	11.15	34.00	38.94	13.47	0.002
7th day	75.50	70.90	17.93	51.00	56.45	20.79	0.021

The balance between the control and treatment groups showed no significant difference ($p > 0.05$) at the 5th day but showed a significant difference on the 7th day ($p < 0.05$). On average the increase in the balance values of treatment and control groups on the 7th day were 33.75 (60.26%) and 22.50 (40.17%) respectively that difference in the value of balance by 11.65 points (20.80%). While the functional ability description between control and treatment group showed a significant difference in both the 5th and 7th day ($p < 0.05$). The average increase in the functional ability of the treatment and control group on the 5th day were 55.05 (55.05%) and 38.94 points (38.94%) and the 7th day were 70.90 (70.90%) and 56.45 (56.46%) respectively. The difference in the value of functional ability on the 5th and 7th day was 16.61 points (16.61%) and 14.95 points (14.95%) respectively.

Discussions

The demographic characteristic of the subjects in this study showed no significant difference between the two groups, the mean age of subjects was 58.35 years, over 67.5% were male, and 55% had right lesions. Similarly, complicating factors statistically showed no significant differences between the two groups despite various complication factors. The highest complication factor was hypertension that has been occurred 55% in the group given EM started at 24 hours and 35% in the group given EM started at 48 hours after ischemic stroke. The second sequence of complication factors was DM and dyslipidemia that has been occurred 5% in the group given EM started at 24 hours and 45% in the group given EM started at 48 hours after ischemic stroke. Meanwhile, the level of stroke severity of both groups is the same, i.e. ASPECTS score was greater than 7. Demographic characteristics showed that all characteristics, including the stroke severity, had no significant differences.

The EM given to the subjects was done as a stimulation to improve neuroplasticity, so that the nerve cells in the brain respond to injury by adapting through structural or functional reorganization to restore function [13] because of the synaptic circuits can be changed by synaptic transmission through change synaptic proteins that can last up to a few minutes [14], [15].

The statistic results showed that the EM between both groups is significantly different in affecting the balance on the 7th day ($p = 0.038$). The level of BBS showed an average increase of balance score of 33.25 for EM at 24 hours and 22.05 for EM at 48 hours with the difference of balance increase was 11.65 points or 20.80%. Meanwhile, the 5th day showed no significant difference between both groups ($p = 0.147$), but it can be seen that there was an increase of balance score in both groups was 22.68 points (40.5%) for EM at 24 hours and 17.33 points

(30.94%) for The EM at 48 hours. This suggests that the 24 hours EM after an ischemic stroke had an impact on the after-stroke healing process.

Furthermore, the functional ability features between the two groups also showed that EM between 24 hours and 48 hours after ischemic stroke gave significant difference on the 7th day ($p = 0.021$) and the 5th day ($p = 0.002$). The level of BI measured on the 7th showed an average increase in the functional ability of 48.00 for the EM at 24 hours and 33.05 for EM at 48 hours. The difference in improvement in functional ability was 14.95 points or 14.95%. While on the 5th day that the level of BI showed an increase of 32.15 points (32.15%) for EM at 24 hours and 15.54 points (15.54%) for EM at 48 hours. That showed an average difference in the increase of functional ability score between both groups was 16.61 points (16.61%). The improvement of functional ability was in line with the improvement in balance. It indicates that the balance both dynamic and static given affect the functional ability. EM provided with clear stage, and appropriate dosages were related to optimising outcomes especially after 3 months post-stroke [1]. Another study [16] that very early mobilisation is not associated with beneficial effect when carried out in patients 24 or 48 hours after the onset of a stroke. There were no significant differences IB score at 3 months, but the length of the hospital stay in the early mobilisation group (24 hours after the attack) was shorter than that in the late mobilisation (48 hours post-attack). There was a possibility of developing improvements from day to day at the beginning of the hospital treatment.

The EM started 24 hours after stroke is associated with the induction of several molecular proteins, e.g. BDNF in the cerebral hemispheric, either lesioned or unlesioned to improve in the event of after ischemic stroke [17]. Increased levels of BDNF on the side of the lesion occur around the neuronal, ependymal, and microglial cells at 24 hours after stroke. Meanwhile, the increase on the side of the unlesioned occurs in the nerve and ependymal cells at 4 to 24 hours after stroke. Increased BDNF also occurs in the cerebral artery and astrocytes 8 days after stroke [18]. If EM is given 48 hours after stroke, it will slow to exploit the expression of several proteins in the brain including BDNF which has an important role as a modulator in the improvement of cell nerve after stroke [19]. BDNF expression will be released after the brain has ischemia, and is a member of the neurotrophin family directly involved in the neurite growth, synaptic transmission, and neurotransmitter synthesis. The EM can improve the ability of BDNF to bind to tropomyosin-receptor kinase B (TrkB) after the event of hypoxia/ischemia [20]. The TrkB is capable of enhancing nerve regeneration through the maturation, growth, and development of nerve cells in the brain, protecting nerves from metabolic disorders, inducing mRNA in the hippocampus, and plasticity of nerve cells that will modulate the survival of nerve cells. The binding between on the BDNF and TrkB may inhibit inflammation, neurotoxicity, and nerve cell apoptosis. EM was also clinically useful in related to the degree of disability which can be seen from the dependency in the daily activities. Exercise can influence nerve plasticity which correlates with intracortical network and motor circuits system that will improve its functional ability.

Although only a few studies discuss the positive effect of EM given 24 hours after a stroke on the experimental animals (rats), they show the neurorestoration process as it does in the results of this study. Some studies report similar results. Ischemic rats with middle cerebral artery occlusion given treadmill can increase expression levels of MK, NGF, PECAM-1, and decrease caspase-3 in the peri-infarct area [21]. The EM started 24 hours in the observed ischemic mice in which it indicated a decrease in the number of apoptosis, inhibition of caspase-3, cleaved caspase-3, an increase in Bcl-2 expression [3]. The rats also showed a decreased volume of infarction and increased motor function. Supported by study, the EM started 24 hours using passive movement in rats with cerebral infarction showed very low caspase-3 and escape latency, while very high Bcl-2 mRNA expression that improved mediation conditions were due to inhibition of apoptotic nerve cells [4]. It provides a change in post-synapse receptor composition, synaptic transmission strength due to synaptic remodelling associated with local cell regulation including mRNA translation, cytoskeleton remodelling, and the role of the receptors both inside and outside synapses [6]. A study [22] on the EM using conscious exercise with mild to moderate intensity at 24 hours after an ischemic stroke can provide neuroprotection in brain injury models. It is related to apoptosis as well as oxidative damage [23]. The EM using the practice of proprioceptive neuromuscular facilitation methods and cognitive exercises starting 6-24 hours after a stroke can improve significant functional ability compared to standard procedure time although the results bring out the same disability after three months [5].

This EM was based on a stroke recovery process that associated with an oedema resolution and return of blood circulation in the penumbra areas that have ischemic, supporting a spontaneous recovery that can be extended a period of resolution from the acute phase to 4 - 6 weeks after stroke which capable of generating adaptive plastic potential that can accelerate the rehabilitation of stroke, when the primary motor cortex (M1) and corticospinal are damaged that the ipsilesional premotor area (PMAs) can be used instead [22].

The EM begins with the provision of stimuli derived from sensory information such as visual, verbal, somatosensory, proprioceptive stimulation, facilitated movements, active participation and active movement targeted movements so that plasticity also

occurs in muscle [15], provides neural improvement and allows for plasticity of neurons as well as motor circuits system due to a series of motion skills using neuro-rehabilitation exercise [24] and conscious physical experience was a great modulation of neural plasticity nervous system in the after stroke [25]. The stimulus that affects neuroplasticity is the basis for the reorganisation of nerve cells including parts of the brain both in the area of the cortex sensory, subcortical, or cerebellum that create the function of balance by the intercortical network [26]. In case that the provision of exercises for postural response was necessary for the balance function. Because the key of EM by exercise is to connect the mind and movement of the limb to inhibition of the inter-hemispheric rebalance, activate the hemisphere and increase motor control particularly on the side lesions [27]. Mobilisation with postural response using posture control exercises, rolling, sitting upright or standing upright to improve balance further enhances the functional ability of after ischemic stroke patients especially for EM at 24 hours after diagnosis of ischemic stroke based on CT Scan.

The concept of EM with progressive exercises that have been formulated by researchers into The TIDIeR as a guide, has been gradually designed starting from the cognitive stages (providing visual, verbal, tactile, proprioceptive stimulus) further upgraded to the associative stages until the movement automatic [28], [29], [30], repeatedly performed, giving feedback [31], with full awareness [32], using functional movements performed automatically resulting in permanent changes [28], [33] and providing functional change.

Some of the limitations of this study are the sample size that was at a minimum so that it is difficult to generalize the results of the study, the characteristics of the patients especially the varied complication factors and the health status of the subjects to control during follow-up and the observation of improving balance and functional ability which was only carried out for 7 days.

The implications of this study are EM with recommended motor learning after 24 hours post-ischemic stroke according to conditions in the criteria, especially for efforts to improve the balance and functional ability of patients with ischemic stroke. It is recommended to observe the expression of nervous growth factor, e.g. BDNF protein after EM started 24 hours after ischemic stroke.

References

1. The AVERT Trial Collaboration Group. Efficacy and safety of very early mobilization within 24 h of stroke onset (AVERT): a randomized controlled trial. Lancet. 2015; 386:5535-46.

2. Zhang ZG, Chopp M, Neurorestorative therapies for stroke: underlying mechanisms and translation to the clinic. Lancet Neurol. 2009; 8(5):491-500. https://doi.org/10.1016/S1474-4422(09)70061-4

3. Zhang P, Zhang Y, Zhang J. Early Exercise Protects against cerebral ischemic injury through inhibiting neuron apoptosis in cortex in rats. Int J Mol Sci. 2013; 14:6074-60891. https://doi.org/10.3390/ijms14036074 PMid:23502470 PMCid:PMC3634421

4. Li M, Peng J, Wang MD, et al. Passive movement improves the learning and memory function of rats with cerebral infarction by inhibiting neuron cell apoptosis. Mol Neurobiol. 2014; 49:216-221. https://doi.org/10.1007/s12035-013-8512-9 PMid:23925702

5. Morrealle M, Marchione P, Pili A, et al. Early versus delayed rehabilitation treatment in hemiplegic patients with ischemic stroke: proprioceptive or cognitive approach? Eur J Phys Rehabil Med. 2015.

6. Nie J, Yang X, Modulation of synaptic plasticity by exercise training as a basis for ischemic stroke rehabilitation. Cel and Mol Neurobiol. 2017; 37:5-16. https://doi.org/10.1007/s10571-016-0348-1 PMid:26910247

7. Rahayu UB, Wibowo S, Setyopranoto I, Development of motor learning implementation for ischemic stroke: finding consensus expert. J Med Sci. 2017; 49(4):200-216.

8. Hoffmann T, Glasziou P, Johnston M. Better reporting of interventions: template for intervention description and replication (TIDieR) checklist and guide better reporting of interventions: template for intervention description and replication (TIDieR). BMJ. 2014; 348:1687. https://doi.org/10.1136/bmj.g1687 PMid:24609605

9. Blum L, Korner BN. Usefulness of the berg balance scale in stroke rehabilitation: a systematic review. Phys Ther. 2008; 88:559-566. https://doi.org/10.2522/ptj.20070205 PMid:18292215

10. Quinn TJ, Langhorne P, Stott DJ. Barthel index for stroke trials: development, properties, and application. Stroke. 2011; 42(4):1146-1151. https://doi.org/10.1161/STROKEAHA.110.598540 PMid:21372310

11. Makizako H, Kobe N, Takano A, et al. Use of the berg balance scale to predict independent gait after stroke: a study of an inpatient population in japan. PM&R. 2015; 7(4):392-399. https://doi.org/10.1016/j.pmrj.2015.01.009 PMid:25633633

12. Hsieh Y, Wang C, Wu S, et al. Establising the minimal clinically important difference of the barthel index in stroke patients. Am Soc Neurorehabil. 2007; 21(3):233-238.

13. Nudo RJ. Neural bases of recovery after brain injury. J Commun Disord. 2011; 44:515-520. https://doi.org/10.1016/j.jcomdis.2011.04.004 PMid:21600588 PMCid:PMC3162095

14. Purves D, Augustine GJ, Fitzpatrick D, et al. Neuroscience. Sinauer Associates Massachusetts. 2004; 582.

15. Calford MB. Dynamic representational plasticity in sensory cortex. Neuroscience. 2002; 111(4):709-738. https://doi.org/10.1016/S0306-4522(02)00022-2

16. Xu T, Yu X, Ou S, Liu X, Yuan J, Chen Y. Efficacy and safety of very early mobilization in patients with acute stroke: a systematic review and meta-analysis. Scientific reports. 2017; 7(1):6550. https://doi.org/10.1038/s41598-017-06871-z PMid:28747763 PMCid:PMC5529532

17. Madinier A, Bertrand N, Rodier M, et al. Ipsilateral versus contralateral spontaneous post-stroke neuroplastic change: involvement of BDNF? Neuroscience. 2013: 231:169-181. https://doi.org/10.1016/j.neuroscience.2012.11.054 PMid:23219910

18. Bejot Y, Tessier AP, Cachia C, et al. Time-dependent contribution of non-neuronal cells to BDNF production after ischemic stroke in rats. Neurochem Int. 2018:102-111.

19. Chen A, Xiong L, Tong Y, et al. The neuroprotective roles of BDNF in hypoxic ischemic brain injury (Review). Biomed Rep. 2013:167-176. https://doi.org/10.3892/br.2012.48 PMid:24648914 PMCid:PMC3956206

20. Kim MW, Bang MS, Han TR, et al. Exercise increased BDNF and trkB in the contralateral hemisphere. Brain Res. 2005:16-21. https://doi.org/10.1016/j.brainres.2005.05.070 PMid:16054599

21. Matsuda F, Sakakima H, Yoshida Y, The effects of early exercise on brain damage and recovery after focal cerebral infarction in rats. Acta Physiol. 2011; 201:275-287. https://doi.org/10.1111/j.1748-1716.2010.02174.x PMCid:PMC3045711

22. Plow EB, Cunningham DA, Varnerin N, et al. Rethinking stimulation of the brain in stroke rehabilitation: why higher motor areas might be better alternatives for patient with greater impairments. Neuroscientist. 2015; 21(3):225-240. https://doi.org/10.1177/1073858414537381 PMid:24951091 PMCid:PMC4440790

23. Xing Y, Yang SD, Dong F, et al. The beneficial role of early exercise training following stroke and possible mechanisms. Life Sci. 2018:32-37. https://doi.org/10.1016/j.lfs.2018.02.018 PMid:29452165

24. Hosp JA, Luft AR. Cortical plasticity during motor learning and recovery after ischemic stroke. Neural Plast 2011. Hindawi Publishing Corporation.

25. Paillard T. Plasticity of the postural function to sport and/or motor experience. J Neurobiorev. 2017; 72:129-152. https://doi.org/10.1016/j.neubiorev.2016.11.015

26. Stein JH, Macho RF, Winstein JC, et al. Stroke recovery & rehabilitation. Demos Medical Publishing. New York.

27. McDermott A, Korner BN. Bilateral arm training in stroke engine intervention. Montreal: McGill University, 2012.

28. Wishart LR, Lee TD, Ezekiel HJ, et al. Application of motor learning principles: The physiotherapy client as a problem-solver. I Concepts. Physiother Can. 2000; 229-232.

29. Halsband U, Lange RK. Motor learning in man: a review of functional and clinical studies. J Physiol. 2006; 99:414-424. https://doi.org/10.1016/j.jphysparis.2006.03.007

30. Taylor JA, Ivry RB. The role of strategies in motor learning. Ann N Y Acad Sci. 2012; 1251:1-12. https://doi.org/10.1111/j.1749-6632.2011.06430.x PMid:22329960 PMCid:PMC4330992

31. Darekar A, McFadyen BJ, Lamontagne A, et al. Efficacy of virtual reality-based intervention on balance and mobility disorders post-stroke: a scoping review. J Neuroeng Rehabil. 2015; 12:46. https://doi.org/10.1186/s12984-015-0035-3 PMid:25957577 PMCid:PMC4425869

32. Lieber RL. Skeletal muscle structure, function and plasticity. The physiological basis of rehabilitation, 2nd ed. Lippincott Williams & Wilkins: London, 2002.

33. Lehto NK, Marley TL, Ezekiel HJ. Application of motor learning principles: the physiotherapy client as a problem-solver. IV. Future directions. Physiother Can. 2001; 109-114.

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Apr 15; 7(7):1093-1096.
https://doi.org/10.3889/oamjms.2019.252
eISSN: 1857-9655
Clinical Science

Diagnostic Value of Platelet–To-Lymphocyte Ratio in Prostate Cancer

Kharisma Prasetya Adhyatma, Syah Mirsya Warli*

Department of Urology, Faculty of Medicine, Sumatera Utara University, Adam Malik Hospital, Medan, Indonesia

Citation: Adhyatma KP, Warli SM. Diagnostic Value of Platelet–To-Lymphocyte Ratio in Prostate Cancer. Open Access Maced J Med Sci. 2019 Apr 15; 7(7):1093-1096. https://doi.org/10.3889/oamjms.2019.252

Keywords: Neutrophil-to-lymphocyte ratio; Platelet-to-lymphocyte ratio; Diagnostic; Prostate cancer

***Correspondence:** Syah Mirsya Warli. Department of Urology, Faculty of Medicine, Sumatera Utara University, Adam Malik Hospital, Medan, Indonesia. E-mail: uro.kharis@gmail.com

Received: 28-Jan-2019; **Revised:** 28-Mar-2019; **Accepted:** 29-Mar-2019; **Online first:** 13-Apr-2019

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Previous studies demonstrated the promising value of platelet-to-lymphocyte (PLR) in prostate cancer.

AIM: This study was conducted to evaluate its pre-biopsy values in predicting prostate cancer.

METHODS: We included all benign prostatic hyperplasia (BPH) and prostate cancer (PCa) patients who underwent a prostate biopsy in Adam Malik Hospital between August 11th 2011 and August 31st 2015. The relationship between pre-biopsy variables which could be affecting the percentage of prostate cancer risk was evaluated, including age, prostate-specific antigen (PSA) level, and prostate volume (EPV). The PLR was calculated from the ratio of related platelets with their absolute lymphocyte counts. The values then analysed to evaluate their associations with the diagnosis of BPH and PCa.

RESULTS: As many as 298 patients consisted of 126 (42.3%) BPH and 172 PCa (57.7%) patients are included in this study. Mean age for both groups are 66.36 ± 7.53 and 67.99 ± 7.48 years old ($p = 0.64$), respectively. There are statistically significant differences noted from PSA (19.28 ± 27.11 vs 40.19 ± 49.39), EPV (49.39 ± 23.51 vs 58.10 ± 30.54), PLR (160.27 ± 98.96 vs 169.55 ± 78.07), and NLR (3.57 ± 3.23 vs 4.22 ± 2.59) features of both groups ($p < 0.05$). The AUC of PLR is 57.9% with a sensitivity of 56.4% and specificity of 55.6% in the cut-off point of 143 ($p = 0.02$). Besides, the NLR cut-off point of 3.08 gives 62.8% AUC with 64.5% sensitivity and 63.5% specificity. We asked for permission from the preceding authors of Indonesian Prostate Cancer Risk Calculator (IPCRC) and calculated its value from 98 randomised patients consist of 45 (45.92%) BPH and 53 (54.08%) PCa. We found a comparable value between PLR/NLR with IPCRC in predicting prostate cancer (AUC of 67.6%, 75.3%, and 68.4%, respectively) with a statistically significant difference of all value in both groups ($p < 0.05$).

CONCLUSIONS: PLR gives promising value in predicting prostate cancer in suspected patients. We suggest a further prospective study to validate its diagnostic values so it can be used as applicable routine calculation.

Introduction

Prostate cancer (PCa) is the second most common cancer worldwide. It accounts for more than 15% of cancer in men, its clinical relevance keeps rising, and 70% of them occurs in developed countries [1], [2]. Prostate biopsy is required for the histopathological diagnosis of prostate cancer, and Trans Rectal Ultrasound Guided procedure remains the gold standard in most countries. Since the biopsy is mostly office procedure and associated with significant complications, various non-invasive strategies have been invented to prevent unnecessary biopsy.

Serum Prostate Specific Antigen (PSA) has been used as the screening standard for patients in suspicion of prostate cancer. In most countries, PSA value of more than 4 ng/ml has been the standard threshold to perform prostate biopsy [1], [2], [3], [4], [5], [6], [7]. But, recent meta-analyses showed that in patients with PSA levels over 4 ng/ml, the positive predictive value of PSA is only 25% [5]. Also, the invasive prostate biopsy may still miss some percentage of cancer, given that up to 20% of men will have prostate cancer in a repeated biopsy [8]. Various imaging and biomolecular marker have been suggested to increase diagnostic accuracy, but none of these methods is available for widespread use, either due to availability issues or the high cost [2], [8].

Over the past decades, our study of the microenvironment of cancer has supported Virchow's hypothesis of the connection between inflammation and cancer. Inflammatory markers have been associated with more aggressive disease [6], [9]. Though small in numbers, previous studies demonstrated a promising value of platelet-to-lymphocyte (PLR) in prostate cancer. Kaynar et al. found an increased level of PLR in PCa compared with that in benign prostatic hyperplasia (BPH) with PSA value greater than 10 ng/ml [10]. A statistically significant higher value of PLR in PCa compared to BPH patients was also demonstrated by Yuksel et al., in 2015 [5]. According to our knowledge, there is still no data on the use of PLR value as a predictor of PCa in Indonesia.

Therefore, this study is conducted to evaluate its pre-biopsy value in predicting PCa.

Material and Methods

Population of Study

This is a diagnostic study with a retrospective design. All patients who underwent a prostate biopsy in Adam Malik General Hospital between August 2011 and August 2015 were included. Data related to prostate cancer prediction factors were collected, and their relationship with malignant pathology was analysed. The factors included were: age, serum PSA value, and estimated prostate volume (EPV). The PLR values were calculated using the routine blood count results, collected right before the biopsy procedures were performed. Histopathology of the biopsy specimen was applied as the gold standard of PCa diagnosis. Patients with irrelevant and incomplete data were excluded from the study.

Variables

Serum PSA was collected from recent laboratory results just before biopsy procedures were performed. We collected EPVs from their initial prostatic Trans Abdominal ultrasound (TAUS) data. Prostate was measured in 3-dimensional aspects, and its volume was estimated with the modified ellipsoid formulation in cm^3 (0.523 [(length x width x height)]. PLR value is a direct ratio of platelets and absolute lymphocyte count which was acquired from the routine blood count at initial assessment.

Analysis

Data input and analyses were performed using SPSS ver 20.0 software. Data will be divided into two groups according to their histopathology of prostate biopsy, the BPH and PCa group. Data related to PCa prediction such as routine blood count and PLR of each group will be distributed in frequency table and analysed for their value in predicting biopsy results with bivariate analysis. A p value of < 0.05 (α = 5%) was considered statistically significant.

Results

Characteristics and Bivariate Analysis

As many as 298 patients consisted of 126 (42.3%) BPH and 172 PCa (57.7%) patients are included in this study. Mean age for both groups are 66.36 ± 7.53 and 67.99 ± 7.48 years old (p = 0.64), respectively. Patient's characteristics and laboratory values are shown in Table 1.

Table 1: Patients Characteristics and Hematologic Parameters

Parameters	BPH (n = 126) Mean ± SD (Median)	PCa (n = 172) Mean ± SD (Median)	p
Age (years)	66.36 ± 7.53	67.99 ± 7.48	0.64*
PSA (ng/dL)	19.28 ± 27.11	40.19 ± 49.39	< 0.0001*
EPV (cm^3)	49.39 ± 23.51	58.10 ± 30.54	0.02*
Hb	12.99 ± 2.00 (13.20)	12.95 ± 2.01 (13.10)	0.754**
Leucocytes Count (x $10^3/mm^3$)	8.67 ± 3.45 (8.11)	9.19 ± 3.29 (8.46)	0.1**
Absolute Lymphocyte Count (x $10^3/mm^3$)	2.09 ± 0.83 (2.02)	2.00 ± 0.76 (1.87)	0.29**
Platelets Count (x $10^3/mm^3$)	286.16 ± 112.24 (266)	311.61 ± 120.81 (294)	0.049**
PLR	160.27 ± 98.96 (128.13)	169.55 ± 78.07 (151.28)	0.02**

*T-test **Mann-Whitney Test

Comparing the laboratory results of both groups, statistically, significant differences were noted from PSA (19.28 ± 27.11 vs 40.19 ± 49.39), EPV (49.39 ± 23.51 vs 58.10 ± 30.54), and PLR (160.27 ± 98.96 vs 169.55 ± 78.07) in each bivariate analysis.

PLR and PSA

We then performed a Receiver Operating Characteristics (ROC) analysis to define the Area under Curve (AUC) of PLR in predicting prostate cancer (Figure 1 and Table 2).

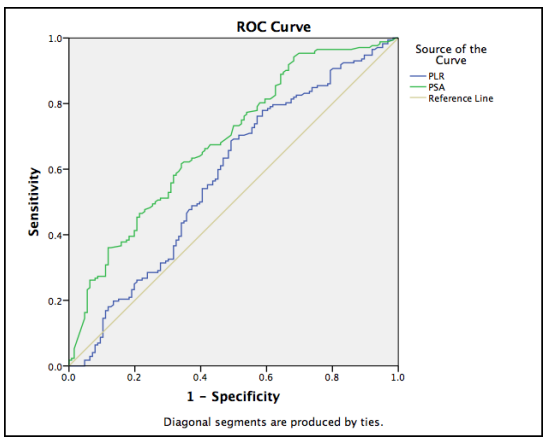


Figure 1: The ROC Curves of PLR and PSA

The AUC of PLR is 57.9% with a sensitivity of 56.4% and specificity of 55.6% in the cut-off point of 143 (p = 0.02).

Table 2: The AUC of PLR and PSA

Parameters	AUC	p
PLR	57.9%	0.02
PSA	68.5%	< 0.0001

PLR and IPCRC

We asked for permission from the preceding authors of Indonesian Prostate Cancer Risk Calculator (IPCRC) and calculated its value from 98 randomised patients which consist of 45 (45.92%) BPH and 53 (54.08%) PCa.

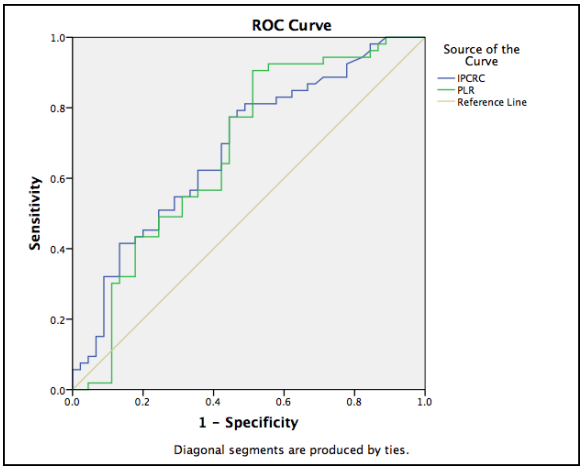


Figure 2: The ROC Curves of PLR and IPCRC Score

We found a comparable value between PLR with IPCRC in predicting prostate cancer (AUC of 67.6% and 68.4%, respectively) with a statistically significant difference was noted between each value (p < 0.05) as shown in Figure 2 and Table 2.

Table 2: The AUC of PLR and IPCRC

Parameters	AUC	p
PLR	67.6%	0.003
IPCRC	68.5%	0.002

Discussion

The body response to cancer parallels with inflammation and wound healings. In 1863, Rudolf Virchow noted leucocytes in neoplastic tissues and suggested a connection between inflammation and cancer. He suggested that the "lymphoreticular infiltration" reflected the origin of cancer at sites of chronic inflammation. Tumour-infiltrating lymphocytes may contribute to cancer growth and spread, and the immunosuppression-associated malignant diseases. In his review in 2001, Balkwill et al. still mentioned the theory of "Tumors: wounds that do not heal" previously showed by Dvorak in 1986. This theory showed how wound healing and tumour stroma formation share many important features. Wound healing is usually self-limiting, but tumours secrete a vascular permeability factor, vascular endothelial growth factor (VEGF), that can lead to persistent extravasation of fibrin and fibronectin and continuous regeneration of extracellular matrix. Platelets in wounds are critical sources of cytokines, especially for transforming growth factor β (TGF-β) and VEGF. Platelet release may also play an important role in angiogenesis. Also, malignant cells secrete proinflammatory cytokines independently [9]. This will be the basis of predicting cancer through platelets count [6], [9]. Our study found a significant difference between platelet counts of PCa and benign prostatic lesions (311.61 ± 120.81 vs 286.16 ± 112.24; p=0.049) which supported the previous theory. But, a previous larger sample study from Yuksel et al., did not show the same results (p = 0.094) [5]. From these findings, we concluded that platelet count could not stand alone as the only predictive marker.

Though inflammatory markers such as lymphocytes were mentioned in previous studies, not all markers are coherent with every cancer. Leucocytes, mainly lymphocytes, are the most prominent marker in many cancers, but not in PCa. Study of Cihan Y, et al. showed that patients with PCa had a lower level of lymphocytes, neutrophils, and a higher level of monocytes with a significant difference in lymphocyte count, compared to healthy controls [11]. McDonald et al., also found that lymphocytes count is significantly lower in patients with elevated PSA compared with patients with PSA below 4 ng/ml [3]. Though this study found that the absolute lymphocyte counts of PCa patients are lower, the difference was not statistically significant compared with a benign group (2.09 ± 0.83 vs 2.00 ± 0.76; p = 0.23).

In this study, though we found a significant difference of platelets count with no statistical difference in lymphocyte count between both groups, the ratio of PLR value gives the event more significant difference (p = 0.02). A similar result was also shown by Yuksel et al., where a significant intergroup statistical difference was found for PLR (p = 0.041) but not for lymphocyte count (p > 0.05) [5]. This also supported by the study of Li et al., who found a statistical difference between PCa and normal/BPH patient (p < 0.05). Kaynar et al., also found that statistically significant value of PLR was observed in PCa and BPH patients with PSA above 10 ng/ml [10].

Yuksel et al. found a statistically significant difference of PLR value between PCa and BPH patients, but not between PCa and prostatitis (p = 0.018 vs p = 0.067). This could be related to the

previous theory of “Tumors: wounds that do not heal” [9]. According to this theory, inflammation cascade, which always happens in the inflammation process, is also continuously happening in tumours without receding. In this study, we put aside the prostatic non-BPH benign lesions to selectively reduce this bias. So we merely compared the histopathologically proven BPH and PCa patients. However, this can only happen in the study with the retrospective design. In the case of prospective design, we cannot conclude whether the prediction of PCa through PLR value can differentiate the histopathology of PCa and prostatitis. Further prospective studies, as well as more predictive marker, are needed.

In conclusion, inflammation cascade, which always happens in the inflammation process, is also continuously happening in tumours without receding. PLR gives promising value as a systemic inflammatory marker in predicting prostate cancer in suspected patients. And in this study, we tried to investigate the applicability in Indonesia. But, if to be applied as routine testing and to selectively decide candidates for prostate biopsy in a patient with PSA value more than 4 ng/ml, this value needs a further prospective trial.

References

1. (IARC) G. Prostate Cancer: Estimated Incidence, Mortality, and Prevalence Worldwide in 2012. IARC, 2012.

2. Gokce MI, Hamidi N, Suer E, Tangal S, Huseynov A, Ibis A. Evaluation of neutrophil-to-lymphocyte ratio prior to prostate biopsy to predict biopsy histology: Results of 1836 patients. Canadian Urological Association journal = Journal de l'Association des urologues du Canada. 2015; 9(11-12):E761-5. https://doi.org/10.5489/cuaj.3091 PMid:26600880 PMCid:PMC4639422

3. McDonald AC, Vira MA, Vidal AC, Gan W, Freedland SJ, Taioli E. Association between systemic inflammatory markers and serum prostate-specific antigen in men without prostatic disease - the 2001-2008 National Health and Nutrition Examination Survey. The Prostate. 2014; 74(5):561-7. https://doi.org/10.1002/pros.22782 PMid:24435840 PMCid:PMC4380881

4. Kawahara T, Fukui S, Sakamaki K, Ito Y, Ito H, Kobayashi N, et al. Neutrophil-to-lymphocyte ratio predicts prostatic carcinoma in men undergoing needle biopsy. Oncotarget. 2015; 6(31):32169-76. https://doi.org/10.18632/oncotarget.5081 PMid:26359354 PMCid:PMC4741667

5. Yuksel OH, Urkmez A, Akan S, Yldirim C, Verit A. Predictive Value of the Platelet-To-Lymphocyte Ratio in Diagnosis of Prostate Cancer. Asian Pacific journal of cancer prevention : APJCP. 2015; 16(15):6407-12. https://doi.org/10.7314/APJCP.2015.16.15.6407 PMid:26434851

6. Sidaway P. Prostate cancer: Platelet-to-lymphocyte ratio predicts prostate cancer prognosis. Nature reviews Urology. 2015; 12(5):238. https://doi.org/10.1038/nrurol.2015.69 PMid:25823375

7. Li F, Hu H, Gu S, Chen X, Sun Q. Platelet to lymphocyte ratio plays an important role in prostate cancer's diagnosis and prognosis. International journal of clinical and experimental medicine. 2015; 8(7):11746-51. PMid:26380014 PMCid:PMC4565397

8. Oh JJ, Kwon O, Lee JK, Byun SS, Lee SE, Lee S, et al. Association of the neutrophil-to-lymphocyte ratio and prostate cancer detection rates in patients via contemporary multi-core prostate biopsy. Asian journal of andrology. 2015. https://doi.org/10.4103/1008-682X.164198

9. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet (London, England). 2001; 357(9255):539-45. https://doi.org/10.1016/S0140-6736(00)04046-0

10. Kaynar M, Yildirim ME, Gul M, Kilic O, Ceylan K, Goktas S. Benign prostatic hyperplasia and prostate cancer differentiation via platelet to lymphocyte ratio. Cancer biomarkers : section A of Disease markers. 2015; 15(3):317-23. https://doi.org/10.3233/CBM-150458 PMid:25586096

11. Cihan YB, Arslan A, Ergul MA. Subtypes of white blood cells in patients with prostate cancer or benign prostatic hyperplasia and healthy individuals. Asian Pacific journal of cancer prevention: APJCP. 2013; 14(8):4779-83. https://doi.org/10.7314/APJCP.2013.14.8.4779 PMid:24083743

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Apr 15; 7(7):1097-1102.
https://doi.org/10.3889/oamjms.2019.228
eISSN: 1857-9655
Clinical Science

Abdominal Compartment Syndrome in Critically Ill Patients

Mohsen Sadeghi[1], Arda Kiani[2], Kambiz Sheikhy[3], Kimia Taghavi[1], Mohsen Farrokhpour[1], Atefeh Abedini[1*]

[1]Chronic Respiratory Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran; [2]Tracheal Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran; [3]Lung Transplantation Research Center, National Research Institute of Tuberculosis and Lung Disease (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran

Citation: Sadeghi M, Kiani A, Sheikh K, Taghavi K, Farrokhpour M, Abedini A. Abdominal Compartment Syndrome in Critically Ill Patients. Open Access Maced J Med Sci. 2019 Apr 15; 7(7):1097-1102. https://doi.org/10.3889/oamjms.2019.228

Keywords: Intra-Abdominal Hypertension; Incidence; Critical Illness; Intensive Care Units; Prognosis; Glasgow Coma Scale; Systemic Inflammatory Response Syndrome; APACHE

***Correspondence:** Atefeh Abedini. Chronic Respiratory Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran. E-mail: dr.abedini110@sbmu.ac.ir

Received: 27-Jan-2019; **Revised:** 03-Apr-2019; **Accepted:** 04-Apr-2019; **Online first:** 13-Apr-2019

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Abdominal compartment syndrome patients suffer severe obstacles such as kidney failure and shock. To evade further complications, identifying the abdominal compartment syndrome (ACS) and Intra-abdominal hypertension (IAH), in critically ill individuals and hospitalised in the intensive care unit (ICU) is obligated.

AIM: The current study intended to study the abdominal compartment syndrome and the concomitant risk factors among hospitalised patients in ICU, by using the Intra-abdominal pressure test.

MATERIAL AND METHODS: One hundred and twenty-five hospitalised patients at ICU entered the current survey. Abdominal pressure was measured by standard intravesical technique. The SPSS 21 analysed the preoperative and intraoperative factors such as demographic records and comorbidities.

RESULTS: Seventy-three (58.4%) participants were males and 52 (41.6%) were women in the mean age of 55.1 ± 18.3 years. Eighty-nine patients (71.2%) showed normal intra-abdominal pressure since 31 patients (24.8%), and 5 patients (4%) developed IAH and ACS. The intra-abdominal pressure (IAP) applied to Glasgow Coma Scale (GCS), Acute Physiology, shock, Systemic Inflammatory Response Syndrome (SIRS), central venous oxygen saturation and Chronic Health Evaluation (APACHE II) score ($P < 0.05$). Patients with high IAP have shown a higher mortality frequency, compared to others ($P < 0.05$).

CONCLUSION: Current findings showed a correlation between IAP hospitalised patients in ICU and shock, SIRS, APACHE II, central venous oxygen saturation and GCS. Intra-abdominal pressure test, as a valuable prognosis test for the abdominal compartment syndrome (ACS) and Intra-abdominal hypertension (IAH), may offer better results when added to the routine medical checkup of ICU patients.

Introduction

Despite improved survival reports following the laparotomy method, the abdominal compartment syndrome (ACS) is quite an expanding matter [1]. Increased intra-abdominal pressure (IAP) points to intra-abdominal hypertension (IAH) that influences the body function in critically ill patients and cause abdominal compartment syndrome (ACS). As stated by the World Society of the Abdominal Compartment Syndrome (WSACS), IAH is defined above 12 mmHg intra-abdominal pressure (IAP). The low-grade IAH is two types. IAP 12–15 mmHg specifies the IAH Grade I and IAP 16–20 mmHg specifies the IAH Grade II [2]. ACS is also defined as an IAP > 20 mmHg with proven signs of failure in new organs, for instance, kidney failure or increasing complications in ventilation [3]. ACS is an intra-abdominal pathology proven from an extra-abdominal source. IAH and ACS happen following reduced abdominal wall compliance and/or enlarged intra-abdominal capacities [2].

The importance of IAP detection in susceptible patients to an IAH and ACS is well known. Intravesical pressure (IVP) measurement is now the gold standard for indirect diagnosis of IAP [4]. IAH is reported in 32.1% of critically ill patients. IAH is also a predictor for mortality and is seen in 30-50% of intensive care hospitalised patients [5].

IAH and ACS develop in critically ill patients, caused by several risk factors such as abdominal

surgery, hypoalbuminemia, trauma, hypoalbuminemia, and high-volume resuscitation [6].

Proper diagnostic techniques can accelerate future researches in evaluating the pathophysiological mechanism of IAH/ACS [5]. Several types of research have been conducted to increase the accuracy of the diagnosis of IAH/ACS, such as the new porcine model of ACS, which was introduced by Shah et al., [7].

Interestingly, there have been several reports on the growing prevalence of ACS reports in the intensive care unit (ICU) and medical ICUs (10.5%) [8]. Therefore, a rapid and right tool to rapid and exact determining the IAH is trustworthy [9].

Based on the literature, IAH characterises a severe disorder with a high incidence in ICU (18%-81%) [6]. However, because of the insidious origination and nonspecific signs of IAH, it has not been accurately studied. Hence, the object of the current study was to test abdominal compartment syndrome and the concomitant risk factors among hospitalised patients in ICU, using the Intra-abdominal pressure test.

Material and Methods

Study design

Patients who referred to the ICU of a tertiary hospital in Tehran because of surgical or non-surgical problems, included in the current study. The mean age for the patients would be > 18 years old. Demographic indexes including age, sex, Glasgow Coma Scale (GCS), BMI, AQA Acute Physiology and Chronic Health Evaluation score (APACHE II) were collected. The exclusion criteria comprised the presence of contraindications for urinary catheterisation (especially in trauma patients) and age < 18. One hundred and twenty-five patients in ICU joined current study [10]. In the current study, disease severity was measured based on APACHE II score (calculated at the time of IAP measurement) and a 12 routine physiologic point score, age, and history of health status that shows the severity of the disease. We considered an increasing score (range 0 to 71) as a risk case of hospital death [11].

Intra-abdominal pressure

The abdominal pressure was measured using the bladder catheter by a standard intravesical method. The catheter clamped and then using a portal aspiration, 25 ccs of hygienic saline was inoculated to the bladder via an attached catheter by an 18-gauge needle to the pressure manometer. Zero of manometer located on mid-maxillary line at the level of the umbilicus. IAH was recorded in Supine and end duration [10]. IAP of ≥ 12 mmHg was determined as hypertension. Also, the IAP of ≥ 12 mmHg + intra-abdominal dysfunctions with/without APP < 60 mmHg were used as ACS. The participants completed and signed informed consent. Each participant was informed about the benefits of the study and personal information kept as secret. The Ethical Committee received.

Data and analysis

Statistical analyses performed by Statistical Package for the Social Sciences (SPSS) (ver. 22.0; SPSS Inc. Chicago, IL, USA) software. The nonparametric Mann-Whitney U test determined the between treatments comparisons. Also, a Student's t-test calculated the differences in the mean, considering a p-value of < 0.05 meant for a significant value.

Results

Tables 1 show demographic data of the study subjects.

Table 1: Anthropometric indexes of participants

	Male	Female
Sex	73 (58.4%)	52 (41.6%)
Age	54.5 ± 18.8	56 ± 17.7

Table 2 shows the body mass index of the study subjects.

Table 2: Body mass index of the study subjects

Underweight	Normal	Overweight	Obese
4.8%	26.4%	52%	16.8%

As seen in Table 3, 73 patients (58.4%) referred to the hospital for surgery while 52 individuals (41.6%) hospitalised for medical problems.

Table 3: The cause of hospitalising in the ICU

Diagnosis	Frequency	Per cent	Diagnosis	Frequency	Per cent
Abdominal mass	2	1.6	Cervix cancer	1	0.8
AKI	3	2.4	CHF	2	1.6
Amputation	2	1.6	Cholangitis	1	0.8
Ascitis	2	1.6	Cholecystectomy	2	1.6
Brain tumor	7	5.6	Cirrhosis	1	0.8
Bronchiectasis	1	0.8	Colectomy	3	2.4
Cerebral aneurysm	1	0.8	COPD	3	2.4
CVA	7	5.6	Electrical injury	1	0.8
DAI	4	3.2	Empyema	1	0.8
DKA	2	1.6	Encephalitis	1	0.8
EDH	1	0.8	Femur FX	6	4.8
Gastric cancer	2	1.6	Intoxication	1	0.8
GIB	3	2.4	LOC	2	1.6
ICH	3	2.4	MI	1	0.8
Intestinal Obstruction	2	1.6	MT	13	10.4
Myasthenia Gravis	2	1.6	Pelvic FX	5	4.0
OSA, OHS	1	0.8	Peritonitis	6	4.8
Ovarian cancer	1	0.8	Pneumonia	7	5.6
Pancreatitis	3	2.4	PTE	2	1.6
Rectal cancer	1	0.8	Spondylodiscitis	1	0.8
SAH	4	3.2	Status Epilepticus	2	1.6
SDH	3	2.4	TTP	1	.8
SLE	1	0.8	Urosepsis	2	1.6
Splenectomy	1	0.8	Vasculitis	1	0.8

Acute Kidney Injury (AKI), Cerebrovascular accident (CVA), Diffuse axonal injury (DAI), Diabetic ketoacidosis (DKA), Subdural hematoma (SDH), chronic obstructive pulmonary disease COPD, Pulmonary Thromboendarterectomy (PTE), Thrombotic Thrombocytopenic Purpura (TTP). Thirty-nine patients (31.2%) had trauma while 86 persons (68.8%) had no earlier trauma. Also, only 34 of the patients (27.2%) had a history of bone fracture. Thirty-nine patients had normal ventilation (31.2%) while 71 patients (56.8%) used a mechanical ventilator and the 15 of them (12%) had the tracheostomy. Table 4 presents the result of blood products transfused for the participants. Those with IAP, IAH, and ACS received 1.3 ± 4, 2.3 ± 2.5 and 7.6 ± 3.2 units of blood products (P < 0.001).

Table 4: Blood analysis report in participants

Blood products	Frequency	Per cent
NO	64	51.2
PC	41	32.8
FFP	2	1.6
PLT	3	2.4
CRYO	2	1.6
PC, FFP	9	7.2
PC, FFP, PLT	3	2.4
PC, FFP, PLT	1	0.8

NO: No blood Products, PC: Packed cell, FFP: Fresh frozen plasma, PLT: Platelet, CRYO: Cryoprecipitate.

As stated by the records, 11 patients had a shock, and 92 experienced none shock. Also, 11 patients among 22 SIRS-positive patients had a shock. At the first visit, patients in the ICU underwent for monitoring the IAH, IAP, and ACS. Table 5 presents the data.

Table 5: The per cent of IAH, IAP and ACS and APACHE II and time duration of hospitalising of the patient in the ICU

	Per cent of patients	APACHE II > 20	APACHE II < 20	the time duration of hospitalise (day)
IAH	9.52	81	8	14 ± 9.7
IAP	90.48	9	22	18.5 ± 5.9
ACS	-	-	5	29 ± 6

ACS: Abdominal compartment syndrome, IAP: Increased intra-abdominal pressure, IAH: Intra-abdominal hypertension.

Table 6 displays the correlation between disease history and IAP in the patients. Based on the results, the concomitant disease had no significant correlation with the IAP (P = 0.09).

Table 6: The correlation between disease history and IAP in the patients

Comorbidity	IAP			Total
	Normal	IAH	ACS	
AF	3	1	0	4
Asthma	2	0	0	2
Cerebral Palsy, Seizure	0	1	0	1
CHF	1	0	0	1
Colon cancer	1	1	0	2
COPD	2	0	0	2
DM	7	5	0	12
DM, Cirrhosis, Rectum adenocarcinoma	0	1	0	1
DM, HLP, Hypothyroidism	1	0	0	1
HBV	1	1	0	2
HLP	1	2	0	3
HTN	7	3	0	10
HTN, DM	5	3	1	9
HTN, DM, CVA	0	1	0	1
HTN, DM, HLP	0	1	0	1
HTN, HLP, BPH, CKD, AF	2	0	0	2
HTN, HLP, DM, IHD	5	0	3	8
IHD	2	0	0	2
MVR, AF	1	0	0	1
NO	45	9	1	55
Osteporosis	1	0	0	1
Polyp	0	1	0	1
PUD	2	1	0	3
Total	89	31	5	125

AF: Atrial fibrillation, CHF: Congestive heart failure, DM: Diabetes mellitus, HLP: hyperlipidemia, HBV: Hepatitis B, HTN: Hypertension, BPH: Benign prostatic hyperplasia, CKD: Chronic kidney disease, IHD: Ischemic heart disease, MVR: Mitral Valve Repair, Cerebrovascular accident (CVA), NO: No blood Products.

As shown in Figure 1, the IAP of patients with trauma was 10.04 ± 5.08 mmHg while it was 9.0504 ± 5.08 mmHg in non-traumatic patients (P = 0.19). Also, the IAH and ACS incidences were 30 and 2% in trauma patients.

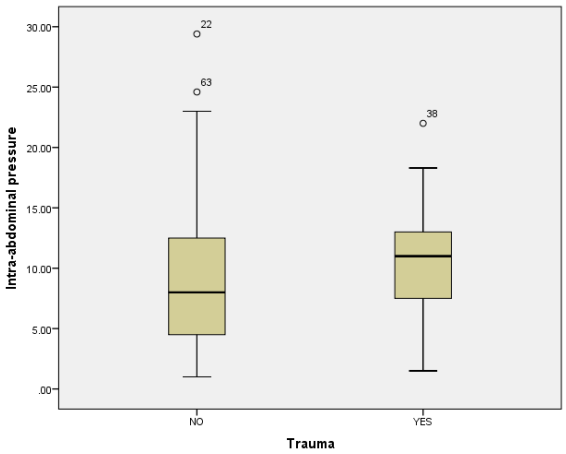


Figure 1: The IAP in patients with or without trauma (IAP: Increased intra-abdominal pressure)

The mean intra-abdominal pressure was 8.3 ± 5 mmHg in non-ventilated patients and 9.4 ± 5.6 mmHg in patients with tracheal intubation. Patients with tracheostomy had an IAP of 11.3 ± 6.3 mmHg (P = 0.15).

There was no significant correlation between positive end-expiratory pressure (PEEP) and intra-abdominal pressure, so that in patients without PEEP, the mean intra-abdominal pressure was 8.4 ± 4.9 mm. In PEEP, three centimetres of intra-abdominal pressure was 4.4 ± 7.9 mm Hg, and at a pressure of 5 cm water, the pressure inside the abdomen was 5.9 ± 9.9 mm Hg and at 7 cm water pressure, intra-abdominal pressure was 4.5 ± 7.7 mm Hg (P = 0.15).

Received fluid in patients with normal intra-abdominal pressure was 1.6 ± 2.2 litres. While the significant different received fluid volume in patients with IAH and patients with ACS was 1.5 ± 2.4 litres and 0.4 ± 4.3 litres respectively (P = 0.002) (Figure 2).

Of the patients with normal intra-abdominal pressure, six patients did not take fluids. While, 33, 16, one, six, 22, one, three and one patients took normal saline, Dextrose saline, 5% dextrose, half-saline, normal saline/dextrose saline, normal saline/5% dextrose, normal saline/half saline, normal saline/5% dextrose, monophagous in turn.

Ten of patients with IAH took normal saline, five took dextrose saline, three took the half-saline, 10 took normal/ dextrose saline, and one patient took normal/half-saline/2% dextrose water/5% amino fusion. Three and two of patients with ACS took normal saline and normal saline/dextrose saline (P = 0.89).

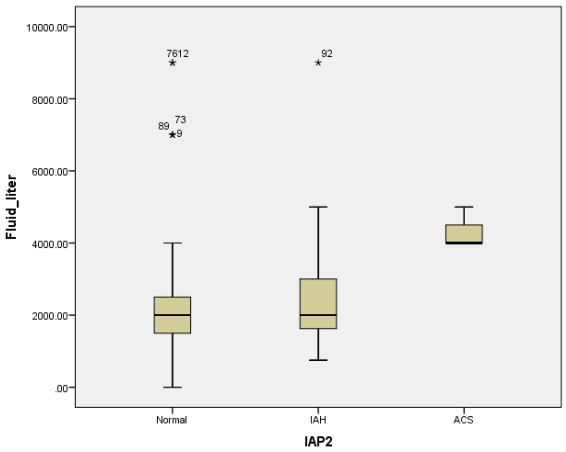


Figure 2: Volume of daily IV fluid in patients (IV: Intravenous)

A significant correlation was documented between homeostasis disorders and IAP in patients (P < 0.001).

Table 7: Correlation between intra-abdominal pressure and homeostasis disorders in patients

Hemostasis	IAP			Total
	Normal	IAH	ACS	
Normal	53	11	0	64
Abnormal PT	14	11	0	25
Abnormal PTT	7	1	0	8
Thrombocytopenia	1	6	2	22
Abnormal PT, PTT	0	2	1	3
PT and Low Plt	1	0	1	2
PT, PTT, Low Plt	0	0	1	1
Total	89	31	5	125

PTT: Partial Thromboplastin Time, PT: Prothrombin Time, PLT: Platelets.

There was no significant correlation between culture types in patients with intra-abdominal pressure (Figure 3) (P = 0.07).

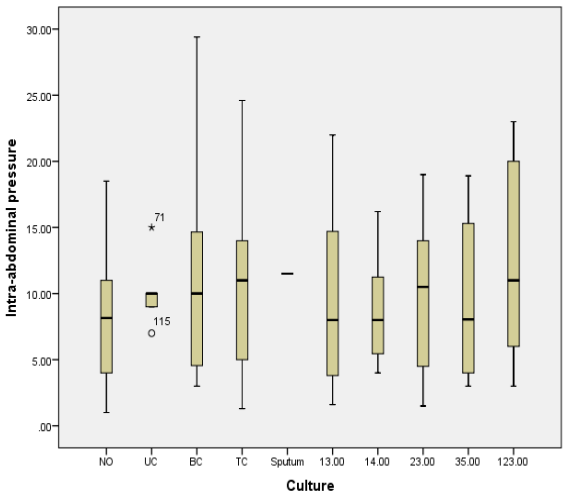


Figure 3: Correlation between culture types in patients with intra-abdominal pressure

In the current study, 13% of the patients with normal intracranial pressure (n = 11) had acidosis, 12% of patients with IAH (n = 4) exhibited acidosis, and 100% of patients with ACS (n = 5) had acidosis (P = 0.001). As presented in Table 8, there was no significant correlation between the type of antibiotic received and intra-abdominal pressure (P = 0.46).

Table 8: Correlation between antibiotic administration and IAP

Antibiotics	IAP			Total
	Normal IAP	IAH	ACS	
Acyclovir	0	1	0	1
Amikacin, Meropenem	2	0	0	2
Cefazoline	2	1	0	3
Cefazoline, Ceftriaxone, Clindamycin	1	0	0	1
Cefotaxime	0	1	0	1
Ceftriaxone, Metronidazole	1	1	0	2
Ciprofloxacine	4	3	0	7
Clindamycin	1	0	0	1
Colistin, Meropenem	3	1	0	4
Colistin, Tazocin	2	0	0	2
Gentamycin	3	1	0	4
Gentamycin, Ciprofloxacin	0	1	0	1
Imipenem, Targocid, Metronidazole	3	2	2	7
Meropenem	6	3	0	9
Metronidazole	5	0	0	5
Metronidazole, Ciprofloxacin	1	0	0	1
NO	32	11	0	43
Vancomycin, Ciprofloxacin, Tazocin	1	0	0	1
Vancomycin, Imipenem, Ciprofloxacin	3	0	0	3
Vancomycin, Meropenem	6	3	0	9
Vancomycin, Meropenem, Ampicillin	1	0	0	1
Vancomycin, Meropenem,Ciprofloxacin	8	1	2	11
Vancomycin, Meropenem, Ciprofloxacin, Colistin, Tazocin	1	0	0	1
Vancomycin, Meropenem, Colistin	2	0	0	2
Vancomycin, Meropenem, Metronidazole	1	1	1	3
Total	89	31	5	125

The SCVO2 in normal IAP patients was 76.8% ± 8.5 while in IAH and ACS patients were 74.6 ± 1 and 59.8% ± 0.8 (P = 0.001). Table 9 displays the correlation between IAP and the identification of the disease. According to the results, a significant correlation detected between IAP and diagnosis of the disease (P = 0.02).

Table 9: Correlation between IAP and diagnosis of the disease

	Normal IAP	IAH	ACS		Normal IAP	IAH	ACS
Abdominal mass	1	0	1	Bronchiectasis	1	0	0
AKI	0	3	0	Cerebral aneurysm	1	0	0
Amputation	2	0	0	Cervix cancer	1	0	0
Ascitis	1	1	0	CHF	2	0	0
Brain tumor	6	1	0	Cholangitis	0	0	1
Cholecystectomy	2	0	0	Colectomy	1	2	0
Cirrhosis	1	0	0	COPD	3	0	0
CVA	6	1	0	Empyema	1	0	0
DAI	4	0	0	Encephalitis	1	0	0
DKA	2	0	0	Femur FX	2	4	0
EDH	1	0	0	Gastric cancer	2	0	0
Electrical injury	1	0	0	GIB	3	0	0
ICH	3	0	0	MT	8	4	1
Intestinal Obstruction	0	2	0	Myasteni Gravis	2	0	0
Intoxication	1	0	0	OSA,OHS	0	1	0
LOC	2	0	0	Ovarian cancer	0	1	0
MI	1	0	0	Pancreatitis	0	3	0
Pelvic FX	2	3	0	SAH	4	0	0
Peritonitis	1	4	1	SDH	2	1	0
Pneumonia	7	0	0	SLE	1	0	0
PTE	2	0	0	Splenectomy	1	0	0
Rectal cancer	0	0	1	Spondylodiscitis	1	0	0
Status Epilepticus	2	0	0	TTP	1	0	0
Urosepsis	2	0	0	Vasculitis	1	0	0

ICH: Intracranial Hemorrhage, LOC: Loss of Consciousness, MI: Myocardial Infarction, PTE: Pulmonary ThromboEmbolism.

As observed in figure 4, the IAP in normal patients detected in 89 patients and among them, 84 survived, but 5 passed away. Furthermore, in IAH patients, 21 survived, and 10 expired. Entire ACS

patients died (P = 0.001). The mean IAP of expired patients was 15.5 ± 6.7 mmHg while in endured patients the IAP was 8.1 ± 4.5 mmHg.

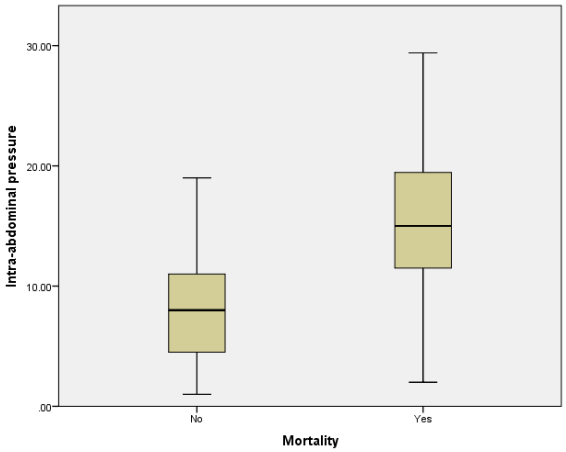


Figure 4: IAP pressure in expired or survived patients (IAP: Increased intra-abdominal pressure)

Discussion

One of the main challenges in Iran is managing the patients with an open abdomen admitted to ICUs. The open abdomen stays at high risk of several complications and organising a trusty closure starts right after hernia. The qualified management in ICU wards controls the feasibility of closure an open abdomen. For that reason, further studies are favourable on the modality of reaching this target. Therefore, the current study intended to test the frequency of the abdominal compartment syndrome and the concomitant risk factors among hospitalised patients in ICU, using the Intra-abdominal pressure test.

Several approaches diminished IAH and to avoid development to the ACS [12]. IAH affects not only abdominal organs but also several organ organisations. It influences lungs, hemodynamics, and cerebral perfusion systems. IAH is the main determining compliance of the mechanically ventilated patients [13]. As observed in this study, shock, SIRS, APACHE II, central venous oxygen saturation and GCS were associated with the IAP. The mortality frequency was higher in patients with advanced IAP. Our findings suggest IAP measurement is necessary for ICU patients to find their prognosis and right intervention. IAH is a source of organ dysfunctions, and ACS is a catastrophic disruption of body physiology that needs urgent treatments [14].

In this study, 39 patients (31.2%) had trauma while 86 persons (68.8%) had no earlier trauma. In this study, the IAP of the patients with normal ventilation was 8.3 ± 5 mmHg. As observed in this study, the IAP for patients with the endotracheal tube and tracheostomy were 9.4 ± 5.6 and 11.3 ± 6.3 mmHg (P = 0.15).

Nowadays IAP is performed as a safe, reliable and reproducible technique [14]. IAH and ACS left significant influences on blood factors of the participants in the current study. To avoid overloading of massive fluid, starting resuscitation is mandatory [15]. IAH takes place in about 50% of critical care patients, 32.1% of which showed IAH and 4.2% develop ACS within the first day of admitting to ICU [16].

Patients with ACS succeed with pharmacological, practical and medical trials [16]. Significant differences detected between antibiotic administration and IAP in the current observation was comparable with this. IAH and ACS are serious threats in ill patients. Nurses in the ICU train to diagnose the IAH and ACS and do the right interventions.

Nursing training has to focus on evidence-based training strategies. Nurses should run standard care dealing with patients at risk of IAH and ACS [17]. Hence, prompt screening is essential to find patients who may show IAH and ACS [18]. IAH links to elevated SOFA scores, high APACHE II, high APACHE III, a further need for mechanical ventilation and insufficient PaO2: FiO2 ratios at admission time. Longer durations of the need for mechanical ventilation and lengths of stay at ICUs in such patients reaffirm the pathophysiological damages of raised ICP [19].

Also, our result was matching the reports that shock, SIRS, APACHE II, central venous oxygen saturation and GCS associated with IAP. The mechanical ventilation is independent predisposing to develop IAH with applying PEEP [20]. Based on the observation in the current study, we think IAP measure in ICU patients may help to define a proper prognosis and complete the intervention. We think further research needed to find new methods for direct investigation of IAH in the patients in ICU.

In conclusion, the pathological features of IAH and ACS are appropriate markers to report systemic disorders and mortality in the ICU. Screening the IAH to monitor the signs of ACS is an economical and valuable way can discover complications in the ICU and may adjust treatment outcomes and reduce hospitality overheads.

Acknowledgement

We thank all the hospital cooperators for helping during this study.

References

1. Hunt L, Frost SA, Hillman K, Newton PJ, Davidson PM. Management of intra-abdominal hypertension and abdominal compartment syndrome: a review. J Trauma Manag Outcomes. 2014; 8(1):2. https://doi.org/10.1186/1752-2897-8-2 PMid:24499574 PMCid:PMC3925290

2. Harhangi BS, Kompanje EJ, Leebeek FW et al. Coagulation disorders after traumatic brain injury. Acta Neurochir. 2008; 150(2):165-75. https://doi.org/10.1007/s00701-007-1475-8 PMid:18166989

3. Krinsley JS. Association between hyperglycemia and increased hospital mortality in a heterogeneous population of critically ill patients. Mayo Clin Proc. 2003; 78(12):1471-1478. https://doi.org/10.4065/78.12.1471 PMid:14661676

4. Rossaint R, Bouillon B, Cerny V, Coats TJ, Duranteau J, Fernández-Mondéjar E, etal. Management of bleeding following major trauma: an updated European guideline. Crit Care. 2010; 14(2):R52. https://doi.org/10.1186/cc8943 PMid:20370902 PMCid:PMC2887168

5. Gavrilovska-Brzanov A, Nikolova Z, Jankulovski N, Sosolceva M, Taleska G, Mojsova-Mijovska M, et al. Evaluation of the effects of elevated intra-abdominal pressure on the respiratory mechanics in mechanically ventilated patients. Maced J Med Sci. 2013; 6(3):261-5.

6. Herlitz J, Thuresson M, Svensson L, Lindqvist J, Lindahl B, Zedigh C, etal. Factors of importance for patients' decision time in acute coronary syndrome. Int J Cardiol. 2010; 141(3):236-42. https://doi.org/10.1016/j.ijcard.2008.11.176 PMid:19136167

7. Nilsson G, Mooe T, Söderström L, Samuelsson E. Pre-hospital delay in patients with first time myocardial infarction: an observational study in a northern Swedish population. BMC Cardiovasc Disord. 2016; 16(1):93. https://doi.org/10.1186/s12872-016-0271-x PMid:27176816 PMCid:PMC4866271

8. Ladwig KH, Meisinger C, Hymer H, Wolf K, Heier M, von Scheidt W, etal. Sex and age specific time patterns and long term time trends of pre-hospital delay of patients presenting with acute ST-segment elevation myocardial infarction. Int J Cardiol. 2011; 152(3):350-5. https://doi.org/10.1016/j.ijcard.2010.08.003 PMid:20813416

9. Braha B, Mahmutaj D, Maxhuni M, Neziri B, Krasniqi S. Correlation of procalcitonin and C-reactive protein with intra-abdominal hypertension in intra-abdominal infections: their predictive role in the progress of the disease. Open Access Maced J Med Sci. 2018; 6(3):479. https://doi.org/10.3889/oamjms.2018.112 PMid:29610604 PMCid:PMC5874369

10. Shafiepour M, Kiani A, Taghavi K, Seifi S, Rezaie MS, Hashemian SM, etal. A Rare Report of Lung Metastasis of the Common Non-Melanotic Skin Cancer. Tanaffos. 2018; 17(1):62. PMid:30116282 PMCid:PMC6087531

11. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. Crit Care Med. 1985; 13(10):818-29. https://doi.org/10.1097/00003246-198510000-00009 PMid:3928249

12. Morejón CD, Barbeito TO. Effect of mechanical ventilation on intra-abdominal pressure in critically ill patients without other risk factors for abdominal hypertension: an observational multicenter epidemiological study. Ann Intensive Care. 2012; 2(1):S22. https://doi.org/10.1186/2110-5820-2-S1-S22 PMid:23281625 PMCid:PMC3527157

13. Van Hee R. Historical highlights in concept and treatment of abdominal compartment syndrome. Acta Clin Belg. 2007; 62(sup1):9-15.

14. Katsios C, Ye C, Hoad N, Piraino T, Soth M, Cook D. Intra-abdominal hypertension in the critically ill: Interrater reliability of bladder pressure measurement. J Crit Care. 2013; 28(5):886-e1. https://doi.org/10.1016/j.jcrc.2013.04.003 PMid:23726386

15. Mahmutaj D, Krasniqi S, Braha B, Limani D, Neziri B. The Predictive Role of Procalcitonin On the Treatment of Intra-Abdominal Infections. Open Access Maced J Med Sci. 2017; 5(7):909. https://doi.org/10.3889/oamjms.2017.194 PMid:29362617 PMCid:PMC5771293

16. Mentula P, Leppäniemi A. Prophylactic open abdomen in patients with postoperative intra-abdominal hypertension. Crit Care. 2010; 14(1):111. https://doi.org/10.1186/cc8207 PMid:20156323 PMCid:PMC2875490

17. Halstrom S, Price P, Thomson R. Environmental mycobacteria as a cause of human infection. Int J Mycobacteriol. 2015; 4(2):81-91. https://doi.org/10.1016/j.ijmyco.2015.03.002 PMid:26972876

18. Strang SG, Van Imhoff DL, Van Lieshout EM, D'Amours SK, Van Waes OJ. Identifying patients at risk for high-grade intra-abdominal hypertension following trauma laparotomy. Injury. 2015; 46(5):843-8. https://doi.org/10.1016/j.injury.2014.12.020 PMid:25805553

19. Abedini A, Kiani A, Taghavi K, Khalili A, Fard AJ, Fadaizadeh L, etal. High-Frequency Jet Ventilation in Nonintubated Patients. Turk Thorac J. 2018; 19(3):127. https://doi.org/10.5152/TurkThoracJ.2018.17025

20. Jacobs RE, Gu P, Chachoua A. Reactivation of pulmonary tuberculosis during cancer treatment. Int J Mycobacteriol. 2015; 4(4):337-40. https://doi.org/10.1016/j.ijmyco.2015.05.015 PMid:26964818

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Apr 15; 7(7):1103-1109.
https://doi.org/10.3889/oamjms.2019.241
eISSN: 1857-9655
Clinical Science

Real World Experience of a Biodegradable Polymer Sirolimus-Eluting Stent (Yukon Choice PC Elite) in Patients with Acute ST-Segment Elevation Myocardial Infarction Undergoing Primary Angioplasty: A Multicentric Observational Study (The Elite India Study)

Bhupendra Verma[1*], Akhilesh Patel[2], Deepak Katyal[3], Vivek Raj Singh[4], Avinash Kumar Singh[5], Amrita Singh[1], Manu Kumar[1], Pratap Nagarkoti[1]

[1]Department of Cardiology, Ujala Superspeciality Hospital, Kashipur, Uttarakhand, India; [2]Abhigya Heart Care Centre, Gorakhpur, Uttar Pradesh, India; [3]Department of Cardiology, Columbia Asia Hospital, Patiala, Punjab, India; [4]Vardhan Heart Clinic, Varanasi, Uttar Pradesh, India; [5]Department of Cardiology, Khushlok Hospital, Bareilly, Uttar Pradesh, India

Citation: Verma B, Patel A, Katyal D, Singh VR, Singh AK, Singh A, Kumar M, Nagarkoti P. Real World Experience of a Biodegradable Polymer Sirolimus-Eluting Stent (Yukon Choice PC Elite) in Patients with Acute ST-Segment Elevation Myocardial Infarction Undergoing Primary Angioplasty: A Multicentric Observational Study (The Elite India Study). Open Access Maced J Med Sci. 2019 Apr 15; 7(7):1103-1109. https://doi.org/10.3889/oamjms.2019.241

Keywords: BPDES; STEMI; Percutaneous coronary intervention; Third generation stents; Drug-eluting stents; Deliverability; Stent thrombosis

***Correspondence:** Bhupendra Verma. Department of Cardiology, Ujala Superspeciality Hospital, Kashipur, Uttarakhand, India. E-mail: bhupendra.269@gmail.com

Received: 30-Jan-2019; **Revised:** 29-Mar-2019; **Accepted:** 30-Mar-2019; **Online first:** 14-Apr-2019

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: The durable polymer drug-eluting stents (DPDES) reduce the risk of repeated target vessel revascularisation (TLR) compared with BMS, but are associated with increased risk of late adverse events. In broadly inclusive populations, the biodegradable-polymer drug-eluting stents (BPDES) have favourable results compared with DPDES in the long term. However, its use in primary angioplasty has not been adequately studied, and data of real-world clinical experience is lacking.

AIM: Aim of this study was to assess the safety and efficacy of Yukon Choice PC Elite sirolimus-eluting stent (a novel BPDES) in STEMI patients undergoing primary angioplasty.

METHODS: We have presented here one-year clinical follow-up data of the Yukon Choice PC Elite sirolimus-eluting stent in patients undergoing primary angioplasty. A total of 636 patients were enrolled in this single arm, prospective observational study from five centres.

RESULTS: This multicentric observational study showed excellent safety and efficacy profile of the novel device at one year follow up. The device-oriented composite endpoint (DOCE) of cardiac death, target-vessel reinfarction, and target-lesion revascularisation (TLR) was 2.7%, and the patient-oriented composite endpoint (POCE) of all-cause death, any myocardial infarction, and any revascularisation was 4.2% at one year. Definite or probable stent thrombosis rate was 0.6%, and no events were recorded beyond 6 months of follow up.

CONCLUSIONS: In patients with STEMI undergoing primary angioplasty, the use of Yukon Choice PC Elite (biodegradable polymer sirolimus-eluting stent) has excellent results at one year. It, therefore, represents an attractive alternative to second generation DES in this high-risk population.

Introduction

Primary percutaneous coronary intervention (PCI) is considered the gold standard of management in ST-segment elevation myocardial infarction (STEMI) [1], [2]. Coronary stenting with a bare-metal stent (BMS) is associated with a lower risk of reinfarction and target vessel revascularisation compared with balloon angioplasty alone [3]. The advent of drug-eluting stents (DES) has further reduced the in-stent restenosis and the risk of repeated target vessel revascularisation compared with BMS [4]. However, the first-generation DES, including the sirolimus and paclitaxel-eluting stents, have increased the risk stent thrombosis over the long term [5], [6]. These late adverse events are related to hypersensitivity reactions to the durable polymer which produces chronic inflammation and thereby delays endothelial healing and favour stent thrombosis [7], [8], [9]. This is especially important in

patients with STEMI because of plaque rupture, high thrombus burden and increased platelet activation [8], [10]. Moreover, there are chronic processes leading to delayed endothelial healing, vessel remodelling and stent malapposition [8], [11].

Second-generation DES were subsequently developed using thinner stent struts, more biocompatible polymers, and a lower dose of newer anti-proliferative agents (Everolimus and Zotarolimus). The most recent addition to the stent technology is the development of biodegradable polymer drug-eluting stents (BPDES), the third generation DES. These newer generation DES with more biocompatible or biodegradable polymer have been shown to have lower thrombogenicity compared to BMS in experimental studies [12]. However, the ideal DES for use in STEMI is still not clear as there is no conclusive evidence of superiority.

BP-DES degrades after completion of drug release and transforms functionally into BMS. This may improve arterial healing by removing the chronic source of inflammation, the durable polymer, present in all current second-generation DES. Consequently, BP-DES appears to be a novel solution for STEMI, by possibly reducing late ischemic events. We report here the one-year outcomes associated with the use of a novel biodegradable polymer DES, the Yukon Choice PC Elite sirolimus-eluting stent (Translumina Therapeutics, Germany), in STEMI population undergoing primary angioplasty. Recent studies with their use in all-comer populations have been encouraging [13], [14], [15]. However, there is a paucity of data regarding real world experience with this device in STEMI patients.

Methods

Patients

This was a prospective observational single arm study from five centres across India. Patients presenting with STEMI were screened for inclusion in the study. Patients were eligible for inclusion if: (1) they presented within 12 hours of symptom onset, or between 12 hours and 24 hours if they had persistent symptoms with evidence of ongoing ischemia; (2) procedures were completed with only Yukon Choice PC Elite DES. Patients were excluded if they had a contraindication to antiplatelet or anticoagulant therapy, undergone CABG, pregnant women and women of childbearing potential and known hypersensitivity to sirolimus, shellac or stainless steel. The study was conducted according to the Helsinki Declaration and the Good Clinical Practice Guidelines. Written informed consent was obtained from each patient before performing the PCI procedure.

Study Device

The Yukon Choice PC Elite stent is a third generation sirolimus-eluting stent (SES) from Translumina Therapeutics (Germany), now being manufactured in India. This is made up of surgical grade stainless steel (87 µm strut thickness) coated with a biodegradable polymer (polylactic acid, PLA) and sirolimus, which releases from the matrix of Resomer 202S and Shellac resin. It has a unique micro-porous stent surface, PEARL surface, which favours better endothelialisation. The drug is coated only abluminally with no drug or polymer on the luminal side of the stent. Sirolimus eluted from the abluminal side controls smooth muscle cell proliferation, while the luminal side promotes endothelialisation as there is no polymer or drug (acts like BMS). It has the least polymeric load of a biodegradable polymer, 1/4th of polymer present in conventional DES. The drug is released in 4-6 weeks, and polymer gets completely degraded in 60-90 days, and it essentially becomes a bare metal stent. Sirolimus has a broad therapeutic profile and flat dose-response curve leading to consistent and homogenous anti-proliferative and immunosuppressant properties. Sirolimus-eluting stents have consistently shown superior outcomes compared to the paclitaxel-eluting stent (PES) [16], [17] and zotarolimus-eluting stent (ZES) [18], whereas comparable safety and efficacy profile to the everolimus-eluting stent (EES) [19], [20]. However, sirolimus is temperature sensitive and get degraded at a temperature of > 30°C into an open-chain isomer (34-hydroxy sirolimus) retaining less than 10% of the immunosuppressive activity [21]. The Yukon Choice PC Elite is a cosmetically improved version of Yukon Choice PC stent, which is specially adapted for conditions of extreme temperatures particularly seen in the tropical regions. It comes with dual packing of the outer polystyrene box and inner aluminium pack. Additionally, it has a highly sensitive temperature monitoring device, the Tag Alert, which uses Sensitech Technology.

Study procedure

All the patients included in the study underwent loading with ticagrelor 180 mg, aspirin 300 mg and atorvastatin 80 mg in the emergency department and shifted to the catheterisation laboratory. The radial approach was the default access site in all patients undergoing primary angioplasty. During the angioplasty procedure, intravenous unfractionated heparin was used for anticoagulation, and glycoprotein IIb/IIIa inhibitors were used as a bail-out. Thrombosuction was used only in cases with large residual thrombus burden after opening the culprit artery with guidewire or balloon. After the intervention, all patients received aspirin indefinitely and clopidogrel, prasugrel or ticagrelor for at least 12 months. Patients remained in

the hospital for at least 48 h. Blood samples were drawn every 24 h for the determination of cardiac markers, blood cell counts and renal function test. Daily recording of ECG was also performed until discharge.

Follow up and study endpoints

Clinical follow-up visits were scheduled at day 14 and monthly after that up to one year. Frequent follow-up visits, telephonic conversation, reminder messages (SMS) and frequent group counselling sessions were organised to ensure better drug compliance, drug/dose adjustment and collecting data regarding adverse events. The primary endpoint of the study was device-oriented composite endpoint (DOCE) of cardiac death, target-vessel reinfarction, and target-lesion revascularisation (TLR) and the co-primary endpoint was patient-oriented composite endpoint (POCE) of all-cause death, any myocardial infarction, and any revascularisation at the 30-day, 6 months and 1-year of follow-up. The secondary endpoint of this study was the incidence of definite or probable stent thrombosis at the 30-day, 6 months and 1-year of follow-up. Additional secondary endpoints were major/minor bleeding and TLR rates.

Definitions

Clinical device success was defined as successful delivery and deployment of the first inserted stent and final diameter stenosis after stenting ≤ 50% by quantitative coronary angiography or visual assessment. Clinical procedure success was defined as clinical device success without the occurrence of serious cardiac events important for ischemia during hospitalisation. Any death was defined as cardiac unless an unequivocal noncardiac cause could be established. Stent thrombosis was defined as acute (< 24 h), subacute (24 h to 30 days), late (> 30 days to 1 year), and very late (> 1 year). It was further defined as per the ARC definition as definite, possible or probable stent thrombosis [22]. Target lesion revascularisation was defined as any clinically indicated repeat percutaneous intervention of the target lesion or bypass surgery of the target vessel performed for restenosis or other complication of the target lesion. Major and minor bleeding was defined using the Thrombolysis in Myocardial Infarction (TIMI) bleeding criteria [22]. Obesity was defined as body mass index > 25 kg/m^2 [23].

Statistical analysis

Descriptive analyses were performed. Categorical variables are expressed as number and percentage of patients. Continuous data are reported as mean ± SD.

Results

Baseline patient characteristics

A total of 636 patients were enrolled in the study. Clinical follow-up was completed in 634 (99.7%) patients at 30 days, 625 (98.2%) at 6 months, and 618 (97.1%) at 1 year, respectively. By the end of one year follow up 14 patients (2.2%) have died, and 4 patients (0.6%) were lost to follow up. The patient follows up is presented in Figure 1.

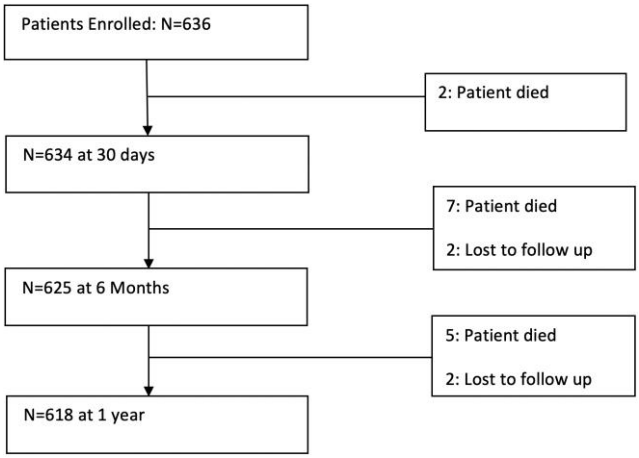


Figure 1: Patient flow and follow up through one year

The mean age was 53.9 years, and 79% of the patients were male. There was a high prevalence of coronary risk factors including hypertension (57.6%), smoking (43.8%), dyslipidaemia (40.8%), obesity (36.9%) and diabetes (20.9%). 3.3% had suffered myocardial infarction before the index event, and 2.8% have earlier undergone PCI. Mean LVEF (left ventricular ejection fraction) was ~47%. 4.8% of patients presented in cardiogenic shock and 16.9% were in Killip class II or more. Baseline characteristics are shown in Table 1.

Table 1: Baseline patient characteristics (N = 636)

Age (years), mean ± SD	53.9 ± 11
Male sex	504 (79.2)
BMI, mean ± SD	26.5 ± 6.2
Cardiovascular risk factors	
Obesity	235 (36.9)
Diabetes	133 (20.9)
Hypertension	368 (57.6)
Dyslipidemia	260 (40.8)
Current smoker	279 (43.8)
Family history of CAD	82 (12.8)
Prior MI	21 (3.3)
Prior PCI	18 (2.8)
LVEF, mean ±SD	46.9 ± 6.2
Cardiogenic shock	31 (4.8)
Killip class >I	108 (16.9)

Lesion and procedural characteristics

The mean door to balloon time was 56 minutes. The radial approach was the primary route of angioplasty (96%). LAD/diagonal was the infarct-related artery in half of the patients followed by RCA

(33%), and LMCA constituted 0.9% of the cases. The majority had single vessel disease (69%), and 6.6% had triple vessel disease. The mean diameter and mean length of the stent were 3.14 mm and 26.15 mm, respectively. Multiple stents were used overall in 16% of patients before hospital discharge and in 4% of patients during the index procedure. Direct stenting was done in 67% of the patients. Thrombosuction was used in 3.3% patients, and bail-out Gp IIb/IIIa inhibitor use was 10.5%. Clopidogrel (79%) was the most commonly prescribed DAPT (dual antiplatelet therapy) along with aspirin followed by ticagrelor (14%) and prasugrel (7%). Compliance to DAPT at one year was 94%. The device success rate was 99.3%, and the procedure success rate was 98.1%. Lesion and procedural characteristics are summarized in Table 2.

Table 2: Lesion and procedural characteristics (N = 636)

Door to balloon (minutes), mean ± SD	56 ± 11
Radial access	612 (96.2)
Infarct related artery	
Left main	6 (0.94)
LAD/Diagonal	309 (48.5)
LCX/Marginal	108 (16.9)
RCA	213 (33.4)
Multivessel disease	193 (30.3)
Reference vessel diameter (mm), mean ±SD	2.91 ± 0.25
Stented length (mm), mean ±SD	26.15 ± 6.35
Stent diameter (mm), mean ±SD	3.14 ± 0.28
Stent implanted per patient	1.18 ± 0.40
Multiple stents used	102 (16)
Thrombosuction	21 (3.3)
Direct stenting	426 (66.9)
Gp IIb/IIIa inhibitor use*	67 (10.5)
DAPT usage	
Clopidogrel	79%
Prasugrel	7%
Ticagrelor	14%
DAPT compliance at 1-year	94%
Device success	99.3%
Procedure success	98.1%

Clinical outcomes

At one-year clinical follow-up, 14 patients (2.2%) had died, including 9 patients (1.4%) who died of cardiac reasons. The device oriented primary endpoint (DOCE) was seen in 1.1% at 30 days, 2% at 6 months and 2.7% at the end of one year.

Table 3: Clinical follow up

Outcomes	30 days	6 months	1 year
All death	3 (0.5)	9 (1.4)	14 (2.2)
Cardiac de	3 (0.5)	7 (1.1)	9 (1.4)
MI (non-fatal)	2 (0.3)	4 (0.6)	5 (0.8)
Target vessel reinfarction	2 (0.3)	3 (0.5)	3 (0.5)
Any revascularization	5 (0.8)	9 (1.4)	11 (1.7)
TLR (clinical driven)	3 (0.5)	5 (0.8)	8 (1.2)
Device oriented endpoint β	7 (1.1)	13 (2)	18 (2.7)
Patient oriented endpoint §	9 (1.4)	19 (3)	26 (4.2)
Stent thrombosis *	3 (0.5)	4 (0.6)	4 (0.6)
Definite	2 (0.3%)	2 (0.3%)	2 (0.3%)
Probable	1 (0.15%)	2 (0.3%)	2 (0.3%)
Major and minor bleeding	3 (0.5)	7 (1.1)	9 (1.5)

The patient-oriented co-primary endpoint (POCE) was 1.4%, 3% and 4.2% at 30 days, 6 months and 1 year respectively. The target lesion re-infarction at one year was 0.5%, and the clinical driven TLR rate was 1.2%. Definite and probable stent thrombosis occurred in 2 patients each till 6 months follow up (total 4 patients, 0.6%). Both of the definite stent thromboses were sub-acute (early stent thrombosis). Beyond 6 months no additional case of stent thrombosis was seen. The major and minor bleeding rates at one year were 1.5%. The clinical outcomes are detailed in Table 3.

Discussion

To our knowledge, this is the largest study reporting the real-world experience of biodegradable polymer sirolimus-eluting stent in the STEMI population undergoing primary angioplasty. This prospective, observational multicentric study showed excellent safety and efficacy profile of Yukon Choice PC Elite with low rates of POCE, DOCE, TLR and overall stent thrombosis rates at one year follow up. The study cohort had a high prevalence of cardiovascular risk factors, cardiogenic shock and poor Killip class, thereby reflecting real clinical practice and testing the stent in a real-world setting. This novel device with enhanced biocompatibility, therefore, appears to be an attractive option in the STEMI population.

The Yukon Choice PC stent (Translumina therapeutics, Hechingen, Germany) consists of a unique microporous stainless-steel stent surface coated abluminally with sirolimus and a PLA biodegradable polymer. This sirolimus-eluting BP-DES has been found non-inferior to permanent polymer SES (Cypher) in the ISAR-TEST-3 study and permanent polymer EES (XIENCE V) or SES (Cypher) in ISAR-TEST-4 [13], [14]. Outcomes were non-inferior even at 3 years and 5 years follow up [24], [25]. The Yukon stent has also been shown to be non-inferior to the PES in diabetic patients (LIPSIA-Yukon trial) [26], [27]. Furthermore, the ISAR-TEST 5 trial showed that this strut had non-inferior results at one year and five years compared to a zotarolimus-eluting stent [28], [29]. The Yukon Choice PC Elite uses the same stent platform and design as Yukon Choice PC used in the ISAR TEST 3 and 4 trials. The major difference is a temperature control system present with the current device, as sirolimus is temperature sensitive.

We have experienced high device and procedural success rate using the Yukon Elite stent (99.3% and 98.1% respectively). This is comparable to a procedural success rate of 97.5% using EES in EXAMINATION Trial [30]. All the operators in this study report to have experienced exceptional manoeuvrability, deliverability and flexibility during stent deployment. Direct stenting was done in 67%, and it failed in 5.6% patients, which is considerably lower than previously reported with 2nd generation

DES (17-18%) [31], [32]. No case of failure of stenting, a mechanical complication related to stent or change over to different DES was seen. Given comparable efficacy of the contemporary DES, deliverability is certainly an important feature in relative superiority of stents. Unfortunately, deliverability data from randomised clinical trials are almost negligible, and therefore real-world experience is critical for determining relative deliverability. The excellent deliverability with Yukon Choice PC stent could be explained by widely spaced, thinner struts (87 μm) and newer improved stent delivery system.

There are limited studies comparing new-generation DES (n-DES) and BMS in the STEMI population. In the recent two trials-COMFORTABLE AMI trial (biolimus-eluting stent with biodegradable polymer versus BMS) and EXAMINATION trial (EES versus BMS) in STEMI-new-generation DES were convincingly superior to BMS [30], [33]. Meta-analysis of these trials showed a significant reduction in rates of target vessel re-infarction, TLR and stent thrombosis in the DES arm [34]. Moreover, this benefit was extended up to 2-year follow-up in both trials [35], [36]. More recently, the 5-year follow-up of EXAMINATION trial demonstrated a reduction in the patient-oriented endpoints, device-oriented endpoints, stent thrombosis and all-cause mortality with DES [37]. Stenting with new-generation DES is thus recommended over BMS for primary PCI [2]. The results of the present study confirm these findings with a favourable 1-year safety and efficacy profile. The overall mortality at one year (2.2%) was lower as compared to COMFORTABLE AMI (3.2%) and EXAMINATION trial (3.5%), and the cardiac death too was much lower (1.4% vs 2.9% and 3.2% respectively). The DOCE in our study was 2.7% at one year as compared to 4.3% in COMFORTABLE AMI, and 5.5% EXAMINATION trial and the POCE was 4.2% vs 8.4% and 11.9% respectively.

The BP-DES were designed to confer benefit particularly in terms of late adverse events compared to durable polymer DES. This is especially applicable in STEMI, where the risk of stent thrombosis is increased due to high thrombus burden, increased platelet activation and inflammation leading to delayed endothelial healing. Indeed, in a pooled analysis of three trials in STEMI, BP-DES led to significant reduction in TLR rates and trend towards reduction of definite or probable stent thrombosis [38]. In our study, the overall stent thrombosis rate was 0.6% (definite 0.3% and or probably 0.3%), with no event seen beyond 6 months. This was lower compared to 2.5% in COMFORTABLE AMI trial and 0.9% in EXAMINATION trial [30], [33]. In ISAR-5 trial, the incidence of definite/probable stent thrombosis was 1.1% in BPDES group and 1.2% in Zotarolimus arm at one year, and there was no stent thrombosis seen beyond one year [29]. Similarly, in COMFORTABLE AMI trial, there was no case of late definite stent thrombosis [35].

Study limitations

The strength of this study was a prospective design with a large number of STEMI patients from multiple centres. However, there were several important limitations too. First, it was an observational study, and a head-to-head comparison is required to confirm the safety and efficacy relative to other newer generation DES. Second, the limited follow up of one year does not allow capturing of very late stent thrombosis. However, recent studies with longer follow up in the STEMI population didn't show any stent thrombosis beyond one year [29], [35]. Third, there may have been underreporting of MI and TLR as follow-up angiography, and routine cardiac bio-marker estimation was not mandatory. However, asymptomatic MI and restenosis are usually associated with less clinical significance, and it is unlikely that they would have affected the final results of this study.

Furthermore, the excellent follow-up results represent indirect evidence of favourable effects of the stent used in the study. Fourth, the favourable results in our study may have been influenced by a greater proportion of direct stenting, shorter door-balloon time, experienced operators, predominantly radial approach and selective use of thrombosuction. Finally, there are reports to suggest that the incidence of stent thrombosis is relatively low in Asian populations [39] and thereby the results may not apply to other populations.

In conclusion, in patients with STEMI undergoing primary angioplasty, the use of Yukon Choice PC Elite (biodegradable polymer sirolimus-eluting stent) has excellent safety and efficacy profile at one year. It may, therefore, represent an attractive solution in this high-risk population. However, long-term follow is needed to determine safety in terms of very late adverse events.

References

1. Keeley EC, Boura JA, Grines CL. Primary angioplasty versus intravenous thrombolytic therapy for acute myocardial infarction: a quantitative review of 23 randomised trials. Lancet. 2003; 361:13-20. https://doi.org/10.1016/S0140-6736(03)12113-7

2. Verma B, Singh A, Saxena AK, Kumar M. Deflated Balloon-Facilitated Direct Stenting in Primary Angioplasty (The DBDS Technique): A Pilot Study. Cardiol Res. 2018; 9(5):284-292. https://doi.org/10.14740/cr770w PMid:30344826 PMCid:PMC6188044

3. Nordmann AJ, Hengstler P, Harr T, Young J, Bucher HC. Clinical outcomes of primary stenting versus balloon angioplasty in patients with myocardial infarction: a meta-analysis of randomized controlled trials. Am J Med 2004; 116(4):253-262. https://doi.org/10.1016/j.amjmed.2003.08.035 PMid:14969654

4. Kastrati A, Dibra A, Spaulding C, Laarman GJ, Menichelli M, Valgimigli M, et al. Meta-analysis of randomized trials on drug-eluting stents vs. bare-metal stents in patients with acute

myocardial infarction. Eur Heart J. 2007; 28(22):2706-2713. https://doi.org/10.1093/eurheartj/ehm402 PMid:17901079

5. De Luca G, Dirksen MT, Spaulding C, Kelbaek H, Schalij M, Thuesen L, et al. Drug-eluting vs bare-metal stents in primary angioplasty: a pooled patient-level meta-analysis of randomized trials. Arch Intern Med. 2012; 172(8):611-621. https://doi.org/10.1001/archinternmed.2012.758 PMid:22529227

6. Kalesan B, Pilgrim T, Heinimann K, Raber L, Stefanini GG, Valgimigli M, et al. Comparison of drug-eluting stents with bare metal stents in patients with ST-segment elevation myocardial infarction. Eur Heart J. 2012; 33:977-87. https://doi.org/10.1093/eurheartj/ehs036 PMid:22362513

7. Byrne RA, Joner M, Kastrati A. Polymer coatings and delayed arterial healing following drug- eluting stent implantation. Minerva Cardioangiol. 2009; 57(5):567-84. PMid:19838148

8. Nakazawa G, Finn AV, Joner M, Ladich E, Kutys R, Mont EK, et al. Delayed arterial healing and increased late stent thrombosis at culprit sites after drug-eluting stent placement for acute myocardial infarction patients: an autopsy study. Circulation. 2008; 118(11):1138-45. https://doi.org/10.1161/CIRCULATIONAHA.107.762047 PMid:18725485

9. Joner M, Finn AV, Farb A, Mont EK, Kolodgie FD, Ladich E, et al. Pathology of drug-eluting stents in humans: delayed healing and late thrombotic risk. J Am Coll Cardiol. 2006; 48:193e202.

10. Gonzalo N, Barlis P, Serruys PW, Garcia-Garcia HM, Onuma Y, Ligthart J, et al. Incomplete stent apposition and delayed tissue coverage are more frequent in drug-eluting stents implanted during primary percutaneous coronary intervention for ST-segment elevation myocardial infarction than in drug-eluting stents implanted for stable/unstable angina: insights from optical coherence tomography. JACC Cardiovasc Interv. 2009; 2(5):445-52. https://doi.org/10.1016/j.jcin.2009.01.012 PMid:19463469

11. Breet NJ, van Werkum JW, Bouman HJ, Kelder JC, Hackeng CM, ten Berg JM. The relationship between platelet reactivity and infarct-related artery patency in patients presenting with a ST-elevation myocardial infarction. Thromb Haemost. 2011; 106(2):331-6. https://doi.org/10.1160/TH10-08-0528

12. Kolandaivelu K, Swaminathan R, Gibson WJ, Kolachalama VB, Nguyen-Ehrenreich KL, Giddings VL, et al. Stent thrombogenicity early in high-risk interventional settings is driven by stent design and deployment and protected by polymer-drug coatings. Circulation. 2011; 123(13):1400-9. https://doi.org/10.1161/CIRCULATIONAHA.110.003210 PMid:21422389 PMCid:PMC3131199

13. Mehilli J, Byrne RA, Wieczorek A, Iijima R, Schulz S, Bruskina O, et al. Randomized trial of three rapamycin-eluting stents with different coating strategies for the reduction of coronary restenosis. Eur Heart J. 2008; 29(16):1975-82. https://doi.org/10.1093/eurheartj/ehn253 PMid:18550554

14. Byrne RA, Kastrati A, Kufner S, Massberg S, Birkmeier KA, Laugwitz KL, et al. Angiographic Results: test efficacy of 3 limus-eluting stents I. Randomized, noninferiority trial of three limus agent-eluting stents with different polymer coatings: the Intracoronary Stenting and Angiographic Results: Test Efficacy of 3 Limus-Eluting Stents (ISAR-TEST-4). Eur Heart J. 2009; 30(20):2441-9. https://doi.org/10.1093/eurheartj/ehp352 PMid:19720642

15. Stefanini GG, Byrne RA, Serruys PW, de Waha A, Meier B, Massberg S, et al. Eur Heart J. 2012; 33(10):1214-22. https://doi.org/10.1093/eurheartj/ehs086 PMid:22447805

16. Schömig A, Dibra A, Windecker S, et al. A meta-analysis of 16 randomized trials of sirolimus-eluting stents versus paclitaxel-eluting stents in patients with coronary artery disease. J Am Coll Cardiol. 2007; 50:1373. https://doi.org/10.1016/j.jacc.2007.06.047 PMid:17903638

17. Stettler C, Wandel S, Allemann S, et al. Outcomes associated with drug-eluting and bare-metal stents: a collaborative network meta-analysis. Lancet. 2007; 370:937. https://doi.org/10.1016/S0140-6736(07)61444-5

18. Rasmussen K, Maeng M, Kaltoft A, et al. Efficacy and safety of zotarolimus-eluting and sirolimus-eluting coronary stents in routine clinical care (SORT OUT III): a randomised controlled superiority trial. Lancet. 2010; 375:1090. https://doi.org/10.1016/S0140-6736(10)60208-5

19. de Waha A, Dibra A, Byrne RA, et al. Everolimus-eluting versus sirolimus-eluting stents: a meta-analysis of randomized trials. Circ Cardiovasc Interv. 2011; 4:371. https://doi.org/10.1161/CIRCINTERVENTIONS.111.963256 PMid:21791671

20. Bangalore S, Kumar S, Fusaro M, et al. Short- and long-term outcomes with drug-eluting and bare-metal coronary stents: a mixed-treatment comparison analysis of 117 762 patient-years of follow-up from randomized trials. Circulation. 2012; 125:2873. https://doi.org/10.1161/CIRCULATIONAHA.112.097014 PMid:22586281

21. Yatscoff RW, Wang P, Chan K, Hicks D, Zimmerman J. Rapamycin: Distribution, pharmacokinetics, and therapeutic range investigations. Ther Drug Monit. 1995; 17:666-671. https://doi.org/10.1097/00007691-199512000-00020 PMid:8588238

22. Cutlip DE, Windecker S, Mehran R, Boam A, Cohen DJ, van Es GA, et al. Clinical end points in coronary stent trials: a case for standardized definitions. Circulation. 2007; 115(17):2344-51. https://doi.org/10.1161/CIRCULATIONAHA.106.685313 PMid:17470709

23. Misra A, Chowbey P, Makkar BM, Vikram NK, Wasir JS, Chadha D, et al. Consensus statement for diagnosis of obesity, abdominal obesity and the metabolic syndrome for Asian Indians and recommendations for physical activity, medical and surgical management. J Assoc Physicians India. 2009; 57:163-70. PMid:19582986

24. Byrne RA, Kastrati A, Massberg S, Wieczorek A, Laugwitz KL, Hadamitzky M, et al. Biodegradable polymer versus permanent polymer drug-eluting stents and everolimus- versus sirolimus-eluting stents in patients with coronary artery disease: 3-year outcomes from a randomized clinical trial.J Am Coll Cardiol. 2011; 58(13):1325-31. https://doi.org/10.1016/j.jacc.2011.06.027 PMid:21920260

25. Kufner S, Byrne RA, Valeskini M, Schulz S, Ibrahim T, Hoppmann P, et al. Five-year outcomes from a trial of three limus-eluting stents with different polymer coatings in patients with coronary artery disease: final results from the ISAR-TEST 4 randomised trial. EuroIntervention. 2016; 11(12):1372-1373. https://doi.org/10.4244/EIJY14M11_02 PMid:25405657

26. Desch S, Schloma D, Möbius-Winkler S, Erbs S, Gielen S, Linke A, et al. Randomized comparison of a polymer-free sirolimus-eluting stent versus a polymer-based paclitaxel-eluting stent in patients with diabetes mellitus: the LIPSIA Yukon trial. JACC Cardiovasc Interv. 2011; 4(4):452-459. https://doi.org/10.1016/j.jcin.2010.11.016 PMid:21511226

27. Stiermaier T, Heinz A, Schloma D, Kleinertz K, Dänschel W, Erbs S, et al. Five-year clinical follow-up of a randomized comparison of a polymer-free sirolimus-eluting stent versus a polymer-based paclitaxel-eluting stent in patients with diabetes mellitus (LIPSIA Yukon trial). Catheter Cardiovasc Interv. 2014; 83(3):418-424. https://doi.org/10.1002/ccd.25131 PMid:23873579

28. Massberg S, Byrne RA, Kastrati A, Schulz S, Pache J, Hausleiter J, et al. Polymer-free sirolimus- and probucol-eluting versus new generation zotarolimus-eluting stents in coronary artery disease: The intracoronary stenting and angiographic results: Test efficacy of sirolimus- and probucol-eluting versus zotarolimus-eluting stents (ISAR-TEST 5) trial. Circulation. 2011; 124:624-632. https://doi.org/10.1161/CIRCULATIONAHA.111.026732 PMid:21768546

29. Colleran R, Kufner S, Harada Y, Giacoppo D, Cassese S, Repp J, et al. Five-year follow-up of polymer-free sirolimus- and probucol-eluting stents versus new generation zotarolimus-eluting stents in patients presenting with st-elevation myocardial infarction. Catheter Cardiovasc Interv. 2017; 89(3):367-374.

https://doi.org/10.1002/ccd.26597 PMid:27377301

30. Sabate M, Cequier A, I-iguez A, Serra A, Hernandez-Antolin R, Mainar V, et al. Everolimus-eluting stent versus bare-metal stent in ST-segment elevation myocardial infarction (EXAMINATION): 1 year results of a randomised controlled trial. Lancet. 2012; 380(9852):1482-90. https://doi.org/10.1016/S0140-6736(12)61223-9

31. Barbato E, Marco J, Wijns W. Direct stenting. Eur Heart J. 2003; 24:394-403. https://doi.org/10.1016/S0195-668X(02)00802-3

32. Remkes WS, Somi S, Roolvink V, Rasoul S, Ottervanger JP, Gosselink AT, et al. Direct drug-eluting stenting to reduce stent restenosis: a randomized comparison of direct stent implantation to conventional stenting with pre-dilation or provisional stenting in elective PCI patients. JACC Cardiovasc Interv. 2014; 7(7):751-8. https://doi.org/10.1016/j.jcin.2014.02.012 PMid:25060017

33. Räber L, Kelbæk H, Ostojic M, Baumbach A, Heg D, Tüller D, et al. Effect of biolimus-eluting stents with biodegradable polymer vs bare-metal stents on cardiovascular events among patients with acute myocardial infarction: the COMFORTABLE AMI randomized trial. JAMA. 2012; 308(8):777-87. https://doi.org/10.1001/jama.2012.10065 PMid:22910755

34. Sabaté M, Räber L, Heg D, Brugaletta S, Kelbaek H, Cequier A, et al. Comparison of newer-generation drug-eluting with bare-metal stents in patients with acute ST-segment elevation myocardial infarction: a pooled analysis of the EXAMINATION (clinical Evaluation of the Xience-V stent in Acute Myocardial INfArcTION) and COMFORTABLE-AMI (Comparison of Biolimus Eluted From an Erodible Stent Coating With Bare Metal Stents in Acute ST-Elevation Myocardial Infarction) trials. JACC Cardiovasc Interv. 2014; 7(1):55-63. https://doi.org/10.1016/j.jcin.2013.07.012 PMid:24332419

35. Räber L, Kelbæk H, Taniwaki M, Ostojic M, Heg D, Baumbach A, et al. Biolimus-eluting stents with biodegradable polymer versus bare-metal stents in acute myocardial infarction: two-year clinical results of the COMFORTABLE AMI trial. Circ Cardiovasc Interv. 2014; 7(3):355-64. https://doi.org/10.1161/CIRCINTERVENTIONS.113.001440 PMid:24847017

36. Sabaté M, Brugaletta S, Cequier A, I-iguez A, Serra A, Hernádez-Antolín R, et al. The EXAMINATION trial (Everolimus-Eluting Stents Versus Bare-Metal Stents in ST-Segment Elevation Myocardial Infarction): 2-year results from a multicenter randomized controlled trial. JACC Cardiovasc Interv. 2014; 7:64-71. https://doi.org/10.1016/j.jcin.2013.09.006 PMid:24332423

37. Sabate M, Brugaletta S, Cequier A, Iniguez A, Serra A, Jimenez-Quevedo P, et al. Clinical outcomes in patients with STsegment elevation myocardial infarction treated with everolimus-eluting stents versus bare-metal stents (EXAMINATION): 5-year results of a randomised trial. Lancet. 2016; 387(10016):357-366. https://doi.org/10.1016/S0140-6736(15)00548-6

38. de Waha A, King LA, Stefanini GG, Byrne RA, Serruys PW, Meier B, et al. Long-term outcomes of biodegradable versus durable polymer drug-eluting stents in patients with acute ST-segment elevation myocardial infarction: a pooled analysis of individual patient data from three randomised trials. EuroIntervention. 2015; 10(12):1425-31. https://doi.org/10.4244/EIJV10I12A247 PMid:24602961

39. Park DW, Park SW, Park KH, Lee BK, Kim YH, Lee CW, et al. Frequency of and risk factors for stent thrombosis after drug-eluting stent implantation during long-term follow-up. Am J Cardiol. 2006; 98(3):352-6. https://doi.org/10.1016/j.amjcard.2006.02.039 PMid:16860022

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Apr 15; 7(7):1110-1113.
https://doi.org/10.3889/oamjms.2019.262
eISSN: 1857-9655
Clinical Science

Thyroid Hormone Profile in Children with Sepsis: Does Euthyroid Sick Syndrome Exist?

Gema Nazri Yanni*, Cynthea Prima Destariani, Arlina Nurbaity Lubis, Melda Deliana

Department of Child Health, Faculty of Medicine, Universitas Sumatera Utara, Haji Adam Malik General Hospital, Medan, Indonesia

Citation: Yanni GN, Destariani CP, Lubis M, Deliana M. Thyroid Hormone Profile in Children with Sepsis: Does Euthyroid Sick Syndrome Exist?. Open Access Maced J Med Sci. 2019 Apr 15; 7(7):1110-1113. https://doi.org/10.3889/oamjms.2019.262

Keywords: Thyroid hormone; Sepsis; Children

***Correspondence:** Gema Nazri Yanni. Department of Child Health, Faculty of Medicine, Universitas Sumatera Utara, Haji Adam Malik General Hospital, Medan, Indonesia. E-mail: gema.nazri.yanni@gmail.com

Received: 07-Mar-2019; **Revised:** 06-Apr-2019; **Accepted:** 09-Apr-2019; **Online first:** 14-Apr-2019

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Alterations in peripheral thyroid hormone metabolism play an eminent role in the development of the euthyroid sick syndrome. Altered solvation may also lead to changes in peripheral thyroid hormones. Data on thyroid hormones in critically ill children remain unclear.

AIM: This study was aimed to evaluate thyroid hormone profile in children with sepsis as well as to assess the association between thyroid level and sepsis outcome.

METHODS: An observational cohort study was conducted in 80 children with sepsis from October 2015 to January 2016 in Haji Adam Malik General Hospital. T3 and T4 level were measured on day 1 and after > 72 hours of sepsis diagnosed.

RESULTS: We recorded length of stay in PICU, patient outcome and analysed the relationship with the chi-square test. Level of T3 and T4 were decreased on day 1 in pediatric sepsis. Of 80 subjects, 57 (71.2%) with low-level T3 and 41 (51.2%) with low T4 were found. The relationship between T3 and T4 level on day 1 with the length of stay were not found (P = 0.500; P = 0.987). There were a significant relationship between level of T3 and T4 with outcome (P = 0.0001; OR 24.706; P = 0.014; OR 3.086). Subject with normal T3 and T4 level had 24 and 3 times life chances compare to lower level.

CONCLUSION: The Euthyroid Sick Syndrome in children with sepsis does exist. There was a significant relationship between T3 and T4 level on day 1 with patient outcome.

Introduction

Sepsis is the most common cause of mortality in infants and children. The incidence of sepsis and septic shock were increasing in the last 30 to 40 years [1]. *World Health Organization* (WHO) reported 70% in eight million children under five years mortalities in developing countries caused by infection diseases which commonly ended in sepsis condition. The incidence of sepsis was 0.56% of 1000 children and 5.6% of 1000 infants with the highest mortality rate as 10.6% [2].

Division of Emergency and Intensive Care Medicine, Department of Child Health, Cipto Mangunkusumo Hospital in 2009 reported incidence of sepsis was 19.3% of 502 patients hospitalised in pediatric intensive care unit (PICU) with mortality rate 10.3% [3]. Mortality rate due to neonatal sepsis in Haji Adam Malik General Hospital in 2008 until 2010 was 32.9% [4].

Sepsis might cause hemodynamic and cardiovascular disorders and hormonal imbalance. A hormonal disorder that often affected in sepsis was thyroid hormones which occur in the form of euthyroid sick syndrome (ESS) or nonthyroidal illness syndrome (NTIS) [5]. Euthyroid sick syndrome (ESS) or nonthyroidal illness syndrome (NTIS) is a condition of decreased thyroid hormone levels without disruption of thyroid hormone function that occurs in severe systemic non-thyroid disease. Changes in thyroid hormone will later result in disruption of oxygen consumption, cardiovascular, sympathetic nerves, respiration, digestive, and hematopoiesis which in turn

will lead to organ system failure and ended in death [6].

The critical disease is characterized by complex and multiple changes in the thyroid pathway. Along with worsening of a critical illness, the decrease occurs in not only triiodothyronine (T3) levels but also thyroxine (T4) and thyroid stimulating hormone (TSH). Decreased levels of T4 and TSH showed an indication of worsening of disease and poor prognosis. The incidence was around 80%, especially in patients with T4 levels < 3 µg/dL. The decreased in thyroid hormone levels is still controversy to date. A study in Greece and the United States reported the decreased level of T4 and TSH affected mortality rate in sepsis and septic shock [7]. A study in the Netherlands showed decreased in T4 that affected mortality, but a study in Belgium showed that T3 was. Decreased in T3 was due to changes in metabolism in thyroid hormones [8], [9], [11].

A study in Cipto Mangunkusumo Hospital in 2014 reported decreased thyroid hormone levels especially T3 in sepsis, while in Semarang showed the decreased of T3 levels were followed by increased of T4 and TSH which was by the definition of ESS. Both studies showed patients with low thyroid hormone levels associated with poor outcome, as measured by pediatric logistic organ dysfunction (PELOD) or pediatric index of mortality (PIM) score.

This study was conducted to evaluate thyroid hormones changes and the outcome in children with sepsis in PICU Haji Adam Malik General Hospital.

Methods

This was a cohort study in 80 patients with sepsis hospitalised in PICU Haji Adam Malik Hospital from October 2015 until January 2016. Thyroid hormone levels were observed on the first day and > 72 hours of admission. Diagnosis of sepsis was made based on the criteria of International Consensus on Pediatric Sepsis 2005.

Table 1: Normal value of T3, T4, and TSH levels [12]

Age	T3(nmol/L)	Age	T4 (nmol/L)	Age	TSH (µIU/L)
1 – 12 months	1.4 – 4	1 – 12 months	77 – 180	1 – 5 months	0.5 – 6
1 – 6 years	1.4 – 3.7	1 – 5 years	58 – 142	6 months – 18 years	0.5 – 4.5
7 – 11 years	1.4 – 3.6	6 – 18 years	58 – 129		
12 – 18 years	1.5 – 3.3				

Inclusion criteria were patients one month until 18 years with a diagnosis of sepsis. Hormone thyroid levels were examined on the first day and > 72 hours. Patients with hypothyroid and hyperthyroid diagnosed by a pediatric endocrinologist before admission to PICU were excluded. Subjects were taken consecutively. Age, gender, PELOD score, length of stay, and outcome were noted. Data were analysed using SPSS version 20. Chi-square test was done to evaluate the association of thyroid hormones and length of stay and outcome. P value < 0.05 was considered statistically significant.

Normal value thyroid hormone levels were shown in Table 1.

Result

During the study period there were 80 patients with sepsis were included (Table 2).

Table 2: Subjects Characteristics

Variable	n = 80
Gender	
Male	46 (57.5%)[a]
Female	34 (42.5%)
Age	7 (1-17)[b]
PELOD score	
High (≥ 20)	31 (38.75%)[a]
Low (< 20)	49 (61.25%)
Length of stay	
< 7 days	37 (46.25%)[a]
≥ 7 days	43 (53.75%)
Outcome	
Alive	38 (47.5%)[a]
Died	42 (52.5%)

[a] categorical data: n (%); [b] numerical data not normal distribution: median (minimum-maximum); [c] numerical data normal distribution: mean ± standard deviation (SD).

Decreased in T3 hormone on the first day of admission was found in 57 patients (71.2%) and decreased in T4 was in 41 patients (51.2%). On the > 72 hours of admission, patients with low T3 hormone levels were decreased to 49 patients (61.2%), on the other side patients with low T4 hormone levels were increased to 41 patients (56.35%), (Table 3).

Table 3: Profile of thyroid hormones

	T3		T4		TSH	
	n (%)	Mean ± SD	n (%)	Mean ± SD	n (%)	Mean ± SD
Day 1						
Normal	23 (28.8%)	1.71 ± 0.53	39 (48.8%)	7.26 ± 1.16	80 (100)	3.49 ± 0.36
Low	57 (71.2%)	0.83 ± 0.22	41 (51.2%)	4.29 ± 1.08	0	0
Day 4						
Normal	31 (38.8%)	1.8 ± 0.46	35 (43.8%)	7.36 ± 1.12	80 (100)	3.41 ± 0.39
Low	49 (61.2%)	0.75 ± 0.27	45 (56.3%)	4.56 ± 1.15	0	0

There was no statistically significant relationship between hormone thyroid levels on the first day of admission and length of stay, where p-value for T3 and T4 were 0.5 and 0.987, respectively (Table 4).

Table 4: Association of T3 and T4 hormone levels and length of stay

	Length of stay		Total	p	OR (95%CI)
Day 1	< 7 days	≥ 7 days			
T3 levels					
Normal	12 (52.2%)	11(47.8%)	23 (100%)	0.500	1.396 (0.529-3.688)
Low	25 (43.9%)	32 (56.1%)	57 (100%)		
T4 levels					
Normal	18 (46.2%)	21 (53.8%)	39 (100%)	0.987	0.992 (0.412-2.391)
Low	19 (46.3%)	22 (53.7%)	41 (100%)		

Thyroid hormone levels on the first day of admission had a statistically significant relationship with sepsis outcome, where a p-value of T3 and T4 were 0.0001 and 0.014 respectively. Patients with normal T3 levels on the first day of admission were 24 times more likely to live than patients with low T3 levels, where patients with normal T4 levels were three times more likely to live than patients with low T4 levels (Table 5).

Table 5: Association of T3 and T4 hormone levels and outcome

Day 1	Outcome		P	OR (95%CI)
	Alive	Died		
T3 levels				
Normal	21 (91.3%)	2 (8.7%)	0.0001*	24.706 (5.205-117.272)
Low	17 (29.8%)	40 (70.2%)		
T4 levels				
Normal	24 (61.5%)	15 (38.5%)	0.014*	3.086 (1.239-7.686)
Low	14 (34.1%)	27 (65.9%)		

Discussion

Thyroid hormones have an important role in the adaptation of metabolic functions to stress and critical illnesses such as sepsis and septic shock. Thyroid hormone will decrease at the onset of infection and will decrease as the disease progresses. Thyroid hormone levels will be normal again after the systemic disease cured [6]. A study in Turkey in 2004 showed decreased T3 and T4 levels in patients with sepsis [13]. Another study in Jakarta 2014 showed thyroid hormones would decrease in sepsis condition, especially T3 levels [14]. In this study, T3 levels were examined on the first day and > 72 hours of admission in 80 patients with sepsis, and decreased levels of T3 and T4 were found on the first day of admission.

At the beginning of illness there will be decreased in T3 levels due to the enzyme 5'-deiodinase defect which converts T4 to T3, decreased number of thyroid receptors mediated by interleukin 1β, the presence of thyroid binding protein inhibitors and increased in TNF-α [15]. Normal T3 levels during the acute phase might be due to a T3 decreased at the beginning of infection (36-72 hours) will return to normal after more than 72 hours, because after 72 hours post infection TSH will increase due to peripheral thyroid hormone secreted by the feedback mechanism of low T3 levels. This mechanism is called the recovery of metabolic activity [16]. Profile of thyroid hormones in this study showed a decreased in T3 and T4 on the first day of admission that described the acute phase of sepsis, and after > 72 hours T3 levels returned to normal in approximately 10% patients (71.2% to 61.2%), but not on T4 levels.

Medications in critically ill patients also affected thyroid function. Medications such as dopamine will lower TSH levels [17]. Dopamine in neonates and children had been reported to suppress the function of the pituitary gland. [14], [18], [19] The use of catecholamines and noradrenaline is also reported to lower TSH [20]. The TSH levels reduction in critically ill patients will be proportional to the decreases in levels of T3 and T4 [17].

A study in Semarang 2014 showed there was no significant relationship between thyroid hormone levels in patients with sepsis and the outcome whether it was improvement or deterioration [21]. A study in Switzerland 2010 reported decreased in T3 could not be used as a prognostic marker [22]. Different results obtained from this study, there were a statistically significant relationship between thyroid hormone levels of T3 and T4 on the first day with sepsis outcome. But, many factors affected the outcome of patients with sepsis, for example, disease severity, nutritional status on admission, and sepsis itself whether it had progressed into severe sepsis [23].

That results were similar to this study, where patients with normal thyroid hormone levels on the first day of admission were more likely to live, normal T3 levels were 24 times, and normal T4 levels were three times more likely to live than low thyroid hormones.

Limitation of this study was no differentiation in the severity of sepsis and did not analyze patients with inotropic drugs which might affect thyroid hormone levels.

In conclusion, this study showed that euthyroid sick syndrome (ESS) was found in patients with sepsis. There was a significant relationship between thyroid hormone levels, T3 and T4, on the first day of admission with sepsis outcome.

References

1. Latief A, Pudjiadi AH, Somasetia DH, Alwy EH, Mulyo GD, Kushartono H. Diagnosis dan tatalaksana sepsis pada anak. Unit Kerja Koordinasi Pediatri Gawat Darurat Ikatan Dokter Anak Indonesia. Jakarta: Ikatan Dokter Anak Indonesia. 2010:1-8.

2. Goldstein B, Giroir B, Randolph A. International pediatric sepsis consensus conference: definitions for sepsis and organ dysfunction in pediatrics. Pediatr Crit Care Med. 2005; 6:2-4. https://doi.org/10.1097/01.PCC.0000149131.72248.E6 PMid:15636651

3. Saraswati DD, Antonius HP, Mulyadi MD, Bambang S, Damayanti RS, Nia K. Faktor Risiko yang Berperan pada Mortalitas Sepsis. Sari Pediatri. 2014; 15:281-9. https://doi.org/10.14238/sp15.5.2014.281-8

4. Sianturi P, Beby SH, Bugis ML, Emil A, Guslihan DT. Gambaran Pola Resistensi Bakteri di Unit Perawatan Neonatus. Sari Pediatri. 2012; 13:431-6. https://doi.org/10.14238/sp13.6.2012.431-6

5. Chopra IJ. Euthyroid sick syndrome: is it a misnomer? J Clin Endocrinol Metab. 1997; 82(2):329-34. https://doi.org/10.1210/jcem.82.2.3745 PMid:9024211

6. Anna GA, Drosos EK, Anastasios MK, Matthew EF. Association between thyroid function tests at baseline and the outcome of

patients with sepsis or septic shock: a systematic review. European Journal of Endocrinology. 2001; 165:147-55.

7. Brinker M, et al. Euthyroid sick syndrome in meningococcal sepsis: the impact of peripheral thyroid hormone metabolism binding proteins. J Clin Endocrinol Metab. 2005; 90:5613-20. https://doi.org/10.1210/jc.2005-0888 PMid:16076941

8. Peeters RP, Wouters PJ, Kaptein E, Toor HV, Visser TJ, Berghe GV. Reduced activation an increased inactivation of thyroid hormone in tissues of critically ill patients. J Clin Endocrinol Metab. 2003; 88:3202-11. https://doi.org/10.1210/jc.2002-022013 PMid:12843166

9. Suvarna JC, Fande CN. Serum thyroid hormone profile in critically children. Indian J Pediatr. 2009; 76:1217-21. https://doi.org/10.1007/s12098-009-0250-7 PMid:19936665

10. Bone RC, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. CHEST. 1992; 101:1644-55. https://doi.org/10.1378/chest.101.6.1644 PMid:1303622

11. Carcillo J A, Planquois J M and Golstein B. Early markers of infection and sepsis in newborn and children. Advances in Sepsis. 2006; 5:118-25.

12. Nicholson JF, Pesce MA. Reference Ranges for Laboratory Tests and Procedures. In: Behrman R E, Kliegman R M and Jenson H B, eds. Nelson Textbook of Pediatrics. 19th ed. Philadelphia: Saunders, 2011:2396-427.

13. Yildizdas D, Neslihan OM, Hacer Y, Ali KT, Yasar S, Bilgin Y. Thyroid Hormone Levels and their Relationship to Survival in Children with Bacterial Sepsis and Septic Shock. J Pediatr Endocrinol Metab. 2004; 171:435-44.

14. Tanurahardja AG, Antonius HP, Pramita GD, Aman P. Thyroid hormone profile and PELOD score in children with sepsis. Paediatr Indones. 2014; 54:245-50. https://doi.org/10.14238/pi54.4.2014.245-50

15. Sakharova OV, Inzucchi SE. Endocrine assesments during critical illness. Crit Care Clin. 2007; 23:467-90. https://doi.org/10.1016/j.ccc.2007.05.007 PMid:17900481

16. Purwanti A, Bambang, Supriatna M. Hubungan kadar tiroid dan skor pediatric index of mortality dengan luaran sepsis pada anak. Medica Hospitalia. 2014; 2:92-7.

17. Rothwell PM, Udwadia ZF, Lawler PG. Thyrotropin concentration predicts outcome in criticall illness. Anaesthesia. 1993; 48:372-6. https://doi.org/10.1111/j.1365-2044.1993.tb07006.x

18. Marks SD. Nonthyroidal illness syndrome in children. Endocrinol. 2009; 38:355-67. https://doi.org/10.1007/s12020-009-9239-2

19. Lodha R, Vivekanandhan S, Sarthi M, Arun S, Kabra SK. Thyroid function in children with sepsis and septic shock. Acta Paediatrica. 2007; 96:406-9. https://doi.org/10.1111/j.1651-2227.2007.00135.x PMid:17407466

20. Fukuda S. Correlation between function of the pituitary-thyroid axis and metabolism cathecolamines by the fetus at delivery. Clin Endocrinol. 1987; 27:331-8. https://doi.org/10.1111/j.1365-2265.1987.tb01159.x

21. Bambang, Asri P, Supriatna. Hormon tiroid pada kondisi anak dengan sepsis. Sari Pediatri. 2014; 16:97-102. https://doi.org/10.14238/sp16.2.2014.97-102

22. Das BK, Agarwal JK, Agarwal PA, Mishra OP. Serum cortisol and thyroid hormone levels in neonates with sepsis. Indian J Pediatr. 2002; 69:663-5. https://doi.org/10.1007/BF02722699 PMid:12356216

23. Haentjens P, Meerhaeghe AV, Velkeniers B. Subclinical thyroid dysfunction and mortality: an estimate of relative and absolute excess all cause mortality based on time to event data from cohort studies. European J Endocrin. 2008; 159:329-41. https://doi.org/10.1530/EJE-08-0110 PMid:18511471

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Apr 15; 7(7):1114-1118.
https://doi.org/10.3889/oamjms.2019.202
eISSN: 1857-9655
Clinical Science

Neonatal Growth, Neurotrophine, Zinc, and Ferritin Concentration in Normal and Iron Deficience Pregnancy: An Observational Analitic Study

Yusrawati Yusrawati[1*], Defrin Defrin[1], Hudila Rifa Karmia[2]

[1]Fetomaternal Division, Obstetrics and Gynecology Department, Medical Faculty of Andalas University, Padang, West Sumatera, Indonesia; [2]Obstetrics and Gynecology Department, Medical Faculty of Andalas University, Padang, West Sumatera, Indonesia

Citation: Yusrawati Y, Defrin D, Karmia HR. Neonatal Growth, Neurotrophins, Zinc, and Ferritin Concentration in Normal and Iron Deficiency Pregnancy: An Observational Analytic Study. Open Access Maced J Med Sci. 2019 Apr 15; 7(7):1114-1118. https://doi.org/10.3889/oamjms.2019.202

Keywords: Ferritin; Neutrophil; Zinc; Neonatal head circumference; Maternal anaemia

***Correspondence:** Yusrawati Yusrawati. Fetomaternal Division, Obstetrics and Gynecology Department, Medical Faculty of Andalas University, Padang, West Sumatera, Indonesia. E-mail: yusrawati65@med.unand.ac.id

Received: 18-Jan-2019; **Revised:** 02-Mar-2019; **Accepted:** 03-Mar-2019; **Online first:** 14-Apr-2019

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Anemia in pregnancy was one of the national problems. Insufficient iron deposits before pregnancy and inadequate iron intake during pregnancy could lead to iron deficiency anaemia in pregnancy, followed by iron deficiency in neonates.

AIM: This study aimed to assess the molecular relationship of maternal iron deficiency with the function of the neonatal central nervous system to know the cognitive aspects of learning ability of children.

METHODS: This study was an observational analytic study with cross-sectional design underwent in RSUP Dr M. Djamil Padang, RSI Ibnu Sina Padang, and RSU BMC Padang. The sample size was 80 pregnant women at term. After a maternal and neonatal physical examination, maternal and umbilical blood samples were obtained to assess maternal ferritin levels and neonates ferritin, neurotrophin and zinc levels using the Enzyme-Linked Immunosorbent Assay (ELISA). Data were analysed using the IBM SPSS Statistics for Windows. The independent sample t-test was performed to assess the relationship for normally distributed data and Mann-Whitney test for abnormal data distribution with significance level $p < 0.05$.

RESULTS: There were differences in mean neonatal ferritin ($p < 0.001$), neonatal neurotrophin ($p < 0.001$), and neonatal zinc ($p < 0.001$) to normal maternal ferritin levels (≥ 15 µg/ml) and low maternal ferritin levels (< 15 µg/ml). The difference in mean neonatal head circumference (0.92; CI95% -0.79-0.98) was associated with neonatal ferritin levels.

CONCLUSIONS: The mean neonatal ferritin, neurotrophin, and zinc levels were found lower in iron deficiency maternal. Maternal iron deficiency correlates with neonate growth, iron deficiency, and neurotrophin expression that affected neonate cognition.

Introduction

Anaemia in pregnancy was one of the national problems because it reflected the socio-economic well-being of the community and greatly affected the quality of human resources. Anaemia in pregnant women was called "potential danger to mother and child" (potentially harmful to mother and child). Therefore, serious attention was needed from all parties to anaemia related to healthcare services [1]. The highest prevalence of anaemia in pregnancy in the world was found in countries of Southeast Asia, Western Mediterranean and Africa. The target of "Global Nutrition Targets 2025" among of which was for mother, infant and child was expected to reduce anaemia by 50% infertile women [2].

Iron plays a role in neurocognitive and neurobehavioral development in the last two-thirds of pregnancy. Research on humans and animals showed that iron deficiency anaemia occurred since intrauterine was associated with the developmental disorders of behavioral development and nerve changes that produce irreversible effects on fetal neurochemistry and neurobiology [3]. Iron is important in the process of erythropoiesis, the formation of hemoglobin, myoglobin, gene transcription, cellular enzyme reactions, and oxidation-reduction reactions.

Iron also plays a role in the process of dendritogenesis, synaptogenesis, neurogenesis, myelination and synthesis of brain neurotransmitters. All of these iron functions are important for the brain to perform its functions, so iron deficiency could lead to behavioural impairment, decreased learning function, and memory [4].

Neurotrophin is a growth factor that plays a role in learning, memory, and behaviour in the hippocampus. Synthesis of neurotrophin requires enzyme and iron. Animal studies had found that iron deficiency early in life caused epigenetic changes that alter chromatin structures and expression of neurotrophin genes, leading to a decrease in the differentiation of the hippocampus neurons and causing behavioural and cognition abnormalities, including decreased memory capacity and increased anxiety. Abnormal behaviour and cognition will persist until adulthood despite adequate treatment [4], [5].

Zinc along with iron is a high concentration in the brain especially the hippocampus and is included in the neurotransmission. Zinc deficiency induces cognitive deficits especially in the process of learning and memory. Some of the zinc is a transferrin transport device, which is also an iron transport vehicle. Under normal circumstances, transferrin saturation is usually less than 50% of iron. When the ratio of iron and zinc is more than 2:1, transferrin available for zinc is reduced, thus inhibiting zinc absorption. The opposite also occurs where high concentrated zinc can inhibit the absorption of iron [6]. Therefore, zinc supplementation was recommended in pregnant women who were given iron supplementation [7]. Low iron status in pregnancy will affect the anthropometric size of newborns. Maternal iron status and pregnancy outcomes showed that serum iron, TIBC, and maternal transferrin saturation levels in third-trimester pregnancies were closely related to birth weight and infant length. The presence of a link between iron deficiency (maternal and neonatal), zinc, and neurotrophin with the central nervous system and neonatal anthropometry had caused authors interest to investigate differences in neonatal growth rates, ferritin levels, neurotrophin, and zinc in pregnancies with normal and low ferritin levels.

This study aimed to assess the molecular relationship of maternal iron deficiency with the function of the neonatal central nervous system to know the cognitive aspects of learning ability of children.

Methods

This was an observational analytic study with a cross-sectional study design. This study was conducted at RSUP. Dr M. Djamil Padang, RSI. Ibnu Sina Padang, RSU. BMC Padang as well as sample examination conducted in Biomedical Laboratory FK Unand in August 2016-November 2016. A sample size of 80 pregnant women at term consisted of 40 samples with normal ferritin (> 15 µg/L) and 40 samples with low ferritin (< 15 µg/L). Sampling was done using consecutive sampling which came antenatal care at RSUP. Dr M. Djamil Padang, RSI. Ibnu Sina Padang, and RSU. BMC Padang.

The population of this study were all pregnant women with at term pregnancy who performed antenatal care on obstetricians' practice at RSUP Dr M. Djamil, RSI. Ibnu Sina Padang and RSU. BMC Padang. The inclusion criteria in this study were term pregnancies, neonates born alive, maternal leukocytes within normal limits, and willing to participate in the study.

After signing informed consent, history, clinical examination and obstetric examination, and ultrasound were performed. Mother's blood sample was taken for haemoglobin (Hb), leukocyte and ferritin examination. After the child was born either pervaginam or Caesarea section, cord blood collection was done to check the levels of ferritin, neurotrophin, and zinc. Neonatal anthropometry was measured after birth, i.e. body weight, body length and head circumference. The maternal serum and neonatal cord serum samples obtained were analysed using the Enzyme-Linked Immunosorbent Assay (ELISA) method.

Data were analysed using the IBM SPSS Statistics for Windows (version 23.0; SPSS, Inc., Chicago, IL, USA). The independent sample t-test was performed to assess the association for normally distributed data and Mann-Whitney test for abnormal data distribution with significance level $p < 0.05$.

Results

Mean of ferritin, neurotrophin, and neonatal zinc levels in normal and low maternal ferritin are presented in Table 1.

Table 1: Mean of Ferritin, Neurotrophin, and Neonatal Zinc Levels in Normal and Low Maternal Ferritin

	Low Maternal Ferritin (< 15 µg/ml) Mean ± SD	Normal Maternal Ferritin (≥ 15 µg/ml) Mean ± SD	CI95%	p* value
Neonates Ferritin Levels (µg/ml)	58.59 ± 2.33	134.68 ± 7.44	53.33-103.07	< 0.001
Neonates Neurotrophin Levels (pg/mL)	1776.20±1.764	3309.82±1.614	780.88-2286.36	< 0.001
Neonates Zinc Levels (mmol/L)	6.72 ± 8.29	14.96 ± 6.40	4.94-11.53	< 0.001

* Distribution of data was normal, the mean difference was analysed using Independent samples t-test; ** Distribution of data was abnormal, the mean difference was analysed using Mann-Whitney Test.

As shown in Table 1, there was a difference of mean ferritin, neutrophil, and zinc of term newborn between normal maternal ferritin (≥ 15 µg/mL) with low maternal ferritin (< 15 µg/ml) of 78.20 µg/mL, 1533.62 pg/mL, and 8.23 mmol/mL, respectively, with $p < 0.05$.

Table 2: Mean of Head Circumference, Body Length, and Neonatal Weight on Normal Neonatal Ferritin and Low Neonatal Ferritin

	Low Neonates Ferritin (< 75µg/ml) Mean ± SD	Normal Neonates Ferritin (≥ 75µg/ml) Mean ± SD	CI95%	p value
Neonates Circumferences (cm)	34 (28-35)	34 (29-36)	-0.79-0.98	0.004**
Neonates Length (cm)	48.22 ± 1.90	48.31 ± 2.06	-07.9-0.98	0.837**
Neonates Weight (gram)	2426 ± 405	3083 ± 464		< 0.001*

* Distribution of data was normal, the mean difference was analysed using Independent samples t-test; ** Distribution of data was abnormal, the mean difference was analysed using Mann-Whitney Test.

In Table 2, shown there was a difference in mean of head circumference, body length, and neonatal weight between normal neonatal ferritin (≥ 75 µg/mL) with low neonatal ferritin (< 75 µg/mL) with $p < 0.05$.

Table 3: Mean Head Circumference, Body Length, and Neonatal Weight in Neonatal Neotrophin Normal and Neonatal Neotrophin Low

	Low Neonates Neurotrophin (< 2000 pg/ml) Mean ± SD	Normal Neonates Neurotrophin (≥ 2000 pg/ml) Mean ± SD	CI95%	p value
Neonates Circumferences (cm)	34 (28-35)	34.3 (32-36)	0.68-2.43	< 0.001**
Neonates Length (cm)	48.11 ± 1.72	48.48 ± 2.27	-0.51-1.26	0.400**
Neonates Weight (gram)	2412 ± 357	3142 ± 463	0.68-2.43	< 0.001*

* Distribution of data was normal, the mean difference was analysed using Independent samples t-test; ** Distribution of data was abnormal, the mean difference was analysed using Mann-Whitney Test.

Table 3, shown there were differences in mean head circumference and weight of term newborn between normal neonatal neurotrophin levels (neurotrophin ≥ 2000 pg/mL) with low neonatal neurotrophin (neurotrophin < 2000 pg/mL) with $p < 0.05$.

Discussion

This study shows a significant difference between low maternal ferritin group (< 15 µg/L) with normal maternal ferritin group (> 15 µg/L). Iron deficiency in the mother reduces iron deposits of the fetus which eventually leads to neonatal iron deficiency [4]. Several previous studies had found that iron deficiency in the fetus and neonate affected neurotrophin expression in the CNS. Neurotrophin has some impacts on areas of the brain that are associated with cognition and behavioural processes in hippocampus, cortex and amygdala areas [3].

Iron deficiency during prenatal and postnatal periods caused decreased levels of neurotrophin and neurogenesis in the dentate hippocampus. This suggested that iron balance is important for the expression of neurotrophin which will help the development of the brain. It may also explain how an iron deficiency in perinatal periods could lead to deficits of behaviour in children [8].

Concentrations of serum ferritin had been used as a standard measure of iron storage in infants, children, and adults. In adults, 1 µg/l serum ferritin is equivalent to 8-10 mg iron stores. In neonates, the ratio between serum ferritin and non-heme iron in the liver is close to 1:2.7 [9]. Perez et al., (2005) found that babies born to mothers with low serum ferritin would tend to have low levels of ferritin as well [10].

The interaction between micronutrients in pregnancy is crucial, where developing fetuses are particularly vulnerable to inappropriate micronutrient status. Excessive and uncontrolled administration of micronutrient supplements during pregnancy may adversely affect other micronutrients because of the competition between each of these micronutrients [11].

Several previous studies had reported that iron supplementation might cause impaired absorption of zinc in the gut. This is due to the barrier of zinc uptake in the intestinal wall. In the state of zinc deficiency, iron metabolism will be disrupted and will lead to iron deficiency anaemia [3]. Therefore, many studies suggested giving zinc along with iron supplementation.

In this study, infants born to mothers with low levels of ferritin had lower zinc levels than infants born to mothers with normal ferritin levels. Research conducted by Jariwala et al., in pregnant women who gave birth at Babha Atomic Research Center Hospital India found a significant correlation between maternal iron levels and maternal zinc levels. Maternal zinc levels would also affect neonatal zinc levels [12].

In this study, it was found that infants with low levels of ferritin had lower zinc levels than infants with normal ferritin levels. Research conducted by Jariwala et al. showed a significant positive correlation between iron levels in neonatal and zinc levels in the neonatal ($p = 0.001$, $r = 0.54$) [11]. Knowing that iron deposits in neonates could be affected by maternal iron status, and with the difference in mean rates of newborn zinc in normal maternal ferritin and low maternal ferritin, it could be concluded that there was an indirect relationship between neonatal zinc levels and neonatal ferritin levels.

Iron deficiency while in the womb and at neonates could disrupt the brain structure, cognitive function, and could result in long-term irreversible motoric and cognitive disorders that cannot be treated with iron supplementation [9]. Neurotrophin plays an important role in neuron protection systems and lowers the risk of neuronal apoptosis. Also,

neurotrophin also plays a role in the growth of axons during development, enhancement of neuron function, neuronal morphological differentiation, and neurotransmitter expression [13]. In this study, there was a significant difference in mean neonatal head circumference between infants with low ferritin levels compared with infants with normal ferritin levels.

Severe deficiency anaemia is associated with poor outcomes such as abortion, preterm delivery, and poor neonatal anthropometry. Previous studies had focused more on the relationship between maternal ferritin levels and anthropometry of newborns. Research conducted by Dalal et al., 2014 had found a significant relationship between the length of the newborn's body and the maternal ferritin level [14]. It is just that the mean difference between maternal ferritin and the length of the newborn's body was not studied in this study

In this study, a significant difference in mean birth weight between infants with normal ferritin levels and infants with low ferritin levels were found. Iron deficiency anaemia in early pregnancy is associated with an increased risk of having a low birth weight baby and preterm birth. In a randomised observational study conducted in Nepal, it was found that the introduction of iron supplements, folic acid, and vitamin A significantly reduced the incidence of low birth weight incidence (19%) when compared with vitamin A supplementation alone [15]. It is known that iron deposits in neonates may be affected by maternal iron status so that infants born to mothers with low ferritin levels will have lower levels of ferritin than babies born to mothers with normal ferritin levels. The significant difference in mean birth weight of the newborns in this study was thought to be indirectly caused by the level of neonatal ferritin which was influenced by maternal ferritin levels.

The head circumference at birth is known to represent brain volume in newborns and had been used in previous studies to measure cognitive function [16]. Veena et al., in 2011 found a positive correlation between birth weight and head circumference with learning ability, long-term memory, and visuospatial ability ($p < 0.05$). There was an association between neonatal neurotrophin and neuronal growth, and the association between cognitive and neonatal head circumference were estimated to influence the findings obtained in this study [16]. Neurotrophin may play an important role in brain development during the antenatal and postnatal periods [13].

Neurotrophin is responsible for modulation of synapse function, neuron cell plasticity, modulation of oligodendrocyte growth, myelin formation, and dendritogenesis how the relationship between the weight and neonatal neurotrophin levels had not been studied previously. Rao et al., a study in 2008 found there was no significant relationship between birth weight and neonatal neurotrophin levels [17]. In this study, however, there was a significant mean difference between newborns who had low neurotrophin levels and high neurotrophin levels ($p < 0.005$).

In conclusion, maternal iron deficiency correlates with neonate growth, iron deficiency, and neurotrophin expression that affected neonate cognition in the future.

Acknowledgement

We want to thank all staffs at RSUP. Dr M. Djamil Padang, RSI. Ibnu Sina Padang, and RSU. BMC Padang which had facilitated us in data collection. We would also like to thank all staffs of Biochemistry Lab, Medical Faculty of UNAND who had facilitated us in the processing of study samples and for all samples of participants who had been willing to participate in this research.

References

1. Vinogradova MA, Tatiana AF, Elena VS, Oleg R, Roman GS, Eugenia SP. Anemia During The Pregnancy: The Management and Outcome Depending on The Etiology. Blood Journal. 2014; 124(21):4839.

2. World Health Organization. Global Nutrition Target 2025. WHO/NMH/NHD/14.3, 2014.

3. Estrada JA, Contreras I, Pliego-Rivero FB, Otero GA. Molecular mechanisms of cognitive impairment in iron deficiency: alterations in brain-derived neurotrophic factor and insulin-like growth factor expression and function in the central nervous system. Nutritional neuroscience. 2014; 17(5):193-206. https://doi.org/10.1179/1476830513Y.0000000084 PMid:24074845

4. Radlowski EC, Johnson RW. Perinatal iron deficiency and neurocognitive development. Frontiers in human neuroscience. 2013; 7:585. https://doi.org/10.3389/fnhum.2013.00585 PMid:24065908 PMCid:PMC3779843

5. Tran PV, Carlson ES, Fretham SJ, Georgieff MK. Early-life iron deficiency anemia alters neurotrophic factor expression and hippocampal neuron differentiation in male rats. The Journal of nutrition. 2008; 138(12):2495-501. https://doi.org/10.3945/jn.108.091553 PMid:19022978 PMCid:PMC2911361

6. Almatsier S. Prinsip Dasar Ilmu Gizi. Jakarta. Gramedia Pustaka Utama. 2009.

7. Almatsier S, Soetardjo S, Soekatri M. Gizi seimbang dalam daur kehidupan. Jakarta: Gramedia pustaka utama. 2011:480.

8. Georgieff MK. The Role of Iron in Neurodevelopment: fetal Iron Deficiency and The Developing Hippocampus. Biochemical Society transactions. 2008; 36(6):1267-71. https://doi.org/10.1042/BST0361267 PMid:19021538 PMCid:PMC2711433

9. Siddappa AM, Rao R, Long JD, Widness JA, Georgieff MK. The assessment of newborn iron stores at birth: a review of the literature and standards for ferritin concentrations. Neonatology. 2007; 92(2):73-82. https://doi.org/10.1159/000100805 PMid:17361090 PMCid:PMC2863301

10. Jaime-Perez JC, Herrera-Garza JL, Gomez-Almaguer D. Sub-optimal fetal iron acquisition under a maternal environment. Arch Med Res. 2005; 36(5):598-602. https://doi.org/10.1016/j.arcmed.2005.03.023 PMid:16099345

11. Upadhyaya C, Mishra S, Ajmera P, Sharma P. Serum iron, copper and zinc status in maternal and cord blood. Indian J Clin Biochem. 2004; 19(2):48-52. https://doi.org/10.1007/BF02894257 PMid:23105456 PMCid:PMC3454214

12. Jariwala M, Suvarna S, Kumar GK, Amin A, Udas AC. Study of the concentration of trace elements Fe, Zn, Cu, Se and their correlation in maternal serum, cord serum and colostrums. Indian J Clin Biochem. 2014; 29(2):181-8. https://doi.org/10.1007/s12291-013-0338-8 PMid:24757300 PMCid:PMC3990806

13. Chouthai NS, Sampers J, Desai N, Smith GM. Changes in neurotrophin levels in umbilical cord blood from infants with different gestational ages and clinical conditions. Pediatr Res. 2003; 53(6):965-9. https://doi.org/10.1203/01.PDR.0000061588.39652.26 PMid:12621105

14. Dalal E, Shah J. A comparative study on outcome of neonates born to anemic mothers versus non anemic mothers. Natl J Med Res. 2014; 4(4):270-3.

15. Shao J, Lou J, Rao R, Georgieff MK, Kaciroti N, Felt BT, Zhao ZY, Lozoff B. Maternal serum ferritin concentration is positively associated with newborn iron stores in women with low ferritin status in late pregnancy. Journal of nutrition. 2012; 142(11):2004-9. https://doi.org/10.3945/jn.112.162362 PMid:23014493 PMCid:PMC3498973

16. Veena SR, Krishnaveni GV, Wills AK, Kurpad AV, Muthayya S, Hill JC, Karat SC, Nagarajaiah KK, Fall CH, Srinivasan K. Association of birthweight and head circumference at birth to cognitive performance in 9-to 10-year-old children in South India: prospective birth cohort study. Pediatr res. 2010; 67(4):424-429. https://doi.org/10.1203/PDR.0b013e3181d00b45 PMid:20032815 PMCid:PMC3073480

17. Rao R, Georgieff MK. Iron in fetal and neonatal nutrition. Semin Fetal Neonatal Med. 2007; 12(1): 54-63. https://doi.org/10.1016/j.siny.2006.10.007 PMid:17157088 PMCid:PMC2048487

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Apr 15; 7(7):1119-1123.
https://doi.org/10.3889/oamjms.2019.223
eISSN: 1857-9655
Clinical Science

Resistance Trend, Antibiotic Utilization and Mortality in Patients with *E. coli* Bacteraemia

Amirreza Najmi[1], Fateme Karimi[1], Vijayanarayan Kunhikatta[1], Muralidhar Varma[2], Sreedharan Nair[1*]

[1]Department of Pharmacy Practice, Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education, Manipal, Karnataka, India; [2]Department of Medicine, Kasturba Medical College, Manipal Academy of Higher Education, Manipal, Karnataka, India

Citation: Najmi A, Karimi F, Kunhikatta V, Varma M, Nair S. Resistance Trend, Antibiotic Utilization and Mortality in Patients with *E. coli* Bacteraemia. Open Access Maced J Med Sci. 2019 Apr 15; 7(7):1119-1123. https://doi.org/10.3889/oamjms.2019.223

Keywords: Resistance pattern; E. coli; ESBL producing; Empirical therapy; Rational antibiotic use; Mortality

***Correspondence:** Sreedharan Nair. Department of Pharmacy Practice, Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education, Manipal, Karnataka. E-mail: nair.sreedhar@manipal.edu

Received: 28-Jan-2019; **Revised:** 18-Mar-2019; **Accepted:** 19-Mar-2019; **Online first:** 14-Apr-2019

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Incidence of bacteraemia and driving concerns about antibiotic resistance is increasing globally. Risk factors for developing antimicrobial resistance are antibiotic overuse, incorrect dosing and extended duration of administration.

AIM: This study was conducted to examine the prescription and susceptibility pattern of antibiotics in bacteraemia patients with ESBL producing and Non-ESBL-producing *E. coli* and their correlation with mortality.

METHODS: Data were collected from medical records of the patients aged 18 years and above, diagnosed with E. coli bacteremia from January 2013 through July 2017. Institutional ethics committee approval was obtained before the study (IEC 483/2017). Cumulative sensitivity/resistance pattern of isolated microorganisms and DDD/100 bed days of prescribed antibiotics were obtained.

RESULTS: 182 cases of *E. coli* bacteraemia were reviewed. 59.9% (n = 109) were male with an age range of 20-90 years. The mortality rate was 24.9% (n = 44). 55.5% (n = 101) of the isolated organisms were ESBL-producing. A high percentage of resistance to cephalosporins and fluoroquinolones were observed among the patients, and most of the identified isolates were sensitive to the aminoglycosides, carbapenems and β-lactam and β-lactamase inhibitor combinations (BLBLIs).

CONCLUSIONS: Frequent utilisation of the high-end antibiotics and increase in microorganism's resistance to different antibiotics can lead to a worrisome level. Local antibiotic resistance data and consumption policy are essential to prevent and slow down this process. We observed a descending resistance trend for amoxicillin-clavulanic acid combination in our setting to both the ESBL producing and non-producing.

Introduction

Antibiotic resistance can be considered as one of the most critical public health issues in the world [1]. There are different risk factors for developing antimicrobial resistance. Antibiotic overuse, incorrect dosing and extended duration of administration can be among the most important ones [1], [2], [3], [4]. We observe a more challenging situation in less developed countries. In several studies, the inappropriate antimicrobial prescription was observed among the primary care physicians [5], [6], [7], [8] and development of bacterial resistance increases the challenges faced by the physicians to treat the patients [9], [10], [11].

Pathogenic *Escherichia coli* (*E. coli*) can cause serious diseases, such as urinary tract infections(UTIs) and bacteraemia [12], [13], [14], and incidence of bacteraemia increasing globally [15]. Bacteria which can usually develop the more serious disease is ESBL (Extended-spectrum β-lactamases) producing *E. coli*. ESBLs are enzymes that can hydrolyse penicillins, aztreonam and cephalosporin's [16]. These ESBL producing bacteria showed lesser acceptable clinical outcome in comparison to susceptible (non-ESBL producing bacteria) bacteria [17], [18], [19], [21], which can be related to delay in appropriate antimicrobial treatment to some extent

[22], [23], [24]. The high rate of clinical successes was observed in existing literature with carbapenems administration for ESBL producing bacteria [25], [26], [27], [28]. On the other hand, the use of Cephalosporins [29] and β-lactam–β-lactamase inhibitor combinations [28], [30] have also been suggested recently with ESBL-positive isolates if MICs are below clinical breakpoints. Moderate to high in vitro activity of piperacillin-tazobactam (PTZ) against ESBLs is suggested by several studies [31], [32], [33]. Equivalence between PTZ and carbapenems in the treatment of ESBL infection was demonstrated in some studies [17].

This study was conducted to examine the prescription and susceptibility pattern of antibiotics in bacteraemia patients with ESBL producing and Non-ESBL-producing *E. coli* and their correlation with mortality. Based on the above background, this study focus on the resistance trend, antibiotic utilisation and mortality in patients with *E. coli* bacteraemia.

Material and Methods

In this cross-sectional study, we reviewed data obtained from 182 patients aged 18 years and above who had experienced bloodstream infection with *E. coli* during five years from Jan 2013 through Dec 2017. The Institutional Ethics Committee approval (IEC 483/2017) was obtained before the start of the study.

Bacteremia was defined as the presence of viable bacteria in the bloodstream [34]. Patients were excluded if the presence of *E. coli* was not confirmed by blood culture testing and through microbiology laboratory report. Antibiotic sensitivity results were obtained from the patient medical record [35].

The culture reports and antibiotic resistance pattern were extracted from online microbiology lab reports database and information on antibiotic prescription from the patient's records. The DDD/100 bed days was calculated for the antibiotics by using the AMC tools software.

The data was analysed by SPSS 20.0 software.

Results

Of the 182 patients who met the inclusion criteria, 59.9% (n = 109) of the patients were male, with age range from 20 to 90 years. The mortality rate was 24.9% (n = 44), and mortality rates trends over time are displayed in Figure 1.

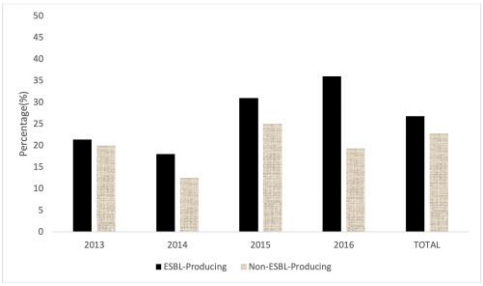


Figure 1: Mortality rate in patients with different types of E.coli isolates over time

The isolated organisms were 55.5% (n = 101) ESBL-producing *E. coli,* and the rest of the organisms were at least sensitive to 4th generation cephalosporins.

Antibiotic sensitivities among the total patient population, viz patients with ESBL producing *E. coli*, patients with non-ESBL producing *E. coli* represented in Table 1.

Table 1: Resistance pattern of different E. coli isolates

Antibiotic name	Non-ESBL producing *E. coli* resistance (%)	ESBL producing *E. coli* resistance (%)	All *E. coli* species resistance (%)
Amikacin	1.3	10.9	6.1
Ampicilin/amoxicillin	77.6	100	90.2
Cefotaxime/ceftriaxone	55.8	100	80.9
Cefuroxime	68.4	100	85.6
Cefazolin/cefadroxil	45.5	100	82.4
Cefpirome/cefepime	2.0	100	68
Ciprofloxacin	55.1	95	77.5
Amoxicillin-clavulanic-acid	24.7	93.1	55.6
Trimethoprim-sulphametoxazole	41.6	54.5	48.9
Gentamicin	21.8	50.5	38
Netilmicin	0.0	18.2	12.1
Imipenem	4.3	6.9	6.1
Piperacillin-tazobactam	8.5	29.7	23
Cefoperazone-sulbactam	2.1	22.8	16.2
Colistin	0	0	0

Among the patients with non-ESBL producing *E. coli*, 45 (55.8%) had an organism resistant to cefotaxime. Sensitivity to the colistin, imipenem, amikacin and netilmicin were observed in most of the ESBL enzyme producing as well as non-producing *E. coli* species. Utilisation pattern of antibiotics in terms of DDD/100 bed-days is depicted in Figure 2.

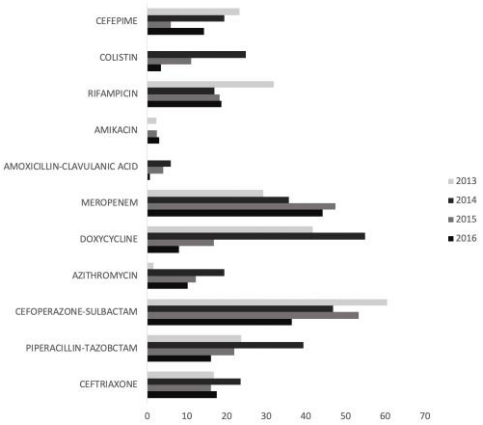


Figure 2: Antibiotic utilisation pattern of patients with E. coli bloodstream infections over time

A group of 40.7% (n = 74) were treated with a cefoperazone-sulbactam combination, and 36.8% (n = 67) were treated with a piperacillin-tazobactam combination and were the most frequently administered antibiotics.

Sixty-two patients (34.1%) received ceftriaxone as empirical therapy. A group of 83.9% (n = 52) of these patients stopped receiving ceftriaxone before the full course of the antibiotic was completed and 35.5% (n = 22) of these patients received a single dose of ceftriaxone after their admission to the hospital. The trend in the change of antibiotic resistance is shown in Figure 3.

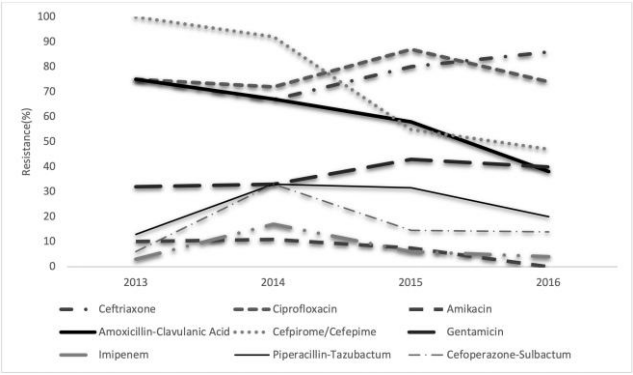


Figure 3: Resistant trends of E. coli isolates to different antibiotics over time

Discussion

ESBL enzyme producing species were dominant in this study, while a lower ratio was reported in other studies [36], [37]. Resistance among *E. coli* species to a variety of antibiotics is increasing. A prior study suggested the use of Ceftriaxone as empirical therapy [29], while we observed an increasing trend of resistance to ceftriaxone among all the reviewed *E. coli* isolates. More than one-third of the patients received ceftriaxone as antimicrobial therapy, and most of them received under-dose medication. This could have affected the increment of cephalosporin's resistance, revealing that added antimicrobial administration control policy may help to overcome antimicrobial resistance.

ESBL producing *E. coli* species were 93.1% susceptible to carbapenems/imipenem which is supported by other studies [25], [26], [27], [28]. A group of 78.2% and 71.3 sensitivity was observed to the cefoperazone-sulbactam and piperacillin-tazobactam combinations respectively which is supported by other studies as well [28], [30]. Aminoglycosides were found to be effective against the entire reviewed *E. coli* isolates. Netilmicin can be recommended as a superior antimicrobial over gentamicin based on its similar activity and higher safety.

The most frequently prescribed antibiotics were cefoperazone-sulbactam, meropenem and piperacillin-tazobactam respectively. Comparison of the DDD/100 bed days results with the trend in resistance pattern of antibiotics over time revealed that a greater number of antibiotics administered in a specific year, more resistance was observed in the same or next year. The behaviour of the resistance pattern of organisms to antibiotics such as amoxicillin-clavulanic acid, cefepime-cefoperazone-sulbactam and piperacillin-tazobactam can support this claim.

The mortality rate of the patients with ESBL producing *E. coli* bloodstream infection was higher in comparison with non-ESBL producing *E. coli* infections, and it supports the prior studies [17], [18], [19], [20], [21]. The difference in the mortality rate of the ESBL producing and non-ESBL producing organisms has constantly been increasing from 2013, and this can reflect the increase in the ineffectiveness of antimicrobial therapy on resistant organism over time.

E. coli infection is the most prevalent bloodstream infection in our study population. Most of these patients received 3rd gen. cephalosporins empirical therapy and treatment usually continue with a β-lactam and β-lactamase inhibitor combination or carbapenems after releasing of culture sensitivity reports. Due to the high resistance of these isolates to cephalosporins, more appropriate empirical therapy can be selected. ESBL enzyme producing species are the most serious bacterial infection, and they should be treated according to evidence-based culture reports. The ability to identify patients at risk for resistant organisms has important implications. Therefore, an appropriate empirical therapy according to local resistance pattern database should be selected.

We observed a descending resistance trend for amoxicillin-clavulanic acid combination in our setting to both the ESBL producing and non-producing *E. coli* isolates, and its use as empirical therapy for bloodstream infections by *E. coli* organism. Despite high utilisation of cefoperazone-sulbactam, piperacillin-tazobactam and meropenem in our patients, the organisms still show a reliable sensitivity toward these antibiotics as shown in the figure3, and this can support the recommendation of other studies for their utilisation [28], [30].

After all, we should express that the pathogenic microorganisms are getting more susceptible to the older and rarely prescribed antibiotics. Local constantly updated microorganism's resistant data is essential in every hospital and must be prepared and used in antimicrobial treatment guidelines to improve the empirical therapies.

References

1. The evolving threat of antimicrobial resistance Options for action WHO Library Cataloguing-in-Publication Data.

2. Hecker MT, Aron DC, Patel NP, Lehmann MK, Donskey CJ. Unnecessary Use of Antimicrobials in Hospitalized Patients. Arch Intern Med. 2003; 163(8):972. https://doi.org/10.1001/archinte.163.8.972 PMid:12719208

3. Solomon DH, Van Houten L, Glynn RJ, Baden L, Curtis K, Schrager H, et al. Academic Detailing to Improve Use of Broad-Spectrum Antibiotics at an Academic Medical Center. Arch Intern Med. 2001; 161(15):1897. https://doi.org/10.1001/archinte.161.15.1897 PMid:11493132

4. McGowan JE. Do intensive hospital antibiotic control programs prevent the spread of antibiotic resistance? Infect Control Hosp Epidemiol. 1994; 15(7):478-83. https://doi.org/10.2307/30148498 PMid:7963440

5. Simpson SA, Wood F, Butler CC. General practitioners' perceptions of antimicrobial resistance: a qualitative study. J Antimicrob Chemother. 2006; 59(2):292-6. https://doi.org/10.1093/jac/dkl467 PMid:17110392

6. Kumar S, Little P, Britten N. Why do general practitioners prescribe antibiotics for sore throat? Grounded theory interview study. BMJ. 2003; 326(7381):138. https://doi.org/10.1136/bmj.326.7381.138 PMid:12531847 PMCid:PMC140007

7. Welschen I, Kuyvenhoven MM, Hoes AW, Verheij TJM. Effectiveness of a multiple intervention to reduce antibiotic prescribing for respiratory tract symptoms in primary care: randomised controlled trial. BMJ. 2004; 329(7463):431. https://doi.org/10.1136/bmj.38182.591238.EB PMid:15297305 PMCid:PMC514206

8. Goossens H, Ferech M, Vander Stichele R, Elseviers M. Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. Lancet. 2005; 365(9459):579-87. https://doi.org/10.1016/S0140-6736(05)70799-6

9. uz Zaman T, Aldrees M, Al Johani SM, Alrodayyan M, Aldughashem FA, Balkhy HH. Multi-drug carbapenem-resistant Klebsiella pneumoniae infection carrying the OXA-48 gene and showing variations in outer membrane protein 36 causing an outbreak in a tertiary care hospital in Riyadh, Saudi Arabia. Int J Infect Dis. 2014; 28:186-92. https://doi.org/10.1016/j.ijid.2014.05.021 PMid:25245001

10. Shibl A, Al-Agamy M, Memish Z, Senok A, Khader SA, Assiri A. The emergence of OXA-48- and NDM-1-positive Klebsiella pneumoniae in Riyadh, Saudi Arabia. Int J Infect Dis. 2013; 17(12):e1130-3. https://doi.org/10.1016/j.ijid.2013.06.016 PMid:24021566

11. Elabd FM, Al-Ayed MSZ, Asaad AM, Alsareii SA, Qureshi MA, Musa HA-A. Molecular characterization of oxacillinases among carbapenem-resistant Acinetobacter baumannii nosocomial isolates in a Saudi hospital. J Infect Public Health. 2015; 8(3):242-7. https://doi.org/10.1016/j.jiph.2014.10.002 PMid:25466594

12. Russo TA, Johnson JR. Medical and economic impact of extraintestinal infections due to Escherichia coli: focus on an increasingly important endemic problem. Microbes Infect. 2003; 5(5):449-56. https://doi.org/10.1016/S1286-4579(03)00049-2

13. Ron EZ. Distribution and evolution of virulence factors in septicemic Escherichia coli. Int J Med Microbiol. 2010; 300(6):367-70. https://doi.org/10.1016/j.ijmm.2010.04.009 PMid:20510649

14. Wiles TJ, Kulesus RR, Mulvey MA. Origins and virulence mechanisms of uropathogenic Escherichia coli. Exp Mol Pathol. 2008; 85(1):11-9. https://doi.org/10.1016/j.yexmp.2008.03.007 PMid:18482721 PMCid:PMC2595135

15. de Kraker MEA, Jarlier V, Monen JCM, Heuer OE, van de Sande N, Grundmann H. The changing epidemiology of bacteraemias in Europe: trends from the European Antimicrobial Resistance Surveillance System. Clin Microbiol Infect. 2013; 19(9):860-8. https://doi.org/10.1111/1469-0691.12028 PMid:23039210

16. Frakking FNJ, Rottier WC, Dorigo-Zetsma JW, van Hattem JM, van Hees BC, Kluytmans JAJW, et al. Appropriateness of empirical treatment and outcome in bacteremia caused by extended-spectrum-β-lactamase-producing bacteria. Antimicrob Agents Chemother. 2013; 57(7):3092-9. https://doi.org/10.1128/AAC.01523-12 PMid:23612198 PMCid:PMC3697326

17. Tamma PD, Savard P, Pál T, Sonnevend Á, Perl TM, Milstone AM. An Outbreak of Extended-Spectrum β-Lactamase-Producing Klebsiella pneumoniae in a Neonatal Intensive Care Unit. Infect Control Hosp Epidemiol. 2012; 33(06):631-4. https://doi.org/10.1086/665715 PMid:22561722

18. Menashe G, Borer A, Yagupsky P, Peled N, Gilad J, Fraser D, et al. Clinical significance and impact on mortality of extended-spectrum beta lactamase-producing Enterobacteriaceae isolates in nosocomial bacteremia. Scand J Infect Dis. 2001; 33(3):188-93. https://doi.org/10.1080/00365540151060806 PMid:11303808

19. Endimiani A, Luzzaro F, Brigante G, Perilli M, Lombardi G, Amicosante G, et al. Proteus mirabilis bloodstream infections: risk factors and treatment outcome related to the expression of extended-spectrum beta-lactamases. Antimicrob Agents Chemother. 2005; 49(7):2598-605. https://doi.org/10.1128/AAC.49.7.2598-2605.2005 PMid:15980325 PMCid:PMC1168714

20. Kang C-I, Kim S-H, Park WB, Lee K-D, Kim H-B, Kim E-C, et al. Bloodstream infections due to extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae: risk factors for mortality and treatment outcome, with special emphasis on antimicrobial therapy. Antimicrob Agents Chemother. 2004; 48(12):4574-81. https://doi.org/10.1128/AAC.48.12.4574-4581.2004 PMid:15561828 PMCid:PMC529180

21. Paterson DL, Ko W-C, Gottberg A Von, Mohapatra S, Casellas JM, Goossens H, et al. International Prospective Study of Klebsiella pneumoniae Bacteremia: Implications of Extended-Spectrum β-Lactamase Production in Nosocomial Infections. Ann Intern Med. 2004; 140(1):26. https://doi.org/10.7326/0003-4819-140-1-200401060-00008 PMid:14706969

22. Anderson DJ, Engemann JJ, Harrell LJ, Carmeli Y, Reller LB, Kaye KS. Predictors of mortality in patients with bloodstream infection due to ceftazidime-resistant Klebsiella pneumoniae. Antimicrob Agents Chemother. 2006; 50(5):1715-20. https://doi.org/10.1128/AAC.50.5.1715-1720.2006 PMid:16641440 PMCid:PMC1472233

23. Hyle EP, Lipworth AD, Zaoutis TE, Nachamkin I, Bilker WB, Lautenbach E. Impact of Inadequate Initial Antimicrobial Therapy on Mortality in Infections Due to Extended-Spectrum β-Lactamase-Producing Enterobacteriaceae. Arch Intern Med. 2005; 165(12):1375. https://doi.org/10.1001/archinte.165.12.1375 PMid:15983286

24. Tumbarello M, Viale P, Viscoli C, Trecarichi EM, Tumietto F, Marchese A, et al. Predictors of Mortality in Bloodstream Infections Caused by Klebsiella pneumoniae Carbapenemase-Producing K. pneumoniae: Importance of Combination Therapy. Clin Infect Dis. 2012; 55(7):943-50. https://doi.org/10.1093/cid/cis588 PMid:22752516

25. Vardakas KZ, Tansarli GS, Rafailidis PI, Falagas ME. Carbapenems versus alternative antibiotics for the treatment of bacteraemia due to Enterobacteriaceae producing extended-spectrum -lactamases: a systematic review and meta-analysis. J Antimicrob Chemother. 2012; 67(12):2793-803. https://doi.org/10.1093/jac/dks301 PMid:22915465

26. Hsu AJ, Tamma PD. Treatment of Multidrug-Resistant Gram-Negative Infections in Children. Clin Infect Dis. 2014; 58(10):1439-48. https://doi.org/10.1093/cid/ciu069 PMid:24501388

27. Paterson DL, Ko W-C, Von Gottberg A, Mohapatra S, Casellas JM, Goossens H, et al. Antibiotic Therapy for Klebsiella pneumoniae Bacteremia: Implications of Production of Extended-Spectrum -Lactamases. Clin Infect Dis. 2004; 39(1):31-7. https://doi.org/10.1086/420816 PMid:15206050

28. Perez F, Bonomo RA. Can We Really Use ss-Lactam/ss-Lactam Inhibitor Combinations for the Treatment of Infections Caused by Extended-Spectrum ss-Lactamase-Producing Bacteria? Clin Infect Dis. 2012; 54(2):175-7. https://doi.org/10.1093/cid/cir793 PMid:22057699

29. Leclercq R, Cantón R, Brown DFJ, Giske CG, Heisig P, MacGowan AP, et al. EUCAST expert rules in antimicrobial susceptibility testing. Clin Microbiol Infect. 2013; 19(2):141-60. https://doi.org/10.1111/j.1469-0691.2011.03703.x PMid:22117544

30. Rodriguez-Bano J, Navarro MD, Retamar P, Picon E, Pascual A. -Lactam/ -Lactam Inhibitor Combinations for the Treatment of Bacteremia Due to Extended-Spectrum -Lactamase-Producing Escherichia coli: A Post Hoc Analysis of Prospective Cohorts. Clin Infect Dis. 2012; 54(2):167-74. https://doi.org/10.1093/cid/cir790 PMid:22057701

31. Hoban DJ, Lascols C, Nicolle LE, Badal R, Bouchillon S, Hackel M, et al. Antimicrobial susceptibility of Enterobacteriaceae, including molecular characterization of extended-spectrum beta-lactamase-producing species, in urinary tract isolates from hospitalized patients in North America and Europe: results from the SMART study 2009-2010. Diagn Microbiol Infect Dis. 2012; 74(1):62-7. https://doi.org/10.1016/j.diagmicrobio.2012.05.024 PMid:22763019

32. Chen Y-H, Hsueh P-R, Badal RE, Hawser SP, Hoban DJ, Bouchillon SK, et al. Antimicrobial susceptibility profiles of aerobic and facultative Gram-negative bacilli isolated from patients with intra-abdominal infections in the Asia-Pacific region according to currently established susceptibility interpretive criteria. J Infect. 2011; 62(4):280-91. https://doi.org/10.1016/j.jinf.2011.02.009 PMid:21382411

33. Marchaim D, Sunkara B, Lephart PR, Gudur UM, Bhargava A, Mynatt RP, et al. Extended-Spectrum β-Lactamase Producers Reported as Susceptible to Piperacillin-Tazobactam, Cefepime, and Cefuroxime in the Era of Lowered Breakpoints and No Confirmatory Tests. Infect Control Hosp Epidemiol. 2012; 33(08):853-5. https://doi.org/10.1086/666632 PMid:22759556

34. Wells BG, Di Piro JT, Schwinghammer TL. CVD. Sepsis and Septic Shock. In: Joseph T. DiPiro, editor. Pharmacotherapy Handbook. 9th ed. Newyork: McGraw-Hill Education, 2014:427-33. PMid:25234570 PMCid:PMC4268415

35. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic Susceptibility Testing by a Standardized Single Disk Method. Am J Clin Pathol. 1966; 45(4_ts):493-6.

36. Horner C, Fawley W, Morris K, Parnell P, Denton M, Wilcox M. Escherichia coli bacteraemia: 2 years of prospective regional surveillance (2010-12). J Antimicrob Chemother. 2014; 69(1):91-100. https://doi.org/10.1093/jac/dkt333 PMid:24003184

37. Hristea A, Olaru ID, Adams-Sapper S, Riley LW. Characterization of ESBL-producing Escherichia coli and Klebsiella pneumoniae from bloodstream infections in three hospitals in Bucharest, Romania: a preliminary study. Infect Dis (Auckl). 2015; 47(1):46-51. https://doi.org/10.3109/00365548.2014.959043 PMid:25365029

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Apr 15; 7(7):1124-1128.
https://doi.org/10.3889/oamjms.2019.250
eISSN: 1857-9655
Clinical Science

Predictors of Caesarean Delivery in Preterm Premature Rupture of Membranes

Samantha Mc Kenzie Stancu[1*], Liran Kobi Ash[2], Cynthia Smeding[3], Maisa Abdullah Alwan[3]

[1]Homerton University Hospital, NHS Foundation Trust, London, United Kingdom; [2]Tel-Aviv Sourasky Medical Center, Tel-Aviv, Israel; [3]Medical University of Warsaw, Warsaw, Poland

Citation: Stancu SM, Ash LK, Smeding C, Alwan MA. Predictors of Caesarean Delivery in Preterm Premature Rupture of Membranes. Open Access Maced J Med Sci. 2019 Apr 15; 7(7):1124-1128. https://doi.org/10.3889/oamjms.2019.250

Keywords: Preterm premature rupture of membranes; Caesarean delivery; Predictors; Outcomes

***Correspondence:** Samantha Mc Kenzie Stancu. Homerton University Hospital NHS Foundation Trust, London, United Kingdom. E-mail: samantha.stancu@gmail.com

Received: 10-Feb-2019; **Revised:** 25-Mar-2019; **Accepted:** 26-Mar-2019; **Online first:** 14-Apr-2019

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Preterm premature rupture of membranes (P-PROM) exerts a tremendous influence on pregnancy prognosis. Additionally, it is a major public health concern, as the cause of up to 40% of all preterm births.

AIM: The objective of this study was to identify predictors of Caesarean Delivery in singleton pregnancies complicated by P-PROM.

MATERIALS AND METHODS: This is a retrospective observational study of all consecutive singleton P-PROM deliveries (24-37 weeks) over an 18 months at a tertiary referral centre. Pertinent data was collected comprising demographics, obstetric history, pregnancy-associated pathology and delivery from electronic patient records. Univariate statistical analysis comprised Odds Ratio, 95% Confidence interval and Chi-square test with subsequent *p*-value with statistical significance set at $p < 0.05$.

RESULTS: A total of 240 women delivered singletons following P-PROM over an 18-month period. Maternal age ranged between 12-41 years with an average age of 28 ± 6.27 years. Vaginal delivery (VD) was the predominant mode of delivery, accounting for 52.9% (n = 127) of deliveries. The following parameters were identified as predictors of Caesarean Section (CS) in P-PROM: vaginal infection ($p = 0.04$), previous CS ($p < 0.0001$), primiparity ($p = 0.004$), gravidity > 5 ($p = 0.009$), university education ($p = 0.0006$) and prenatal care ($p < 0.0001$).

CONCLUSION: The advantage of CS over vaginal delivery is expedited delivery of the distressed fetus, while that of vaginal delivery entails avoiding postoperative morbidity. However, large multicentric randomised-controlled studies are needed to elucidate this dilemma definitively.

Introduction

Preterm premature rupture of membranes (P-PROM) exerts a tremendous influence on pregnancy prognosis. This pregnancy complication not only jeopardises maternal and neonatal outcomes, but is a major public health concern due to its association with preterm birth [1], [2], [3], [4]. Although P-PROM only complicates between 2-3% of pregnancies, it is the single most common identifiable cause of preterm birth, responsible for up to 40% of all preterm births [5], [6].

P-PROM is defined as membrane rupture between 24 and 37 + 6 weeks of gestation. The WHO classification of P-PROM encompasses membrane rupture during the pre-viable period (< 24 weeks), the extreme (24-28 weeks), the very early (28-31 weeks), the moderate (32-34 weeks) and the late (35-37 weeks) preterm period. The clinical presentation, severity and management differ according to gestational age [7].

The aetiology of P-PROM is multifactorial, influenced by maternal physiology, environmental factors and genetics. The most prominent risk factors associated with P-PROM comprise a previous history of P-PROM, previous preterm birth, genital infection, cigarette smoking, multiple pregnancy, polyhydramnios, cervical incontinence, antepartum

hemorrhage, invasive prenatal procedures and connective tissue disease [8], [9], [10], [11], [12], [13], [14], [15], [16], [17].

The most intrepid debate regarding the therapeutic conduit of P-PROM continues to spark opposing views concerning the optimum time and mode of delivery. Pregnancy prolongation combats prematurity-associated pathology but increases the risk of chorioamnionitis [18]. Conservative management refers to antibioprophylaxis, tocolysis and fetal pulmonary maturation before 34 weeks [19], [20], [21].

Interventional management refers to amnioinfusion and fibrin glue sealing, both of which are targeted for second-trimester rupture [22], [23]. Induction of labour (IOL) in P-PROM is indicated once the pregnancy reaches 34 weeks, without the necessity of checking for fetal lung maturity [24].

P-PROM carries a 20-fold recurrence risk. As such, the importance of preventing P-PROM should be a common goal for professional Obstetric societies worldwide [25].

The objective of this study was to identify predictors of Caesarean Delivery in singleton pregnancies complicated by P-PROM.

Material and Methods

This is a retrospective observational study of all consecutive singleton P-PROM deliveries (24-37 weeks) over an 18 months at a tertiary referral centre. Electronic patient records were retrieved from the hospital computer system. Pertinent data was collected comprising demographics, obstetric history, pregnancy-associated pathology and delivery.

Statistical analysis encompassed both descriptive and analytical statistics with parametric and non-parametric tests. The descriptive statistical analysis was performed for numerical parameters using Microsoft Excel 2010 functions including mean, median, range (minimum-maximum) and standard deviation. The analytical statistical analysis included univariate tests comprising Chi-square and subsequent *p*-value for parametric variables. Odds Ratio with 95% Confidence Interval was used to evaluate potential risk factors. Statistical significance was set at $p < 0.05$.

This study was carried out according to STROBE guidelines.

No ethical approval was necessary for this study since this analysis consisted of pre-existing computer records, based solely on the routinely collected information, with a cohort represented as a de-identified data set.

Results

In the 18 months between January 2016 and June 2017, a total of 240 women delivered singletons following P-PROM. A total of 239 live births were registered, yielding a 99.6% live birth rate. Maternal age ranged between 12-41 years with an average age of 28 ± 6.27 years. Some two-thirds (n = 153) of women resided in an urban environment. The most common level of education was high school (n = 96, 40%). Sixty-five percent (n = 157) of women had prenatal care since the first trimester.

Gravidity ranged between 1-19 with an average of three. Parity ranged between 1-11, with an average of two. Forty-five percent (n = 108) of women were primiparous while 51.2% (n = 123) were multiparous and 3.8% (n = 9) were grand multiparas. Gestational age ranged between 24-37 weeks with an average of 35.1 ± 2.76 weeks. The highest proportion of preterm neonates were delivered during the late preterm period (n = 170, 70.8%) as demonstrated in Table 1. The most frequent gestational age at admission was 36 weeks, accounting for 35.4% of the cohort.

Table 1: Preterm Delivery According to the WHO Classification

Preterm Delivery Intervals	Number	Percentage (%)
Extremely preterm (24-28 w)	12	5%
Very preterm (29-32 w)	22	9.2%
Moderate preterm (33-35 w)	36	15%
Late preterm (36-37 w)	170	70.8%
Total	240	100%

Presentation to hospital following membrane rupture ranged between 10 minutes and 24 days. The largest proportion of women: 27% (n = 64) presented to the hospital between 61-120 minutes of membrane rupture, as shown in Table 2. Two women (0.8%) were pyrexial upon presentation, which was subsequently diagnosed as chorioamnionitis.

Table 2: Interval Between Membrane Rupture and Hospital Admission

Rupture to Admission Interval	Number	Percentage (%)
10-30 minutes	25	10.4
31-60 minutes	56	23.3
61-120 minutes	64	26.7
2-4 hours	56	23.3
4-6 hours	12	5
6-10 hours	9	3.8
10-16 hours	6	2.5
16-24 hours	3	1.3
>24 hours	9	3.8
Total	240	100%

Gestational diabetes mellitus (GDM) accounted for the sole pregnancy-associated pathology (PAP) present in this cohort (n = 10, 4.2%). Eighteen percent (n = 44) of patients presented with significant anaemia upon admission (below the threshold for physiological dilutional anaemia of pregnancy: Hb < 10.5 g/dL). Excessive weight gain in pregnancy (weight gain > 15 kilograms (kg) accounted for 18% (n = 45) of the study cohort and ranged between 15-33 kg with an average of 19.8 ± 4.09 kg.

Fifteen percent (n = 37) of women were diagnosed with a vaginal infection upon admission. *Candida albicans* was the most common causative microorganism (n = 12, 32.4%), followed by 11 (29.7%) cases of Group B Streptococcus (*S. agalactiae*). Nine (3.8%) patients had a concurrent urinary tract infection (UTI). *Escherichia coli (E. coli)* was the common causative microorganism accounting for two-thirds (n = 6) of uropathogens. Amniotic fluid cultures were taken from 107 women (44.5%), 96 (89.7%) of which were monomicrobial. Again, *Candida albicans* was the most frequent microorganism isolated (n = 35, 32.7%). The most common latency interval was 2-4 hours (n = 38, 15.8%), with a range of: 60 minutes-26 days.

Vaginal delivery (VD) was the predominant mode of delivery accounting for 52.9% (n = 127) of deliveries. Of the 36 women who had a previous Caesarean Section (CS), two (5.5%) opted for a vaginal birth after cesarean (VBAC) (OR: 289, 95% CI: 238.45-2171.66, p < 0.0001). Statistically significant differences were obtained between modes of delivery at weeks 31 and 36 of gestation. Caesarean delivery dominated at 31 weeks, whereas the opposite trend was observed at 36 weeks (Table 3).

Table 3: Gestational Age at Delivery According to Mode of Delivery

Gestational Age	CS (n = 113)	NVD (n = 127)	Total	Odds Ratio	95% CI	*p*-value
24	0 (0%)	2 (1.6%)	2 (0.8%)	0.22	0. 01 – 4.65	0.33
25	1 (0.9%)	1 (0.8%)	2 (0.8%)	1.12	0. 06 – 18.19	0.93
27	2 (1.8%)	3 (2.4%)	5 (2.1%)	0.74	0.12 – 4.53	0.74
28	2 (1.8%)	1 (0.8%)	3 (1.25%)	2.27	0.20 – 25.37	0.50
29	1 (0.9%)	0 (0%)	1 (0.4%)	3.4	0.43 – 84.30	0.45
30	1 (0.9%)	1 (0.8%)	2 (0.8%)	1.12	0. 06 – 18.19	0.93
31	8 (7.1%)	0 (0%)	8 (3.3%)	20.54	1.17 – 360.13	0. 03
32	5 (4.4%)	5 (3.9%)	10 (4.2%)	1.12	0. 31 – 4. 00	0.85
33	2 (1.8%)	3 (2.4%)	5 (2.1%)	0.74	0.12 – 4.53	0.74
34	8 (7.1%)	5 (3.9%)	13 (5.4%)	1.85	0.59 – 5.85	0.28
35	12 (10.6%)	5 (3.9%)	17 (7.08%)	2.89	0.98 – 3.50	0. 05
36	30 (27.4%)	56 (44.1%)	86 (35.8%)	0.45	0.26 -0.79	0. 005
37	41 (36.3%)	45 (35.4%)	86 (35.8%)	1. 03	0.61 – 1.76	0.89
Total	113	127	240	-	-	-

Table 4 illustrates that vaginal infection, gravidity > 5, primiparity, prenatal care and university education are predictors of CS.

Table 4: Predictors of Delivery by Caesarean Section for P-PROM

Characteristic (n = 240)	CS (n = 113)	VD (n = 127)	Odds Ratio	95% CI	p-value
Age > 35	24 (55.9%)	19 (44.1%)	1.53	0.78 – 2.97	0.20
Urban residence	77 (68%)	76 (60%)	1.43	0.84 – 2.44	0.18
Gravidity > 5	10 (8.8%)	27 (21.3%)	0.35	0.16 – 0.70	0. 009
Primiparity	62 (55%)	46 (36%)	2.14	1.27 – 3.59	0. 004
Prenatal care	93 (82%)	64 (50%)	4.57	2.52 – 8.30	< 0.0001
University education	33 (29%)	14 (11%)	3.32	1.67 – 6.62	0. 0006
GDM	8 (80%)	2 (20%)	4.76	0.98 – 22.91	0. 05
Anaemia	22 (50%)	22 (50%)	1.15	0.59 – 2.21	0.66
Excessive weight gain of pregnancy	24 (21%)	21 (17%)	1.36	0.71 – 2.60	0.35
Vaginal infection	23 (20%)	14 (11%)	2. 02	1. 00 – 4.23	0. 04
UTI	4 (44.4%)	5 (66.6%)	0.89	0.23 – 3.41	0.87
+ amniotic fluid culture	51 (47.7%)	56 (52.3%)	1. 04	0.62 – 1.73	0.87
Time to delivery > 120 min	28 (43.7%)	36 (56.3%)	0.82	0.46 – 1.48	0.53
Delivery < 34/40	23 (60.5%)	15 (39.5%)	1.90	0.94 – 3.87	0. 07
Chorioamnionitis	2 (100%)	0 (0%)	1.12	0.15 – 8.12	0.90

Discussion

P-PROM is not only a critical pregnancy complication, but also a public health concern due to its strong association with PTB [26], [27].

There is an ongoing debate regarding the optimum mode and timing of delivery in P-PROM [28], [29], [30], [31], [32], [33]. The optimum time for delivery that guarantees to avoiding both chorioamnionitis and preventing the consequences of prematurity has yet to be identified [30].

The most common indications for an emergency CS include placental abruption, cord prolapse and systemic chorioamnionitis with fetal distress. Although CS is often considered a life-saving procedure for both the mother and the fetus in the setting of chorioamnionitis, an intra-amniotic infection increases the risk of subsequent surgical site infection, endometritis, visceral injury due to tissue friability, thrombotic events and hospital-acquired infections [34].

Our rate of CS: 52.9% (n = 113) is significantly higher than that of 28% in Ibishi et al.'s prospective study examining modes of delivery in P-PROM [33]. Kayiga et al., obtained a similar rate of CS compared to Ibisha et al.,: 30.5% compared to 28% [28]. Pasquier et al., reported the most comparable rate of CS to the present study, even slightly higher at 58.7% [35].

Kayiga et al., did not find a statistically significant difference in perinatal mortality between the two modes of delivery. However, CS was associated with an increased incidence of maternal postpartum infections, death and admission to the special care baby unit. Kayiga et al., concluded that although there was no statistically significant difference in perinatal-neonatal mortality, vaginal delivery is a safer mode of delivery as it carries lower rates of maternal and perinatal morbidity compared to CS [28].

The following parameters were identified as predictors of CS in P-PROM: vaginal infection (p = 0.04), previous CS (p < 0.0001), primiparity (p = 0.004), gravidity > 5 (p = 0.009), university education (p = 0.0006) and prenatal care (p < 0.0001).

VBAC is not routinely offered to women delivering in Romanian public hospitals since they are not staffed with a dedicated obstetric anaesthetist to attend as a matter of urgency in the event of uterine rupture already at the stage of dehiscence to prevent fetal demise.

The incidence of chorioamnionitis was exceedingly low in the present study: 0.8% compared to 4.8% in Kayiga et al., prospective study [28].

From this cohort, 35% (n = 84) of women did not seek prenatal care. The reasons encompassed remote geographical location, low socioeconomic

status, in addition to a low level of education. Only a single patient with university education did not seek prenatal care compared to 82 without university education (OR: 29.01, 95% CI: 3.92 – 214.47, p = 0.001).

The reasons behind high gravidity in this cohort comprise a low level of maternal education, low socioeconomic status, a lack of national educational programme regarding family planning, as well as the absence of a national limit of the number of elective pregnancy terminations.

The limitations of this study comprise its retrospective nature, small sample size, uneven distribution among categories of prematurity and the lack of implementation of IOL into this centre's practice.

In conclusion, P-PROM exerts a tremendous influence on pregnancy prognosis. The objective of this study was to identify predictors of Caesarean Delivery in singleton pregnancies complicated by P-PROM.

The following parameters were identified as predictors of CS in P-PROM: vaginal infection (p = 0.04), previous CS (p < 0.0001), primiparity (p = 0.004), gravidity > 5 (p = 0.009), university education (p = 0.0006) and prenatal care (p < 0.0001).

The advantage of CS over vaginal delivery is expedited delivery of the distressed fetus, while that of vaginal delivery is the avoidance of postoperative morbidity. However, large multicentric randomised-controlled studies are needed to definitively elucidate this dilemma.

References

1. Smith NC, Roberts DE. Preterm Prelabour Rupture of Membranes. RCOG. 2010; 2006(44):1-12.

2. Yücel N, Yücel O, Yekeler H. The relationship between umbilical artery Doppler findings,fetal biophysical score and placental inflammation in cases of premature rupture of membranes. Acta Obs Gynecol Scand. 1997; 76:532-535. https://doi.org/10.3109/00016349709024578

3. Knight M, Kenyon S, Brocklehurst P et al. Saving Lives, Improving Mothers' Care - Lessons learned to inform future maternity care from the UK and Ireland Confidential Enquiries into Maternal Deaths and Morbidity 2009- 12. MBRRACE UK National Perinatal Epidemiology Unit, University of Oxford, 2012:1-120.

4. Abir G, Akdagli S, Butwick A et al. Clinical and microbiological features of maternal sepsis : a retrospective study. Int J Obstet Anesth. 2017; 29:26-33. https://doi.org/10.1016/j.ijoa.2016.09.003 PMid:27793427

5. Harper MA, Byington RP, Espeland MA et al. Pregnancy-related death and health care services. Obstet Gynecol. 2003; 102:273-278. PMid:12907099

6. Bergholt T, Stenderup JK, Vedsted-Jakobsen A et al. Intraoperative surgical complications during cesarean section: an observational study of the incidence and risk factors. Acta Obs Gynecol Scand. 2003; 82(3):251-256. https://doi.org/10.1034/j.1600-0412.2003.00095.x

7. Goldenberg RL, Culhane JF, Iams J et al. Epidemiology and causes of preterm birth. Lancet. 2008; 371:75-84. https://doi.org/10.1016/S0140-6736(08)60074-4

8. Odibo AO, Gray DL, Dicke JM, et al. Revisiting the Fetal Loss Rate After Second-Trimester Genetic Amniocentesis. Obstet Gynecol. 2008; 111(3):589-595. https://doi.org/10.1097/AOG.0b013e318162eb53 PMid:18310360

9. Duff P. Preterm premature (prelabor) rupture of membranes. Uptodate, 2017:1-30. PMid:28595730

10. Mercer BM. Preterm premature rupture of the membranes: current approaches to evaluation and management. Obstet Gynecol Clin North Am. 2005; 32:411-425. https://doi.org/10.1016/j.ogc.2005.03.003 PMid:16125041

11. Mercer BM, Goldenberg RL, Moawad AH et al. The preterm prediction study: effect of gestational age and cause of preterm birth on subsequent obstetric outcome. Natl Inst Child Health Hum Dev Matern Med Units Network Am J Obstet Gynecol. 1999; 181:1216-1221. https://doi.org/10.1016/S0002-9378(99)70111-0

12. Lee T, Carpenter MW, Heber WW et al. Preterm premature rupture of membranes: risks of recurrent complications in the next pregnancy among a population based sample of gravid women. Am J Obstet Gynecol. 2003; (1):188-209. https://doi.org/10.1067/mob.2003.115

13. Ekwo EE, Gosselink CA, Moawad A. Unfavorable outcome in penultimate pregnancy and premature rupture of membranes in successive pregnancy. Obstet Gynecol. 1992; 80:166-172. PMid:1635725

14. Parry S, Strauss JF. Premature rupture of the fetal membranes. N Engl J Med. 1998; 338(10):663-670. https://doi.org/10.1056/NEJM199803053381006 PMid:9486996

15. Williams MA, Mittendorf R, Stubblefield PG et al. Cigarettes, coffee, and preterm premature rupture of the membranes. Am J Epidemiol. 1992; 135(8):895-903. https://doi.org/10.1093/oxfordjournals.aje.a116385 PMid:1585902

16. Kyrklund-Blomberg NB, Cnattingius S. Preterm birth and maternal smoking: risks related to gestational age and onset of delivery. Am J Obstet Gynecol. 1998; 179(4):1051-1055. https://doi.org/10.1016/S0002-9378(98)70214-5

17. Harger JH, Hsing AW, Tuomala RE et al. Risk factors for preterm premature rupture of fetal membranes: a multicenter casecontrol study. Am J Obstet Gynecol. 1990; 163:130-137. https://doi.org/10.1016/S0002-9378(11)90686-3

18. Van Der Ham DP, Vijgen SM, Nijhuis JG et al. Induction of labor versus expectant management in women with preterm prelabor rupture of membranes between 34 and 37 weeks: a randomized controlled trial. PLoS Med. 2012; 9(4):e1001208. https://doi.org/10.1371/journal.pmed.1001208 PMid:22545024 PMCid:PMC3335867

19. Newton ER. Chorioamnionitis and intraamniotic infection. Clin Obstet Gynecol. 1993; 36(4):795-808. https://doi.org/10.1097/00003081-199312000-00004 PMid:8293582

20. Taylor J, Garite TJ. Premature rupture of membranes before fetal viability. Obstet Gynecol. 1984; 64(5):615-620. PMid:6333658

21. Mercer BM. Preterm Premature Rupture of the Membranes. Science. 2003; 101(1):178-193.

22. Lewis DF, Robichaux AG, Jaekle RK et al. Expectant management of preterm premature rupture of membranes and nonvertex presentation: what are the risks? Am J Obstet Gynecol. 2007; 196(6):566-e1-6.

23. Gramellini D, Fieni S, Kaihura C et al. Antepartum amnioinfusion: a review. J Matern Fetal Neonatal Med. 2003; 14:291-296. https://doi.org/10.1080/jmf.14.5.291.296 PMid:14986801

24. Richter J, Henry A, Ryan G et al. Amniopatch procedure after previable iatrogenic rupture of the membranes: a two-center review. Prenat Diagn. 2013; 33(4):391-396.

https://doi.org/10.1002/pd.4080

25. Stafford I, Didly GA Clark SL et al. Visually estimated and calculated blood loss in vaginal cesarean delivery. Am J Obstet Gynecol. 2008; 199(5):519.e1-7. https://doi.org/10.1016/j.ajog.2008.04.049 PMid:18639209

26. Spinnato JA, Shaver DC, Bray EM et al. Preterm premature rupture of the membranes with fetal pulmonary maturity present: a prospective study. Obstet Gynecol. 1987; 69:196-201. https://doi.org/10.1016/0020-7292(87)90096-8

27. Copper RL, Goldenberg RL, Creasy RK. A multicenter study of preterm birth weight and gestational age-specific neonatal mortality. Am J Obstet Gynecol. 1993; 168:78-84. https://doi.org/10.1016/S0002-9378(12)90889-3

28. Kayiga H, Lester F, Amuge PM et al. Impact of mode of delivery on pregnancy outcomes in women with preterm premature rupture of membranes after 28 weeks of gestation in a low-resource setting: A prospective cohort study. PLoS One. 2018. https://doi.org/10.1371/journal.pone.0190388 PMid:29320516 PMCid:PMC5761877

29. Chakraborty B, Mandal T, and Chakraborty S. Outcome of prelabor rupture of membranes in a tertiary care center in west Bengal. Indian Journal of Clinical Practice. 2013; 24(7).

30. Mousiolis A, Papantoniou N, Mesogitis S et al. Optimum mode of delivery in gestations complicated by preterm premature rupture of the membranes. J Maternal Fetal Neonatal Med. 2012; 25(7):1044-1049. https://doi.org/10.3109/14767058.2011.614659 PMid:21854136

31. Tahir S, Aleem M, and Aziz R. Incidence & outcome of preterm-premature rupture of membranes. Pakistan Journal of Medical Sciences. 2002; 18(1):26-32.

32. Eleje GU, Ezebialu IU, Umeobika JC et al. Pre-labour rupture of membranes at term: a review of management in a health care institution. Afrimedic Journal 2010; 1(2):10-14.

33. Ibishi VA, Isjanovska RD. Prelabour Rupture of Membranes: Mode of Delivery and Outcome. Open Access Maced J Med Sci. 2015; 3(2):237-240. https://doi.org/10.3889/oamjms.2015.037 PMid:27275227 PMCid:PMC4877859

34. Robinson JN, Norwitz E. Preterm birth: Risk factors and interventions for risk reduction. UpToDate. 2017.

35. Pasquier JC, Rabilloud M, Picaud JC et al. A prospective population-based study of 598 cases of PPROM between 24 and 34 weeks' gestation: description, management, and mortality (DOMINOS cohort). European Journal of Obstetrics & Gynecology and Reproductive Biology. 2005; 121(2):164-170. https://doi.org/10.1016/j.ejogrb.2004.12.015 PMid:16054957

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Apr 15; 7(7):1129-1132.
https://doi.org/10.3889/oamjms.2019.239
eISSN: 1857-9655
Clinical Science

The Effect of Supplementation of Calcium on Prevention of Pre - Eclampsia in Pregnant Women at Kuta Baro Community Health Center Aceh Besar, Indonesia

Anita Anita*, Nurlaili Ramli

Department of Midwifery, Polytechnic of Health-Ministry of Health, Aceh, Indonesia

Citation: Anita A, Ramli N. The Effect of Supplementation of Calcium on Prevention of Pre - Eclampsia in Pregnant Women at Kuta Baro Community Health Center Aceh Besar, Indonesia. Open Access Maced J Med Sci. 2019 Apr 15; 7(7):1129-1132. https://doi.org/10.3889/oamjms.2019.239

Keywords: Supplementation of Calcium; Pre-eclampsia; Pregnant Women; Community Health Center

***Correspondence:** Anita Anita. Department of Midwifery, Polytechnic of Health-Ministry of Health, Aceh, Indonesia. E-mail: anita@poltekkesaceh.ac.id

Received: 09-Feb-2019; **Revised:** 21-Mar-2019; **Accepted:** 22-Mar-2019; **Online first:** 14-Apr-2019

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Pre-eclampsia is a major cause of maternal death in Indonesia. Prevention of the incidence of pre-eclampsia is believed as one of the optional treatment that can be done by consuming calcium supplementation

AIM: This study aimed to investigate procalcitonin levels in non-small cell lung cancer patients.

METHODS: Observational study with prospective cohort design with a quantitative approach was conducted to see the validity and re ability of the question. The population was pregnant women in Kutabaro Health Center in 2018 who were pregnant women > 20 weeks from the appropriate inclusion and exclusion criteria of a total of 29 respondents. The intervention group who were given calcium and about 58 pregnant mothers were assigned for the controlled group. The sampling technique was the Multistage sampling method/ sampling, using purposive sampling and Total Population. Analysis of data using uni-variable, bi-variable and multiple variables. The statistical test used Chi-Square (χ^2) at the significance level of $p < 0.05$. To see the magnitude of the risk with the Relative Risk (RR) with a 95% confidence interval (CI).

RESULTS: A total of 68 lung cancer patients fulfilled the criteria of this study, 55 men (80.9%) and 13 women (19.1%). The highest percentage of cytology/histopathology type found was adenocarcinoma (80.9%), and 60.3% of those were diagnosed in stage IV. An increased procalcitonin level (greater than 0.01 ng/mL) occurred in 80.9% of Non-Small Cell Lung Cancer (NSCLC) patients. It appears that the higher the stage of lung cancer, the lower procalcitonin levels would be, although it was not statistically significant. There was no association between lung cancer subtype with procalcitonin levels.

CONCLUSION: An increased level of procalcitonin may be an indication not only for infection but also for NonSmall Cell Lung Cancer.

Introduction

One of-of the Millennium Development Goals (MDGs) is to improve the health of pregnant women by reducing maternal mortality (RMM). Indonesian MDGs is reported the still high maternal mortality rate. The maternal mortality rate in Indonesia is still high at 359 per 100 000 live births [1]. The maternal mortality is dominated by three main causes, namely hypertension in pregnancy, bleeding and infection. Hypertension in pregnancy has increased in proportion, from 20% in 2007 to 30% in 2012 [2]. Hypertension in pregnancy (HDK) including pre-eclampsia is a major cause of maternal mortality worldwide [3]. Prevention of the incidence of pre-eclampsia can be done with calcium supplementation. Calcium supplementation can reduce the risk of pre-eclampsia and prevent preterm birth [4].

The World Health Organization (WHO) has recommended calcium supplementation 1500-2000 mg/day in populations with low calcium intake as part of antenatal care (ANC) as prevention of pre-eclampsia in pregnant women, especially in pregnant women who have a high risk of hypertension [5]. Some regions in Indonesia provide calcium supplements to pregnant women when conducting pregnancy examinations to health centres. The implementation of calcium supplementation in the community health centre is not always the same in

terms of the time and amount of calcium supplements. Some research results in developing countries generally have low calcium intake. Research in Cameroon showed that as many as 94.6% of pregnant women had inadequate calcium intake, the average intake of pregnant women only fulfilled the recommended 62.3% calcium nutrient adequacy rate (RDA) [6].

Research in Thailand showed that as many as 55% of pregnant women had inadequate calcium intake with an average calcium intake of 493.2 mg/day, equivalent to 61.7% of the calcium RDA of Thai people [7]. Research in Peru shows the prevalence of pregnant women who have an inadequate calcium intake of 86% [8]. Research in Indonesia after the monetary crisis showed that the average calcium intake of pregnant women was 360 ± 140 mg/day and 96% of pregnant women had inadequate calcium intake [9]. Calcium intake in pregnant women in developing countries is generally very low due to diet [10]. In contrast to developed countries which generally have high calcium intake due to the production and consumption of dairy products [11]. Some regions in Indonesia have calcium supplementation programs for pregnant women, one of which is the large Aceh District. The implementation of calcium supplementation in health centres in large Aceh is not always the same in terms of the time and amount of calcium supplements. Prevention of the incidence of pre-eclampsia can be done by giving calcium supplements by doctors, and midwives, especially in rural areas. The results of observations and data from the Aceh Besar Health Service showed the highest cases of pregnant women who experienced pre-eclampsia in the Kutabaro community health centre. In 2018 at the Pukesmas Kutabaro District of Aceh, there were 29 cases of pregnant women who had pre-eclampsia, 2 of whom were cases of eclampsia. The case of pregnant women who experience pre-eclampsia is increasing in the following year.

Pre-eclampsia events contribute to maternal deaths. Data from the Aceh Provincial Health Office in 2018 showed that the maternal mortality rate (MMR) in Aceh Province was 135/100,000 births [12]. Giving calcium is an effort to support the prevention of pre-eclampsia and prevent maternal mortality. Based on the background of the problem above, this study aims to determine the effect of Supplement calcium to prevent Pre-eclampsia in pregnant women at the Kuta Baro Health Center, Aceh Besar.

Material and Methods

To get the data for this research, it used the observational technique with a quantitative approach and a prospective cohort research design. It was conducted from July to November 2018 at the Kuta Baro Community Health Center. The population in this study were pregnant women who had a gestational age of more than 25 weeks, totalling 60 participants and 30 were assigned as the intervention group and 30 others as the controlled group. The local midwives/village officials who monitored calcium consumption in pregnant women are 30 participants. The population in this study were pregnant women who were at the Kuta Baro Health Center in 2018. The samples in this study were pregnant women with gestational age > 20 weeks, according to inclusion and exclusion criteria.

The number of samples in this study was 30 respondents for the group given calcium with a dose of 1000 mg and 30 respondents who were given calcium at a dose of 500 mg (ratio 1:1) — data analysis using chi-square statistical approach to the analysis of bivariate and multivariate regression analysis.

Results

Uni-variate Analysis

Table 1 shows the average calcium in the blood before intervention amounted to 9.1 with the lowest level 8 and the highest 10.2 while the average calcium in the blood after the intervention was 9.9 with the lowest grade value of 8.8 and the highest 13.5. 100% of pregnant women did not experience the occurrence of pre-eclampsia at the Kuta Baro Health Center, Aceh Besar.

Table 1: Characteristics of Respondents in the study of the Effect of Giving Calcium Supplements by local midwives on calcium levels to prevent pre-eclampsia in pregnant women

Variable		N mean ± SD	% Min-max
Calcium levels in blood	Before	9.1 ± 0.7	8 - 10.2
	After	9.9 ± 0.8	8.8 - 13.5
Pre-eclampsia	Yes	0	0
	Not	60	100
Education	High	34	56.7
	Low	26	43.3
Parity	At risk	25	41.7
	Not Risky	35	58.3
Mother's age	At risk	13	21.7
	Not Risky	47	78.3
Points antenatal care (ANC)	Midwife	56	93.3
	Hospital	4	6.7
Blood pressure	Sistole	116.0 ± 8.3	88 -138
	Diastole	73.3 ± 7.9	50 - 90
Frequency of *antenatal care* (ANC)		2.3 ± 0.5	1 - 3

Bi-variate Analysis

Data analysis is to see differences in the average effect of giving calcium supplements by midwives on calcium levels for the prevention of pre-eclampsia in pregnant women using a paired t-test. The effect of external variables on calcium levels in the blood of pregnant women using the independent t-test.

Table 2: Paired t-test Effect of Calcium Supplement on calcium levels for the prevention of pre-eclampsia in pregnant women between the intervention and control groups

Variable	Calcium		Difference	t	p
	Before	After			
	mean ± SD	mean ± SD			
Group Intervention	8.9 ± 0.7	9.8 ± 0.8	0.8	5.6	0.0001
Control	9.3 ± 0.6	10.0 ± 0.8	0.7	4.3	0.0002

The results of the analysis using a paired t-test showed that there was a significant change in the average blood calcium levels before and after intervention in the intervention group with a p-value of 0,0001. In the control group, there was a significant difference in the level of calcium in the blood before and after the intervention (p = 0,0002).

Table 3: Results of independent t-test variables outside the effect of giving calcium supplements by midwives on calcium levels to prevent pre-eclampsia in pregnant women

Variable		n	Calcium	Difference	T	p
			mean ± SD			
Group	Intervention	30	0.8 ± 0.84	0.1	0.41	0.68
	Control	30	0.7 ± 0.9			
Education	High	34	0.9 ± 1.1	0.2	1.0	0.32
	Low	26	0.7 ± 0.6			
Parity	At risk	25	0.9 ± 1.1	0.1	0.6	0.57
	Not risky	35	0.8 ± 0.8			
Mother's age	At risk	47	0.9 ± 1.0	1.3	1.1	0.29
	Not risky	13	0.6 ± 0.9			
ANC Frequency	> 2	19	0.9 ± 1.1	0.3	1.1	0.29
	≤ 2	41	0.7 ± 0.8			

Table 3 shows there is no difference in the average calcium in blood levels in the external variables for both The results of the study using paired t-test showed that there were differences in the average calcium levels before and after calcium supplementation in the intervention group and the controlled group ($p \leq 0.05$). The results of the study also showed that 100% of the study sample did not experience pre-eclampsia. The difference between groups of pregnant women who received calcium supplements amounted to 1000 mg with 500 mg which was equal to 0.1 mg and statistically the value of $p \geq 0.05$. The results showed an increase in blood calcium levels after calcium was given in the intervention group and the control group.

However, there was no difference in the increase in calcium levels in the blood of pregnant women between mothers who received 500 mg and 1000 mg calcium in the intervention and control groups. The results also showed that there was no relationship between the characteristics of participants with an increase in calcium levels in the mother's blood.

Discussion

The results of the meta-analysis show that calcium supplementation during pregnancy effectively reduces the occurrence of pre-eclampsia [13], [15].

Pregnant and breastfeeding women need more calcium than non-pregnant women. Calcium in pregnant women can support the formation of bones and teeth and fetal joints. Calcium deficiency in pregnant women can cause calcium requirements taken from calcium reserves in the mother's bones; this has the potential to cause osteoporosis in the mother. Giving calcium by midwives to pregnant women adequately is strongly recommended because it can prevent the occurrence of pre-eclampsia [14]. The results of the study conducted from a cohort study that there are several factors that can cause pre-eclampsia, namely diabetes, multiple pregnancy, nulliparous, family history of eclampsia, high body mass index and maternal age more than 40 years [15].

The results of the study showed that mothers at risk did not cause pre-eclampsia in the mother. The results of this study are different from the research conducted by Astuti, that the age of more than 30 years is at risk of experiencing pre-eclampsia, as well as mothers with a history of pre-eclampsia who have a 3 times greater risk of experiencing severe eclampsia compared with mothers who do not have a pre-eclampsia history 16]. Some studies suggest calcium supplements for pregnant women to prevent pre-eclampsia, especially in the second and third trimester of pregnancy, especially in low social-economic areas [17]. Calcium deficiency can increase the risk of pre-eclampsia, especially in young women. Calcium supplements can reduce the incidence of pre-eclampsia, although the effect of consumption of calcium supplements depends on the age and social-economic status of the mother, especially in areas with low calcium consumption geography [18].

In conclusion, there was a significant change in the average blood calcium levels before and after calcium supplements were given in the intervention and control groups ($p < 0.05$). Supplementation Calcium in Pregnant Women at Kuta Baro Community Health Center can prevent the occurrence of pre-eclampsia.

References

1. [BPS] Badan Pusat Statistik. Survei demografi dan kesehatan Indonesia [laporan]. Jakarta (ID): Badan Pusat Statistik, 2012.

2. Kementerian Kesehatan Republik Indonesia. Profil Kesehatan Indonesia Tahun 2013. Jakarta: Kementerian Kesehatan Republik Indonesia, 2014.

3. Say L, Chou D, Gemmill A, Tunçalp Ö, Moller AB, Daniels J, Gülmezoglu AM, Temmerman M, Alkema L. Global causes of maternal death: A WHO systematic analysis. Lancet Glob Health. 2014; 2(6):e323-33. https://doi.org/10.1016/S2214-109X(14)70227-X

4. Camargo EB, Moraes LF, Souza CM, Akutsu R, Barreto JM, da

Silva EM, et al. Survey of Calcium Supplementation to Prevent Preeklampsia: The Gap between Evidence and Practice in Brazil. BMC Pregnancy and Childbirth. 2013; 206(13):1-7.

5. WHO. World Health Statistics 2013. Geneva: World Health Organization, 2013.

6. Agueh VD, Tugoué MF, Sossa C, Métonnou C, Azandjemè C, Paraiso NM, Ouendo ME, Ouédraogo LT, Makoutodé M. Dietary calcium intake and associated factors among pregnant women in southern Benin in 2014. Food and Nutrition Sciences. 2015; 6(11):945-954. https://doi.org/10.4236/fns.2015.611098

7. Sukchan P, Liabsuetrakul T, Chongsuvivatwong V, Songwathana P, Sornsrivichai V, Kuning M. Inadequacy of nutrients intake among pregnant women in the Deep South of Thailand. BMC Public Health. 2010; 10(572):1-8. https://doi.org/10.1186/1471-2458-10-572

8. Sacco LM, Caulfield LE, Zavaleta N, Retamozo L. Dietary pattern and usual nutrient intakes of Peruvian women during pregnancy. European Journal of Clinical Nutrition. 2003; 57(11):1492-1497. https://doi.org/10.1038/sj.ejcn.1601716 PMid:14576764

9. Hartini TN, Winkvist A, Lindholm L, Stenlund H, Persson V, Nurdiati DS, Surjono A. Nutrient Intake and Iron Status of Urban Poor and Rural Poor without Access to Rice Fields Are Affected by the Emerging Economic Crisis: The Case of Pregnant Indonesian Women. European Journal of Clinical Nutrition. 2003; 57(5):654-666. https://doi.org/10.1038/sj.ejcn.1601595 PMid:12771966

10. Cheng Y, Dibley MJ, Zhang X, Zeng L, Yan H. Assessment of Dietary Intake among Pregnant Women in a Rural Area of Western CHINA. BMC Public Health. 2009; 9(222):1-9. https://doi.org/10.1186/1471-2458-9-222

11. Harville E, Schramm M, Watt-Morse M, Chantala K, Anderson J, Hertz-Picciotto I. Calcium Intake during Pregnancy among White and African-American Pregnant Women in the United States. The Journal of the American College of Nutrition. 2004; 23(1): 43-50. https://doi.org/10.1080/07315724.2004.10719341 PMid:14963052

12. Dinkes Aceh. Profil Kesehatan Aceh 2018. Banda Aceh. Dinkes Aceh, 2018.

13. Hofmeyr Y, Dully L, Ataalah A. Dietary calcium supplementation for prevention of pre-eclampsia and related problem: a systemic review and commentary. BJOG. 2007; 114(8):933-43. https://doi.org/10.1111/j.1471-0528.2007.01389.x PMid:17565614

14. Sukonppong, Phopong. Serum calcium and serum magnesium in normal and preeclampsia pregnancy. Arch Gynecol Obst. 2007; 214(1):12-16.

15. Agus, P, Jhon MF. Metabolisme kalsium, Buku Ajar ilmu penyakit ; jilid III edisi V : Balai penerbit FKUI, 2009.

16. Astuti, SL. Analisis faktor resiko terjadinya preeklampsia berat pada ibu hamil trimester 3. Jurnal Terpadu Ilmu Kesehatan. 2013; 2(2):41-155.

17. Bennett, Zuzannah, et al. Association of ontario midwives, Hypertensive disordes of pregnancy, clinical practice guideline No.15. Toronto, Kanada, 2012. : http://www.aom.on.ca/Health_Care_Professionals/Clinical_Practice_Guidelines/.2012;

18. Punthumapol C, Kittichotpanich B. Serum calcium, magnesium and uric acid in preeclampsia and normal pregnancy. J Med Assoc Thai. 2008; 91(7):968. PMid:18839833

19. Mohieldein AH, Dokem AA, Osman YH, Idris HM. Serum calcium level as a marker of pregnancy-induced hypertension. Sudan Journal of Medical Sciences. 2007; 2(4):245-8.

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Apr 15; 7(7):1133-1137.
https://doi.org/10.3889/oamjms.2019.248
eISSN: 1857-9655
Clinical Science

Arthroscopic Assisted Reduction and Internal Fixation of Tibial Plateau Fractures

Sherif Hamdy Mohamed Zawam*, Ahmed Mahmoud Gad

Faculty of Medicine, Cairo University, Cairo, Egypt

Citation: Zawam SHM, Gad AM. Arthroscopic Assisted Reduction and Internal Fixation of Tibial Plateau Fractures. Open Access Maced J Med Sci. 2019 Apr 15; 7(7):1133-1137. https://doi.org/10.3889/oamjms.2019.248

Keywords: Arthroscopic assisted; Tibial plateau fractures; ORIF; Schatzker

***Correspondence:** Sherif Hamdy Mohamed Zawam. Faculty of Medicine, Cairo University, Cairo, Egypt. E-mail: herifzawam@gmail.com

Received: 07-Feb-2019; **Revised:** 25-Mar-2019; **Accepted:** 26-Mar-2019; **Online first:** 14-Apr-2019

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Tibial plateau fractures present an important entity in orthopaedic fractures. Arthroscopic-assisted reduction and internal fixation is a good alternative to ORIF as it has the advantage of direct visualisation of the articular surface of the plateau, direct assessment of the reduction of the articular surface, and managing any associated intra-articular pathology.

AIM: Our study aim is to determine the results of arthroscopic assisted reduction and internal fixation of tibial plateau fractures.

METHODS: This study involved 25 patients with tibial plateau fractures presenting to the emergency department of Cairo University Hospitals between the periods of November 2016 and May 2017. The patients were followed up for an average of 14 months (11-18 months). According to Schatzker's classification, five patients had type I, eleven had type II, and nine patients had type III fractures.

RESULTS: The average time to full union in Schatzker type I was 9.1 weeks, in type II was 10.2 weeks, and in type III it was 9.4. The mean clinical Rasmussen score among the 25 patients was 26 (range, 24-30). A group of 19 patients (76%) had excellent results, (4 type I, 8 types II, and 7 types III) 6 patients (24 %) had good results (1 type I, 3 types II, 2 types III). Radiologic results were excellent in (14 cases) 56.0% and good results (11 cases) 44%.

CONCLUSION: Arthroscopic assisted reduction and fixation of tibial plateau fractures have the advantages of checking the adequacy of reduction, avoiding the need for detachment of the meniscus, and allowing for accurate diagnosis and management of associated knee injuries. Therefore, we recommend that arthroscopic assisted reduction and fixation of tibial plateau fractures should be used more often.

Introduction

Tibial plateau fractures present an important entity in orthopaedic fractures. They may be associated with other knee injuries such as meniscal tears, cruciate ligaments injuries and collateral ligaments damage [1], [2].

The role of conservative treatment is limited in the management of fractures of the tibial plateau. Open reduction and internal fixation (ORIF) is the standard treatment option. Several complications may arise from ORIF, such as wound complications and infection [3].

Arthroscopic-assisted reduction, and internal fixation is a good alternative to ORIF as it has the advantage of direct visualisation of the articular surface of the plateau, direct assessment of the reduction of the articular surface, and managing any associated intra-articular pathology. Several studies have adopted arthroscopic assisted reduction of tibial plateau fractures with good results [4].

Our study aim is to determine the results of arthroscopic assisted reduction and internal fixation of tibial plateau fractures.

Methods

This study involved 25 patients with tibial plateau fractures presenting to Kasr El Ainy Hospitals between the period of November 2016 and May 2017. The patients were followed up for an average of 14 months (11-18 months). Twelve patients sustained

their injuries from road traffic accidents (RTA), eight patients fell from a height, and five patients had sports injuries.

There were 16 males (64%) and 9 females (36%). The age ranged from 19 to 55 years with a mean age of 38.8 years. According to Schatzker's classification, five patients had type I, eleven had type II, and nine patients had type III fractures [5].

Full medical history was obtained from the patients. Then, a detailed physical examination was performed to assess the local skin condition and the neurovascular status of the affected limb. Plain x-rays and CT scan were ordered to assess the type of the fracture, the degree of depression, joint widening, and plan the way of management. Also, the patients were checked for any associated fractures. Operative indications included step-off of the articular surface more than 2-3 mm, widening of the tibial condyles more than 5 mm or varus/valgus instability more than 10 degrees [6].

Patients with open physis, open or pathological fractures were excluded. The patients were put in a back slab initially to stabilise the fracture site and allow the soft tissues to heal. The patients were admitted and given "low molecular weight heparin" to protect them against deep venous thrombosis. When oedema and the skin condition improve, the patients were prepared for surgery.

The procedure was performed under spinal anaesthesia putting the patients in supine position. Pre-operatively, the prophylactic antibiotic was given before tourniquet inflation. Knee ligaments were examined under anaesthesia. The standard anterolateral portal was used for viewing, and the anteromedial portal was used for manipulation. Evacuation of the organised intra-articular hematoma was done first to improve the visibility (Figure 1).

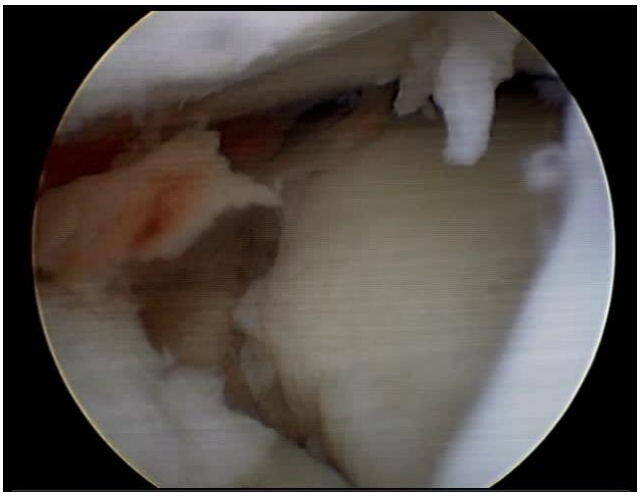


Figure 1: The shape of the fracture before reduction (after removal of the hematoma)

Then, an examination of the intraarticular structures was performed. A "figure of 4 positions" was used during examination and reduction of lateral plateau fractures as by this we open the joint space and improve the visualisation.

Prolene suture loops or retractors were used to retract the lateral meniscus to expose the lateral tibial plateau and the fracture site. This avoids the detachment of the meniscus that occurs with an open approach.

In Schatzker Type I Fractures, K-wires (Kirschner wires) were used to manipulate the split fragment to be used as joy-stick for reduction. When the fracture was reduced, K-wires for cannulated screws were used to temporarily fix the fracture, until the cannulated screws were inserted. Any other associated pathology was dealt with accordingly.

The reduction of Schatzker Type II Fractures was more difficult than Type I or typed III, as the fracture pattern consists of depressed parts and split parts, especially if the fracture was comminuted.

First, the fracture pattern was studied well using the arthroscopy to identify the number of fragments, the degree of depression, split and depressed parts. Then A.C.L guide was used over the depressed part to pass a guide wire from the medial or lateral side of the knee.

The position of the wire was checked using the arthroscope and the image intensifier. After that, a window was created around the K-wire using the mosaicplasty set (Figure 2).

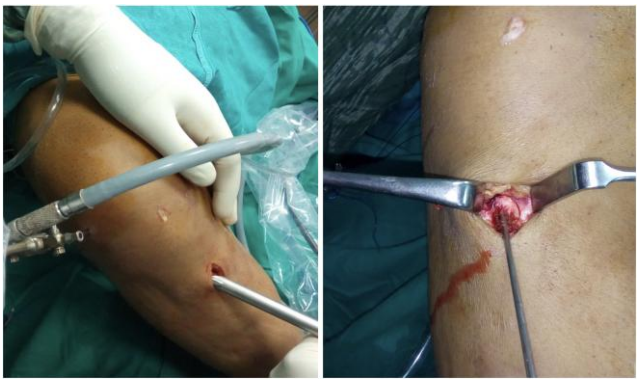


Figure 2: the window was created around the K-wire using the mosaicplasty set

Then, using an impactor, and through the created cortical window, the depressed parts were gently elevated until they reach the level of the surrounding normal plateau. Intra-articular fine-tuning was done to adjust the level of the depressed part. The split parts were manipulated using K-wires.

Then, temporary stabilisation of the fracture was achieved using subchondral K-wires until iliac crest graft was impacted to fill the defects. The accuracy of the reduction was checked both arthroscopically and using the image intensifier (Figure 3).

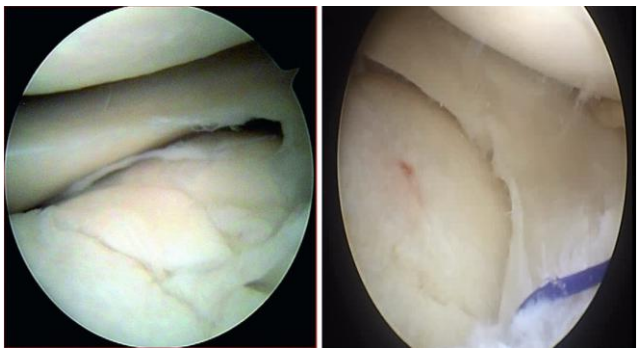


Figure 3: The shape of the articular surface after reduction in 2 patients

Final fixation of the fracture was done in 2 ways. Cannulated screws were used in cases with no comminution or when the fracture pattern was relatively stable after reduction (Figure 4).

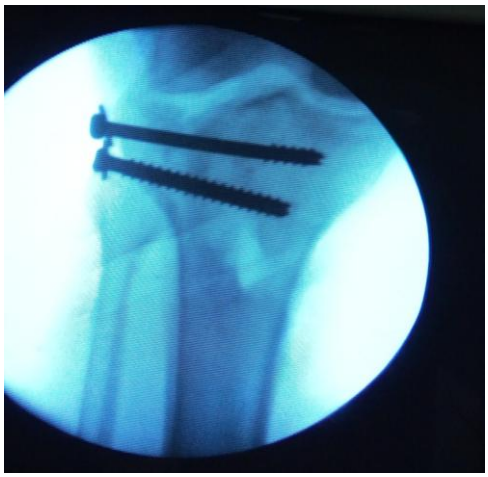


Figure 4: Intraoperative photo from the image intensifier after fracture reduction and fixation by 2 screws

While, in the presence of a comminuted articular surface, or unstable fracture pattern, plate and screws were used for fixation as this added more stability (Figure 5). They were inserted using minimal skin incisions. Iliac crest graft was used in 21 cases.

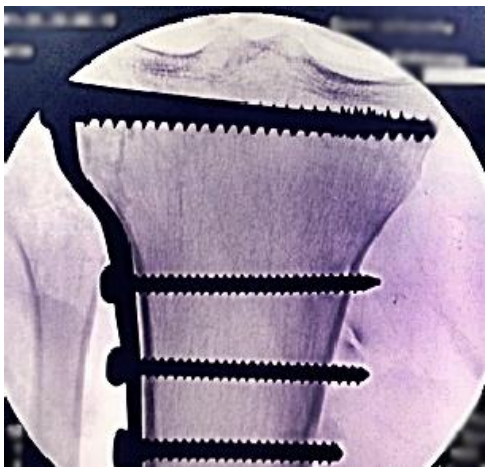


Figure 5: Intraoperative photo from the image intensifier after fracture reduction and fixation by plate and screws

The way of management of Schatzker type III fractures was similar to that of type II in many ways, but it was easier. As in Schatzker type III fractures, there is an only central depression in the lateral plateau. The A.C.L guide was used, the depressed fragment was elevated, iliac crest bone graft was used, and lastly, the fracture was fixed with two cannulated screws with washers.

The patients received postoperative antibiotic prophylaxis for 4 days. Oral anticoagulation was given and continued until partial weight bearing was allowed. The patients were followed up weekly to check the wound until sutures were removed after 2 weeks. Then, the patients were followed up clinically and radiologically in each monthly visit. Partial weight bearing was allowed after an average of eight weeks. Full weight bearing was allowed after the full union was observed radiologically and clinically.

Results

The patients were followed up for an average of 14 months (11-18 months). The average time to full union in Schatzker type I was 9.1 weeks, in type II was 10.2 weeks, and in type III it was 9.4.

This was checked both clinically and radiologically. Clinically, the patient was examined for the absence of tenderness at the fracture site and during weight bearing. Radiologically, the x-rays were checked for the continuity of the cortical bone and absence of defects in the fracture site (Figure 6).

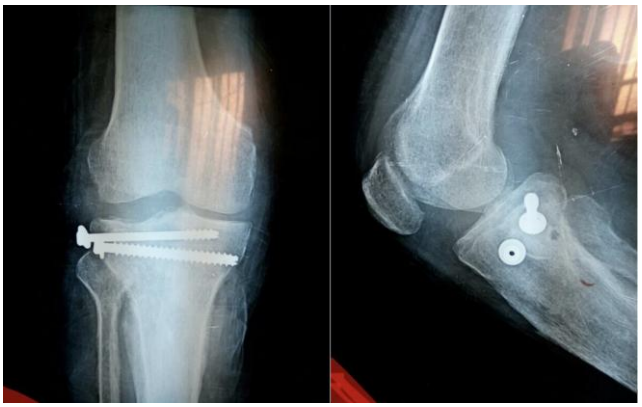


Figure 6: Xrays (anteroposterior and lateral views) after 1 year

The mean clinical Rasmussen score among the 25 patients was 26 (range, 24-30). Nineteen patients (76%) had excellent results, (4 type I, 8 types II, and 7 types III), and 6 patients (24 %) had good results (1 type I, 3 types II ,2 types III). Radiologic results were excellent in (14 cases) 56 % and good in (11 cases) 44%.

Regarding the associated conditions, there

were 8 patients with lateral meniscus lesions, 2 patients with medial meniscus lesions, and 2 patients with partial A.C.L rupture.The torn menisci were sutured using outside in or all-inside techniques, in case of meniscal-capsular separation. In the case of central tears, partial meniscectomy was done. The partial A.C.L injuries were treated conservatively.

Regarding the complications, one patient had a superficial infection, which was treated by antibiotics and repeated dressings. Another case was complicated by articular surface depression (4 mm) due to early weight bearing against the instructions and the patient refused surgical revision.

A group of 20 patients (80%) were satisfied and returned to their previous work before the injury, three reported mild satisfaction, and 2 were dissatisfied.

Discussion

Fractures of the tibial plateau represent 1% of all types of fractures. They should be properly managed. Otherwise, several complications may occur. They may be associated with meniscal tears, cruciate ligaments injuries or collateral ligaments damage [7].

Conservative treatment has a limited role in treating fractures of the tibial plateau. Open reduction and internal fixation is the standard treatment option. Several complications may arise from ORIF, such as wound complications and infection. Ideal management should aim at anatomic reduction and rigid internal fixation. This will allow early mobilisation preventing joint stiffness [8].

Minimally invasive methods include arthroscopic assisted reduction and internal fixation avoiding opening the fracture site, large skin incisions and detachment of the menisci [9].

Fowble et al., performed a retrospective study comparing the results of arthroscopic assisted reduction and fixation to the standard open approach. The group treated with the arthroscopic technique showed superior results compared to the open group [10].

Dall'Oca et al., compared the results of patients treated with arthroscopic assisted reduction and internal fixation to those of patients treated with the open method. The study was conducted on one hundred patients. They were followed up for seventy-three months on average. There were no differences between the results of both groups in patients with Schatzker type I fractures. However, in type II, III and IV the arthroscopic group showed better results when compared to the open group. In fractures with type V and VI classification, the results of both groups were poor, but the arthroscopic group had fewer infection rates [11].

Our study involved 25 patients with tibial plateau fractures presenting to Kasr El Ainy Hospitals between the period of November 2016 and May 2017.

In our study, we used the arthroscope first to assess intra-articular structures, examine for any associated pathology and study the fracture pattern and components. Using the A.C.L guide, a wire was inserted into the fracture site. A cortical window was created around the wire. Then using an impactor, the depressed segment was elevated and carefully reduced. Iliac crest graft was used and impacted under the fracture site.

Split fragments could be manipulated and reduced using k-wires as Joysticks. The reduction was checked fluoroscopically and arthroscopically and fixed using cannulated screws or plates and screws. This combination between the arthroscope and the image intensifier allows the more precise anatomical reduction, as the fracture was visualised from inside and outside. This minimises the size of the incision and the surgical dissection. Also, this avoids the detachment of the meniscus that occurs with the open approach as prolene suture loops or retractors were used to retract the lateral meniscus to expose the lateral tibial plateau and the fracture site [12].

The average time to full union in Schatzker type I was 9.1 weeks, in type II was 10.2 weeks, and in type III it was 9.4. According to the clinical Rasmussen score, nineteen patients (76%) had excellent results, (4 type I, 8 types II, and 7 types III), and 6 patients (24%) had good results (1 type I, 3 types II, 2 types III). Radiologic results were excellent in 14 cases (56 %) and good in 11 cases (44%). There were no patients who had fair or poor results.

These were considered high compared to the other studies that adopted similar techniques. This may be because our study was conducted only on Schatzker Type I, II, and III only.

Regarding the complications, in the study conducted by Pogliacomi F, et al., two patients (11%) were complicated by joint depression. In Roerdink, et al., 9 patients (30%) was complicated by secondary displacement, but without affecting the outcome. Also, his study was conducted only on patients more than 55 years old. In our study, one patient had a superficial infection, which was treated by antibiotics and repeated dressings. Another case was complicated by articular surface depression (4 mm) due to early weight bearing against the instructions and the patient refused surgical revision.

Our average follows up period was 14 months which is acceptable in comparison to the other studies, as most of the complications appear on the first three to six months. The following table compares our results to other similar studies (Table 1).

Table 1: Comparison between our results and other similar studies

	Pogliacomi et al. [13]	Roerdink et al. [14]	Our study
Number of patients	18 patients	30 patients	25 patients
Average follow up period	12 months	36 months	14 months
Schatzker classification	I-IV	I-VI	I-III
Mean Clinical (Rasmussen Score)	45% excellent 37% good 12%fair 6% poor	40% excellent, 40% good 10% fair 10% poor	76% excellent 24 % good
Mean Radiographic (Rasmussen Score)	29 % excellent 39% good 32% fair 16% were poor	13.3% excellent, 33.3% good, 33.3% were fair 20% poor	56 % excellent 44% good
Post-operative Complications	Two patients (11%)	Nine patients (30%)	Two cases (8%)

In conclusion, arthroscopic assisted reduction and fixation of tibial plateau fractures is not a commonly used technique in management in spite of its benefits. It is not a surgically demanding technique; it requires basic arthroscopic surgical skills and an average learning curve.

Its main advantage is checking the adequacy of reduction, avoiding the need for detachment of the meniscus, and allows for accurate diagnosis and management of associated knee injuries. Therefore, we recommend that Arthroscopic assisted reduction and fixation of tibial plateau fractures should be used more often.

Author Contribution

Sherif Hamdy Mohamed Zawam: literature search, data collection and performing the operative technique, data analysis. Ahmed Mahmoud Gad: Revision, Editing.

References

1. Jennings JE. Arthroscopic management of tibial plateau fractures. Arthroscopy: The Journal of Arthroscopic & Related Surgery. 1985; 1(3):160-8. https://doi.org/10.1016/S0749-8063(85)80003-7

2. Caspari RB, Hutton PM, Whipple TL, Meyers JF. The role of arthroscopy in the management of tibial plateau fractures. Arthroscopy: The Journal of Arthroscopic & Related Surgery. 1985; 1(2):76-82. https://doi.org/10.1016/S0749-8063(85)80035-9

3. Tilkeridis K, Kiziridis G, Tottas S, Kougioumtzis I, Riziotis G. Arthroscopically Assisted Fixation of the Tibial Plateau Fractures. J Bone Res. 2018; 6(188):2. https://doi.org/10.4172/2572-4916.1000188

4. Chan YS, Yuan LJ, Hung SS, Wang CJ, Yu SW, Chen CY, Chao EK, Lee MS. Arthroscopic-assisted reduction with bilateral buttress plate fixation of complex tibial plateau fractures. Arthroscopy: The Journal of Arthroscopic & Related Surgery. 2003; 19(9):974-84. https://doi.org/10.1016/j.arthro.2003.09.038 PMid:14608317

5. Burdin G. Arthroscopic management of tibial plateau fractures: surgical technique. Orthopaedics & Traumatology: Surgery & Research. 2013; 99(1):S208-18. https://doi.org/10.1016/j.otsr.2012.11.011 PMid:23347755

6. Honkonen S. Indications for surgical treatment of tibia condylar fractures. Clin Orthop. 1994; 302:199-205. PMid:8168301

7. Hohl M. Part I: Fractures of the proximal tibia and fibula. In: Rockwood C, Green D, Bucholz R, eds. Rockwood and Green's Fractures in Adults. Vol 2. 3rd ed. Philadel¬phia, PA: JB Lippincott, 1992:1725-1757.

8. Kampa J, Dunlay R, Sikka R, Swiontkowski M. Arthroscopic-assisted fixation of tibial plateau fractures: patient-reported postoperative activity levels. Orthopaedics. 2016; 39(3):e486-91. https://doi.org/10.3928/01477447-20160427-03 PMid:27135456

9. Hartigan DE, McCarthy MA, Krych AJ, Levy BA. Arthroscopic-assisted reduction and percutaneous fixation of tibial plateau fractures. Arthroscopy techniques. 2015; 4(1):e51-5. https://doi.org/10.1016/j.eats.2014.11.002 PMid:25973374 PMCid:PMC4427637

10. The fowble CD, Zimmer JW, Schepsis AA. The role of arthroscopy in the assessment and treatment of tibial plateau fractures. Arthroscopy: The Journal of Arthroscopic & Related Surgery. 1993; 9(5):584-90. https://doi.org/10.1016/S0749-8063(05)80410-4

11. Dall'Oca C, Maluta T, Lavini F, Bondi M, Micheloni GM, Bartolozzi P. Tibial plateau fractures: compared outcomes between ARIF and ORIF. Strategies in Trauma and Limb Reconstruction. 2012; 7(3):163-75. https://doi.org/10.1007/s11751-012-0148-1 PMid:23086660 PMCid:PMC3482433

12. Siegler J, Galissier B, Marcheix PS, Charissoux JL, Mabit C, Arnaud JP. Percutaneous fixation of tibial plateau fractures under arthroscopy: a medium term perspective. Orthopaedics & Traumatology: Surgery & Research. 2011; 97(1):44-50. https://doi.org/10.1016/j.otsr.2010.08.005

13. Pogliacomi F, Verdano MA, Frattini M. Combined arthroscopic and radioscopic management of tibial plateau fractures: report of 18 clinical cases. Acta Bio Medica Atenei Parmensis. 2005 76(2):107-14. PMid:16350556

14. Roerdink WH, Oskam J, Vierhout PA. Arthroscopically assisted osteosynthesis of tibial plateau fractures in patients older than 55 years. Arthroscopy: The Journal of Arthroscopic & Related Surgery. 2001; 17(8):826-31. https://doi.org/10.1016/S0749-8063(01)90005-2

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Apr 15; 7(7):1138-1142.
https://doi.org/10.3889/oamjms.2019.267
eISSN: 1857-9655
Clinical Science

Role of Serotonin and Dopamine in Psoriasis: A Case-Control Study

Made Wardhana*, Martina Windari, Nila Puspasari, Nyoman Suryawati

Dermatology and Venereology Department, Faculty of Medicine, Udayana University, Sanglah General Hospital, Bali, Indonesia

Citation: Wardhana M, Windari M, Puspasari N, Suryawati N. Role of Serotonin and Dopamine in Psoriasis: A Case-Control Study. Open Access Maced J Med Sci. 2019 Apr 15; 7(7):1138-1142. https://doi.org/10.3889/oamjms.2019.267

Keywords: Psoriasis; Proinflammatory; Cytokines; Serotonin; Dopamine; Regulation

***Correspondence:** Made Wardhana. Dermatology and Venereology Department, Faculty of Medicine, Udayana University - Sanglah General Hospital, Bali, Indonesia. E-mail: wardhanamade@unud.ac.id

Received: 15-Feb-2019; **Revised:** 03-Apr-2019; **Accepted:** 04-Apr-2019; **Online first:** 14-Apr-2019

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Psoriasis is a chronic inflammatory disease mediated by the immune system with increased proliferation of keratinocytes. The exact cause is unknown but as a multifactor, such as infection, trauma and psychological stress have been thought to play a role in its pathophysiology. Dopamine and serotonin are believed to have a strong role in stress conditions and also directly play a role in psoriasis.

AIM: This study aimed to evaluate the role of dopamine, serotonin, and psychological stress in psoriasis.

METHODS: This study used a case-control design involving 30 patients with psoriasis (as a case group) and 30 healthy controls in the Dermatology and Venereology Polyclinic of Sanglah General Hospital Denpasar during the period December 2016 to February 2017. All samples were taken for venous blood examination serum dopamine and serotonin and analysed using the ELISA method. Statistical analysis using an independent t-test, partial correlation, receiver operator characteristic (ROC) curve, and logistic regression model.

RESULT: There were significant differences in serotonin, dopamine, and stress index levels between groups with psoriasis and non-psoriasis (102.68 ± 25.44 Vs. 154.17 ± 20.90; $p < 0.001$), (437.13 ± 164.83 Vs. 138.11 ± 89.51; $p < 0.001$), and (138.5 ± 27.80 Vs. 92.55 ± 42.97; $p < 0.001$). Significant negative correlation was found between serotonin level and stress index ($r = -0.366$; $p = 0.016$) and between serotonin and dopamine ($r = -0.634$; $p < 0.001$) but a positive correlation was found between dopamine and stress index ($r = 0.459$; $p = 0.042$). Serotonin and dopamine showed that it could be used as a biochemical predictive model for psoriasis (AUC > 0.7). Multivariable risk analysis model high serum dopamine was the most important risk factor for the occurrence of psoriasis (adjusted OR: 7.8; 95% CI: 3.45-15.57; $p = 0.024$)

CONCLUSION: Serotonin and dopamine have a significant role in the pathophysiology of the occurrence of psoriasis, and psychological stress can affect psoriasis through its influence on serotonin and dopamine.

Introduction

Psoriasis is an autoimmune chronic-residual skin inflammatory disease, characterised by hyperproliferation of keratinocytes with erythema plaques, hyperkeratosis, and silvery-coated scales — symmetrical distribution of predilection areas of the extensor, scalp, lumbosacral region. The exact cause is unknown but several predisposing factors such as genetics, environmental factors, trauma, infection, drugs and psychological stress [1], [2]. The prevalence of psoriasis is 2% of the world population, but in America and Canada, it is 4.6% and 4.7%. Whereas in Asia around 0.4-0.7% [1], [3]. The number of new cases of psoriasis in our center tends to be rising from years to years.

Currently, several studies prove psychological and neuroendocrine stress that can affect immune responses with clinical manifestations such as atopic dermatitis, alopecia, acne vulgaris, psoriasis, etc. Zangeneh and Fazeli found all stress hormones, catecholamines, corticotrophin releasing hormones (CRH), dopamine significantly increased in psoriasis. Interferon-gamma (IFN-γ) is a proinflammatory cytokine synthesised by T helper 1 cell (Th1), has been known to play a critical role in the pathogenesis of psoriasis. Psychological stressors lead to the dominant role of Th1 cells resulting in excessive IFN-γ synthesis [4], [5].

Dopamine is a neurotransmitter which

together with norepinephrine and epinephrine, is called catecholamine [6], [7]. Catecholamines function to regulate nerve function, neuroendocrine and immune systems. Dopamine can increase the activity of keratinocytes, which play a role in the release of cytokines and chemokines [7], [8]. Some studies show that cells in the immune system can be affected by dopamine. Dopamine agonists stimulate the production of interleukin (IL)-6 and IL-8, where IL-6 and IL-8 play a role in the proliferation and differentiation of epidermal cells, besides IL-8 also stimulates chemotaxis of neutrophils and promotes acute inflammation. In keratinocyte cells of psoriasis patients found a high level of IL-8 expression and high dopamine serum receptors and can result in autoimmunity [8], [9].

Serotonin (5 hydroxytryptamines; 5-HT) is a neurotransmitter whose effect is mediated through different receptor interactions and consists of 14 subtypes [10]. Serotonin is known as a neurotransmitter in the central nervous system and is involved in many processes including cognition and memory. In the other side, serotonin plays an essential role in vasoconstriction, heart rate in the cardiovascular and gastrointestinal systems [11]. Psychological stress can also reduce the synthesis of Serotonin and can affect many immunological processes and cause a decrease in proinflammatory cytokines such as tumour necrosis factor (TNF-α) [12], [13]. TNF-α plays an important role in the pathogenesis of psoriasis, selective activation of serotonin receptors with the 2,5-dimethoxy-4-iodoamphetamine antagonist (DOI) produces a strong blockade of pro-inflammatory cytokines such as the Interleukin-6 cytokine (Il-6) and Interleukin-1b (IL-1b), then DOI antagonist also prevents TNF-α so and increase IL-6 level. If there is a decrease in serotonin, there will be an increase in proinflammatory cytokines that form the basis of the role mechanism of the inflammatory process in psoriasis [11], [12], [13].

Based on this perspective, we would like to evaluate the role psychological stress causes a rise in dopamine and a decrease in serotonin reduction as a risk factor for psoriasis.

Material and Method

Study design and subject

The study used a case-control design that took place in the Dermatology and Venereology Polyclinics in Sanglah General Hospital Denpasar, Bali-Indonesia during the period December 2016 to February 2017. The case group consisted of new or old case psoriasis who had not received any systemic treatment or two weeks had stopped treatment. Control is a healthy person or does not suffer from other allergic skin diseases. The two groups after being informed were asked to sign the informed consent sheet. Socio-demographic characteristics such as age, gender, body mass index were recorded and also the clinical type of psoriasis, clinical form of psoriasis and severity were calculated by the Psoriasis Area Severity Index (PASI) [14]. Stress index was evaluated using questions from the Social Readjustment Rating Scale by assessing a live event from an index of 10 – 200 [15]. Both groups performed venous blood collection to examine serum dopamine and serotonin using the method ELISA.

Statistical analysis

Statistical analysis was performed using SPSS version 25.0 for windows. Partial correlation was used to find a correlation between stress index, dopamine level and serotonin level by controlling age, gender, and body mass index. Independent sample t-test or Mann Whitney U test was used to compare numerical variables such as serotonin serum, serum dopamine, and stress index in patients with psoriasis and non-psoriasis group. Receiver operator curve (ROC) analysis and risk analysis model using logistic regression were performed to determine the role of serotonin and dopamine in psoriasis. All tests were considered significant if the value of $p < 0.05$.

Result

Subject characteristics

There were no significant differences in sex, age, body mass index between patients with psoriasis and non-psoriasis group ($p > 0.05$). However, the group of people with psoriasis tends to have a family history of psoriasis (59%). In the case group (psoriasis person) there were more people with a moderate psoriasis category (44%) based on the PASI category, with clinical types of maculopapular (64%), and type I psoriasis (70%) (Table 1).

Table 1: Subject characteristics

Characteristics	Case (n = 60) Psoriasis	Control (n = 60) Non-Psoriasis	p-value
Gender (n, %)			
Male	48 (49%)	50 (51%)	0.058
Female	12 (54%)	10 (46%)	
Age (years) (Mean ± SD)	45.54 ± 11.2	46.21 ± 0.68	0.562
Family history of psoriasis			
Yes	20 (72%)	4 (28%)	0.003*
No	40 (44%)	26 (56%)	
BMI (n, %)			
High (> 25 kg/m^2)	32 (59%)	22 (41%)	0.063
Normal (≤ 25 kg/m^2)	28 (43%)	38 (57%)	
Type of Psoriasis (n, %)			
Type I	42 (70%)	-	
Type II	18 (30%)	-	
Psoriasis area severity index category (PASI) (n, %)			
Mild	20 (34%)	-	
Moderate	26 (44%)	-	
Severe	14 (22%)	-	
Clinical type of psoriasis (n, %)			
Maculo-papular	38 (64%)	-	
Gutate	16 (27%)	-	
Erythrodermic psoriasis	6 (9%)	-	

*Significant ($p < 0.05$).

Differences of stress index, serotonin level, and dopamine level between psoriasis and non-psoriasis group

There were significant differences in serotonin levels between groups with psoriasis (102.68 ± 25.44) and non-psoriasis (154.17 ± 20.90) ($p < 0.001$), a person with psoriasis had lower serum serotonin levels compared to a non-psoriasis person. There were significant differences in dopamine levels between groups with psoriasis (437.13 ± 164.83) and non-psoriasis (138.11 ± 89.51) ($p < 0.001$), a person with psoriasis had higher serum dopamine levels compared to non-psoriasis. There were significant differences in stress index between groups with psoriasis (138.5 ± 27.80) and non-psoriasis (92.55 ± 42.97) ($p < 0.001$), a person with psoriasis had a higher stress index compared to non-psoriasis (Table 2).

Table 2: Comparison of stress index, serotonin level, and dopamine level, between psoriasis and non-psoriasis group

Variable	Mean ± SD (Psoriasis)	Mean ± SD (Non-Psoriasis)	Mean differences	95% CI	p
Dopamine serum (ng/ml)	437.13 ± 164.83	138.11 ± 89.51	299.06	230.50-367.04	< 0.001
Serotonin serum (ng/ml)	102.68 ± 25.44	154.17 ± 20.90	51.49	39.14-63.82	< 0.001
Stress index	138.5 ± 27.80	92.55 ± 42.97	45.95	27.24-64.65	< 0.001

Correlation between stress, serotonin, and dopamine

A correlation test was carried out using partial correlation test by controlling the variables of age, sex, body mass index to determine the correlation between stress index, dopamine level, and serotonin level. There was a significant negative correlation between serotonin level and stress index ($r = -0.366$; $p = 0.016$), a significant negative correlation between serotonin and dopamine ($r = -0.634$; $p < 0.001$). This shows an inverse correlation between serotonin and dopamine and also between serotonin and stress index. However, there is a moderate positive correlation between dopamine and stress index ($r = 0.459$; $p = 0.042$), this indicates that the higher stress index will be followed by an increase in serum dopamine (Table 3).

Table 3: Correlation between stress index, dopamine level, and serotonin level after controlling for age, gender and body mass index

Variable	Stress Index		
	n	r (coefficient correlation)	p
Serotonin level	120	-0.366	0.016
Dopamine level		0.458	0.042
	Dopamine Level		
	n	r (coefficient correlation)	p
Serotonin level	120	-0.634	< 0.001

A predictive model of serotonin and dopamine as a biochemical marker in psoriasis

Predictive models were carried out using analysis of receiver operator characteristics curve (ROC) on serotonin and dopamine parameters in psoriasis and non-psoriasis groups as the dependent variable in this study. Serotonin and dopamine showed that it could be used as a biochemical predictive model for psoriasis (AUC > 0.7) (Figure 1).

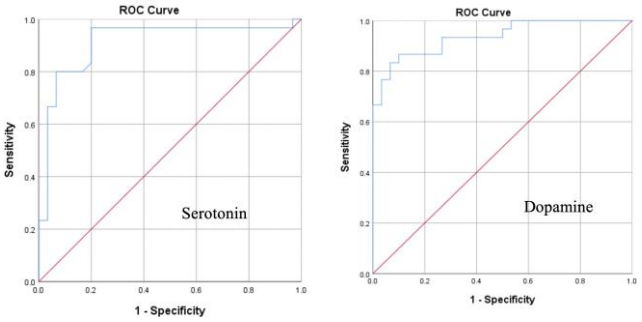


Figure 1: ROC analysis of serotonin (left) and dopamine (right) as a biochemical marker in psoriasis

Dopamine has sensitivity, specificity, and cut-off values of 87%, 90%, and 320 ng/ml respectively and serotonin has a sensitivity value of 94%, specificity of 64%, and a cut-off value of 141 ng/ml (Table 4).

Table 4: Area under the curve (AUC), cut-off value, sensitivity, and specificity of serotonin and dopamine in psoriasis

Parameter	AUC	95% CI	Cutt-off value	Sensitivity	Specificity	p
Serotonin	0.912	0.82-0.94	141 ng/ml	94%	64%	< 0.001
Dopamine	0.937	0.87-0.98	320 ng/ml	87%	90%	< 0.001

Risk analysis model for serotonin and dopamine in psoriasis

Risk analysis is based on the cut-off values of ROC of serum dopamine and serotonin, for serum dopamine values higher than 320 ng/ml classified as high dopamine serum levels, and lower ones are referred to as normal values. Then for serum serotonin, a value lower than cut off value is calcified as a low serum level, and a higher value is classified as normal.

Table 5: Risk analysis model of serotonin and dopamine level in psoriasis

Parameter		Univariable Model					Multivariable Model		
		Psoriasis	Non-Psoriasis	OR	95% CI	p	Adjusted OR	95% CI	P
		n (%)	n (%)						
Serotonin	Low	42 (70%)	14 (24%)	7.7	2.4-24.4	0.023*	5.4	2.23- 8.56	0.016*
	Normal	18 (30%)	46 (76%)						
Dopamine	High	50 (83%)	16 (27%)	15.6	4.7-30.8	0.019*	7.8	3.45- 15.57	0.024*
	Normal	10 (17%)	44 (73%)						

*Significant ($p < 0.05$).

In univariable risk analysis mode low serotonin level (OR: 7.7; 95% CI: 2.4-24.4; $p = 0.023$) and high dopamine levels (OR: 15.6; 95% CI: 4.7-30.8; $p = 0.019$) proved to be a risk factor of psoriasis. In a multivariable risk analysis model, high serum dopamine was the most important risk factor for the occurrence of psoriasis (adjusted OR: 7.8; 95% CI: 3.45-15.57; $p = 0.024$). People with high-level serum

dopamine have a risk of 7.8 times higher to develop psoriasis compared to a person who has low serum dopamine level (Table 5).

Discussion

It has been widely known that stress has a close relationship with immunity in the human body [16]. In general, chronic stress can dampen the adaptive immune system and also innate immunity (natural killer cell activity), increasing the proinflammatory cytokine cascade. This occurs through activation of the hypothalamus-pituitary-adrenal axis of the autonomic nervous system [12], [17]. It should be noted that the chronic increase in proinflammatory cytokines can act as a mechanism of feedback to the central nervous system (CNS) and have significant consequences on an individual's psychological well-being. In people with psoriasis, it is very common for a decrease in quality of life, depressed mood disorders, a tendency for a higher stress, social stigma, and problems in work caused by psoriasis itself. This condition makes psoriasis not something that is caused by immunology, but it is also characterised by an issue of mental disorders such as stress and depression [12], [16], [17].

Many studies have provided an increase in proinflammatory mediators in chronic stress disorders; these include increases in IL-1, IL-6, and TNF-α in the blood and cerebrospinal fluid [18], [19]. The proinflammatory cytokines in the central nervous system can affect the metabolism of monoamine neurotransmitters (dopamine, serotonin, and norepinephrine) which causes a decrease in availability, synthesis, and increased uptake of neurotransmitters that have a potential effect on inflammation and development of chronic stress into mood disorders [19]. Apart from that, proinflammatory cytokines such as TNF-α, IL-1, and IL-6 which are released by macrophages due to chronic inflammation and pro-inflammatory mediators produced by keratinocytes on the skin that experience psoriasis can reach the brain by penetrating the blood-brain barrier [12], [18]. Proinflammatory cytokines that have arrived in the brain will trigger a sickness behaviour (fatigue, fever, somnolence) which will then mediate the onset of depression and ongoing stress [10], [12]. Through this mechanism, it can be seen that stress affects inflammation and can trigger psoriasis as well as vice versa so that this will become a chain of never-ending circling pathophysiology [12], [20], [21]. It was also found in our study that stress correlated with dopamine levels which had a significant role in the occurrence of psoriasis.

Serotonin (5-HT) is a neurotransmitter involved in many regulatory processes in the body and biological functions, psychological processes in the CNS, and also the role of peripheral tissue [21], [22]. In vivo studies, serotonin plays a role in cell signalling, and modulation of the peripheral immune system includes T cells, mast cell macrophages, dendritic cells, and platelets [23]. Specifically, the receptors of serotonin are expressed in T cells, where the result of sensitisation is the release of proinflammatory cytokines because of the response of serotonin secreted by mast cells which binds to the 5HT-2 receptor [24]. The response also affects the maturation and proliferation of T cells. The ratio of Th1: Th2 is also affected by serotonin, a change in ratio <1 is associated with a higher risk of autoimmune disease. Serotonin receptors are also expressed in CD8 cells, monocytes, dendritic cells, mast cells [18], [25]. A decrease in serotonin will cause an increase in production of inflammatory mediators such as TNF-α, IFN-γ, IL-1β, IL-6, IL-8 which will carry out activation on nuclear factor-KB and induce activation of keratinocytes and trigger deterioration of keratinocytes and worsening of psoriasis symptom [13], [26], [27]. This is consistent with the findings of this study that low serotonin is a risk factor for psoriasis.

It is well known that skin diseases such as psoriasis and atopic dermatitis are strongly influenced by the incidence of stress and dopamine has a very close relationship in the configuration of stress. Dopamine is an important neurotransmitter in the central nervous system and locomotive control, cognition, emotion, immunology, and neuroendocrine secretion [21]. Study by Mori et al. in the mouse animal model (in-vitro) shows that the intervention of dopamine 1 (D1) receptor antagonist (SCH 23390) decreases the expression of IL-4, IFN-γ observed in 3-hour conditions (rapid phase immunological reactions) and 24 hours (slow phase immune reaction) on rat skin [28]. It can be seen that D1 like receptors are selectively expressed in Th2 cells, which suggests that dopamine can trigger the differentiation of Th2 cells. This indicates that D1-like dopamine receptor can induce degranulation of mast cells and trigger the release of proinflammatory cytokines. The role of dopamine in controlling Th2 cells in modulating the immune system is very important in psoriasis [5], [28]. In this study found a significant association between high dopamine levels as a risk factor for psoriasis.

The limitation in this study is that apart from psychological stress parameters, this study did not examine depression scale experienced in the study group, then the limited number of samples became a lack of generalisation to the population of the results of this study.

In conclusion, serotonin and dopamine have a significant role in psoriasis, and psychological stress can affect serotonin and dopamine levels which have an indirect effect on psoriasis. More detailed research on the biomolecular mechanism of the role of serotonin and dopamine using a larger number of samples is needed.

Acknowledgements

I would like to thank our patients from the Dermatology and Venereology Polyclinic and all dermatology and venereology resident in Sanglah General Hospital for their technical help.

References

1. Takeshita J, Grewal S, Langan SM, Mehta NN, Ogdie A, Voorhees SV, et al. Psoriasis and comorbid disease: epidemiology. Journal of the American Academy of Dermatology. 2017; 76(3):377-390. https://doi.org/10.1016/j.jaad.2016.07.064 PMid:28212759 PMCid:PMC5731650

2. Nuzzo S, Feliciani S, Cortelazzi S, Fabrizi G, Pagliarello C. Immunopathogeneis of psoriasis: Emphasis on the role of Th17 cells. International Trends In Immunity Journal. 2014; 2(3):111-115.

3. Slavenka J, Milena R, Jelena M, Natasa M, Janko J, and Bosiljka D. Relevance of Psychosomatic Factors in Psoriasis: A Case-control Study. Acta Derm Venereol. 2009; 89:364-368. https://doi.org/10.2340/00015555-0669 PMid:19688147

4. Zangeneh FZ, Fazeli A. The significace of stress hormones in psoriasis. Acta Medica Iranica. 2008; 46(6):485-488.

5. Besser MJ, Ganor Y, Levite M. Dopamine by itself activates either D2, D3 or D1/D5 dopaminergic receptors in normal human T-cells and triggers the selective secretion of either IL-10, TNFalpha or both. J Neuroimmunol. 2005; 169:161-71. https://doi.org/10.1016/j.jneuroim.2005.07.013 PMid:16150496

6. McKenna F, McLaughlin PJ, Lewis BJ, Sibbring GC, Cummerson JA, Jones BD, et al. Dopamine receptor expression on humanT- and B-lymphocytes, monocytes, neutrophils, eosinophils and NK cells: a flow cytometric study. J Neuroimmunol. 2002; 132:34-40. https://doi.org/10.1016/S0165-5728(02)00280-1

7. Pani L, Porcella A, and Gessa GL. The role of stress in the pathophysiology of the dopaminergic system. Molecular Phychiatry. 2005; 16:14-21.

8. Parrado AC, Canellada A, Gentile T, and Rey-Roldan EB. Dopamine Agonist Upregulate IL-6 and IL-8 Production in Human Keratinocytes. Neuroimmunomodulation. 2012; 19:359-66. https://doi.org/10.1159/000342140 PMid:23051896

9. Thorslund K, El-Nour H, Nordlind K. The serotonin transporter protein is expressed in psoriasis, where it may play a role in regulating apoptosis. Arch Dermatol Res. 2009; 301:449-457. https://doi.org/10.1007/s00403-009-0933-y PMid:19263059

10. Martino M, Rocchi G, Escelsior A, and Fornaro M. Immunomodulation Mechanism of Antidepressants: Interactions between Serotonin/Norepinephrine Balance and Th1/Th2 Balance. Current Neuropharmacology. 2012; 10:97-123. https://doi.org/10.2174/157015912800604542 PMid:23204981 PMCid:PMC3386509

11. Fouad YSF, Bakry OA. Immunohistochemical Evaluation of Role of Serotonin in Pathogenesis of Psoriasis. Journal of Clinical and Diagnostic Research. 2016; 10(10):5-9.

12. Moynihan J, Reider E, Tausk F. Psychoneuroimmunology: the example of psoriasis. G Ital Dermatol Venereol. 2010; 145(2):221-228. PMid:20467396 PMCid:PMC3801168

13. Ronpirin C and Tencomnao T. Psoriasis: A review of the role of serotonergic system. African Journal of Biotechnology. 2010; 9(11):1528-1534. https://doi.org/10.5897/AJB10.020

14. Schmitt J, and Wozel G. The Psoriasis Area and Severity Index Is the Adequate Criterion to Define Severity in Chronic Plaque-Type Psoriasis. Dermatology. 2005; 10:194-199. https://doi.org/10.1159/000083509 PMid:15785046

15. Holmes TH, Rahe RH. The Social Readjustment Rating Scale. Journal of Psychosomatic Research. 1967; 11:213-218. https://doi.org/10.1016/0022-3999(67)90010-4

16. Parra GS, Dauden E. Psoriasis and depression: the role of inflammation. Actas Dermosifiliogr. 2019; 110(1):12-19.

17. Ferreira BI, Abreu JL, Reis JP, Figueiredo AM. Psoriasis and associated psychiatric disorder: a systematic review on etiopathogenesis and clinical correlation. J Clin Aesthet Dermatol. 2016; 9(6):36-43. PMid:27386050 PMCid:PMC4928455

18. Thorslund K, Nour H, Nordlind K. The serotonin transporter protein is expressed in psoriasis, where it may play a role in regulating apoptosis. Arch Dermatol Res. 2010; 301(6):449-57. https://doi.org/10.1007/s00403-009-0933-y PMid:19263059

19. O'Connell PJ, Wang X, Leon-Ponte M. A novel form of immune signaling revealed by transmission of the inflammatory mediator serotonin between dendritic cells and T cells. Blood. 2006; 107:1010-1117. https://doi.org/10.1182/blood-2005-07-2903 PMid:16223770 PMCid:PMC1895901

20. Cowen PJ. Cortisol, serotonin, and depression, all stressed out. British Journal of Psychiatry. 2002; 180:99-100. https://doi.org/10.1192/bjp.180.2.99 PMid:11823315

21. Maria K, Elizabeth J, Matti AL, Michalis M, Marios M. Noradrenaline, Dopamine, Serotonin: Different Effects Of Psychological Stress On Brain Biogenic Amines In Mice And Rats. Pharmacological Research. 2000; 41(3):344-348.

22. Wu H, Denna TH, Storkersen JN, Gerriets V. Beyond a neurotransmitter: the role of serotonin in inflammation and immunity. Pharmacology Research. 2019; 140:100-114. https://doi.org/10.1016/j.phrs.2018.06.015 PMid:29953943

23. Yuan XQ, Qiu G, Liu XJ, Liu S, Wu Y, Wang X, et al. Fluoxetine promotes remission in acute experimental autoimmune encephalomyelitis in rats. Neuroimmunomodulation. 2012; 19(4):201-208. https://doi.org/10.1159/000334095 PMid:22441536

24. Taler M, Gil AD, Korob I, Weizman A. The immunomodulation effect of the antidepressant sertraline in an experimental autoimmune encephalomyelitis mouse model of multiple sclerosis. Neuroimmunomodulation. 2011; 18(2):117-122. https://doi.org/10.1159/000321634 PMid:21088435

25. Brig D Saldanha, Maj N Kumar, K Srivastava. Serum Serotonin Abnormality in Depression. MJAFI. 2009; 65:108-112. https://doi.org/10.1016/S0377-1237(09)80120-2

26. Isabelle CT, Anne-France PB, Homer D. Differential effect of serotonin on cytokine production in lipopolysaccharide-stimulated human peripheral blood mononuclear cells:involvement of 5-hydroxytryptamine2A receptors. International Immunology. 2003; 15(2):233-240. https://doi.org/10.1093/intimm/dxg027

27. Shajib MS, Khan WI. The role of serotonin and its receptors in activation of immune responses and inflammation. Acta Physiol. 2014; 3:1-14.

28. Mori T, Kabashima K, Fukamachi S, Kuroda E, Sakabe JI, Kobayashi M, et al. D1-like dopamine receptors antagonist inhibits cutaneous immune reaction mediated by Th2 and mast cells. Journal of Dermatological Science. 2013; 71:37-44. https://doi.org/10.1016/j.jdermsci.2013.03.008 PMid:23639699

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Apr 15; 7(7):1143-1147.
https://doi.org/10.3889/oamjms.2019.277
eISSN: 1857-9655
Clinical Science

Chest Ultrasound in Predication of Weaning Failure

Soliman Belal Soliman*, Faheem Ragab, Randa Aly Soliman, Ayman Gaber, Ahmed Kamal

Critical Care Medicine Department, Cairo University, Cairo, Egypt

Citation: Soliman SB, Ragab F, Soliman RA, Gaber A, Kamal A. Chest Ultrasound in Predication of Weaning Failure. Open Access Maced J Med Sci. 2019 Apr 15; 7(7):1143-1147. https://doi.org/10.3889/oamjms.2019.277

Keywords: mechanical ventilation; chest ultrasonography; Doppler tissue imaging; lung ultrasound

***Correspondence:** Soliman Belal Soliman. Critical Care Medicine Department, Cairo University, Cairo, Egypt. E-mail: solimanbelal@kasralainy.edu.eg

Received: 26-Feb-2019; **Revised:** 04-Apr-2019; **Accepted:** 05-Apr-2019; **Online first:** 14-Apr-2019

Funding: This research did not receive any financial support

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

Abstract

AIM: Failure of weaning from mechanical ventilation (MV) is a common problem that faces the intensivist despite having some prediction indices. Application of chest ultrasonography (US) may help in weaning and prediction of its outcome.

METHODS: 100 patients on invasive MV fulfilling criteria of weaning shifted to spontaneous breathing trial (SBT) (using PSV 8 cm H_2O) for 1 hour. Weaning failure was defined as; Failed SBT, reintubation and/or ventilation or death within 48 hours. Echocardiography was used to get Ejection fraction, E/A ratio, Doppler tissue imaging (DTI) &, lung ultrasound (LUS) was used to assess LUS score, diaphragm ultrasound was used to assess diaphragmatic thickening fraction (DTF).

RESULTS: Mean age 57.1 ± 14.5, 62% were males. Weaning was successful in 80% of patients. LUS score was significantly higher in the failed weaning group: (10.8 ± 4.2) vs (16.5 ± 4.2 cm), (p: 0.001). (DTF) Was significantly higher in the successful weaning group: (43.0 ± 10.7) vs (28.9 ± 2.8 cm), (p: 0.001). DTF can predict successful weaning using Receiver operating characteristic (ROC) curves with the cutoff value: ≥ 29.5 with sensitivity 88.0% and specificity 80.0% with a p-value < 0.001.LUS score can predict weaning failure by using a ROC curve with cutoff value: ≥ 15.5 with sensitivity 70.0% and specificity 82.5 % with a p-value < 0.001.)

CONCLUSION: The use of bedside chest US (to assess lung and diaphragm) of great benefit throughout the weaning process.

Introduction

Mechanical ventilation (MV) is one of the most common interventions in critical care. Weaning failure from MV occurred in 10-20% of patients [1], [2].

Timing is crucial when deciding if a patient can be successfully extubated. Both premature discontinuation and unnecessary delay of MV weaning have associated with poor outcome [3].

One of the major causes of weaning failure is the imbalance between the load on the diaphragm and its ability to cope with it [4], [5], [6], [7]. There is rising evidence that diaphragmatic dysfunction has a critical role in ventilator dependency. Diaphragm thickness calculated at end inspiration is related to maximal inspiratory pressure. Diaphragm US can be used to evaluate diaphragmatic dysfunction [7], [8], [9], [10]. Shifting a patient from MV to spontaneous breathing may be associated with lung aeration loss(derecruitment), which can cause weaning failure, [11], [12]. Lung ultrasound is an emerging and increasingly used tool to investigate both in a semi-quantitative and quantitative way lung aeration during MV [12].

Patients and Methods

A prospective observational study was conducted on one hundred patients admitted to the department of critical care medicine, faculty of medicine, Cairo University, from January 2016 to July 2017. The study was approved by the Ethical committee of Cairo University. Written informed consent was obtained by first degree relatives.

Patients

One hundred consecutive mechanically ventilated patients for more than 48 hours were included when the underlying cause that had required intubation was resolved, making the patient candidate to a first 1-hour SBT [10]. Exclusion criteria were patients aged < 18 years, patients with tracheostomy, cardiac arrhythmias, Left ventricular systolic dysfunction (LVEF < 50%) [22], diaphragmatic paralysis Neuromuscular disorders for example: Guillain barre syndrome and Myasthenia gravis, Severe ICU-acquired neuromyopathy, chronic obstructive pulmonary disease with forced expiratory volume < 50% of the predicted value and history of pneumonectomy or chronic lung disease

Methods

All patients were subjected to the following:

1-Detailed history is taking. Full physical examination and Laboratory investigations (Complete blood count, Coagulation profile, Arterial blood gases, Liver functions (ALT), Kidney functions (creatinine), blood glucose), bedside twelve leads ECG and chest x-ray.

2-Mechanical ventilation. All patients were intubated and mechanically ventilated under volume controlled ventilation using a Puritan Bennet 840 ventilator, and they were observed till improvement of their conditions and became eligible to enter the SBT for weaning [23]. Patients were included in the weaning trial if they met the weaning criteria [11].

3-SBT protocol. Patients were put on PS/CPAP trial (pressure support 8 cm H_2O, CPAP 5 cm H_2O for one hour and they were extubated if they have succeeded in the trial. Failure of the weaning process was defined as a failed SBT or the need for reintubation within 48 hours following extubation [11], [24], so all patients included in the study were observed for 48 hours after the SBT. The following weaning indices were recorded for all patients during the SBT: Tidal volume (TV), Respiratory rate (RR), ABG: Po_2, So_2 % and Po_2/Fio_2, Rapid shallow breathing index: (RSBI (f/VT) = Respiratory rate/tidal volume [25]. A threshold of ≤ 105 is a must to continue the SBT [25].

Indicators of weaning failure were recorded:

a) objective indices: tachycardia more than 140 beats/min, tachypnea more than 35 breaths/min, use of accessory respiratory muscles, systolic arterial pressure more than 200 or less than 80 mmHg, hypoxemia: SO_2 less than 90%, acidosis, Arrhythmia [11];

b) subjective indices: Agitation, disturbed conscious level, increased work of breathing. SBT without the presence of the above signs was considered successful, and patients were extubated [11].

4-Echocardiographic study. Transthoracic Doppler echocardiographic examination was 30 minutes after the start of the SBT using TOSHIBA ACUSON X 300, with a probe 3.5 MHZ was used to assess the LV systolic function using 2D and modified Simpson's method. LV diastolic function was assessed by measuring mitral inflow velocity E and A waves and velocities of mitral annulus (Ea) using Doppler tissue imaging.

5-Lung ultrasound. (LUS) Was performed using a 2- to 4-MHz convex probe [16]. LUS was performed by the same trained physician at the end of the SBT. An LUS score has been produced to provide quantifiable comparable measures of changes in aeration [17], [18]. This score originates from the conversion of LUS patterns into numeric values, Four aeration patterns by ultrasound were defined [17], [18]: 1) normal aeration (N): presence of lung sliding with A-lines or less than two isolated B lines; 2) moderate loss of lung aeration: multiple B lines (B1 lines); 3) severe loss of lung aeration: multiple fused B lines (B2 lines); and 4) lung consolidation (C), the presence of a dynamic air bronchograms and tissue pattern , N = 0, B1 lines = 1, B2 lines = 2, C = 3. The final score, ranging from 0 to 36, is the sum of the values, from 0 to 3, assigned to the LUS patterns visualised in each of the 12 regions examined.

6-Diaphragmatic ultrasound. Diaphragm ultrasound was performed using a 10 MHz linear probe; Each diaphragm was evaluated by B-mode and M-mode after 30 min of the SBT [26]. The diaphragm was seen by placing the transducer, in the eighth intercostal space, perpendicular to the chest wall between the anterior axillary and the mid axillary lines, to see the zone of apposition of the muscle below the costophrenic sinus [27], [28]. The diaphragm was imaged as a structure with three layers, including two parallel echoic lines (the peritoneal membrane and the diaphragmatic pleura) and a hypo echoic structure between them (the muscle itself) [27], [28]. The patient was then instructed to perform maximum deep inspiration and then maximum exhalation [27], [28]. On each frozen B-mode image, the diaphragm thickness was measured from the middle of the pleural line to the middle of the peritoneal line. Then, the diaphragmatic thickening fraction (DTF) was calculated as a percentage from the following formula: Thickness at end inspiration − Thickness at end expiration/Thickness at end expiration [27], [28]. The same steps were done using the M mode.

Statistical methods

Data were coded and entered using the statistical package SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 22. Data were summarised using mean and standard

deviation in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. Comparisons between quantitative variables were made using the non-parametric Mann-Whitney tests. For comparing serial measurements within each patient in each group, the non-parametric Wilcoxon signed rank test was used. ROC curve was constructed with an area under curve analysis performed to detect the best cutoff value for detection of the success of 1st SBT. P-values less than 0.05 were considered as statistically significant.

Results

The studied patients were divided into two groups: group 1, 80 patients with successful weaning; group 2, 20 patients with failure of weaning.

Table 1: Patients characteristics

	Group 1	Group 2	P value
Demographic data			
Age (years)(mean)	56.4	59.9	0.383
Sex (male/female)	80 (52/28)	20 (10/10)	0.301
Causes of mechanical ventilation:			
Respiratory failure (62%)	14	48	
Cardiac cause (12 %)	2	10	0.542
DCL (16%)	4	12	
Hemodynamic support (10%)	0	10	
2D echocardiographic data:			
LVEDD (mm)(mean)	48.5	52.6	0.25
LVESD (mm) (mean)	38	39	0.82
LVEF (%) (mean)	56.2%	52.7%	0.06
Ventilator parameters before the SBT in both groups:			
Frequency	20.7 ± 3.6	24 ± 3.9	0.012
Tidal volume	484.2 ± 69.1	409.0 ± 82.7	.005
Minute ventilation	9788.2 ± 1438.6	9725.0 ± 1191.2	.899
Ventilator parameters before the SBT in both groups:			
Frequency	25.4 ± 2.3	29.6 ± 3.6	0.001
Tidal volume	416.4 ± 70	346 ± 76.9	0.008
Minute ventilation	10387 ± 1479	9898 ± 1441	0.351
Baseline hemodynamic data (pre-SBT): Systolic BP (mmHg)			
Heart rate (beats/min)	123 ± 15.9	122.5 ± 30.4	0.934
	97.9 ± 6.9	106.7 ± 5.6	0.001

LVEDD left ventricular end-diastolic diameter, LVESD left ventricular end-systolic diameter, LVEF left ventricular ejection fraction.

LUS score was significantly lower in the group (1) than group (2) (10.8 ± 4.2 vs 16.5 ± 4.2 cm, P: 0.001). DTF was significantly higher in group (1) than group (2) (43.0 ± 10.7vs 28.9 ± 2.8 cm, P: 0.001).Duration of mechanical ventilation was significantly higher in-group (2) than group (1) (8.8 ± 0.6 vs 6.0 ± 1.4 cm, P: 0.001).

DTF can predict successful weaning with a cut-off point ≥ 29.5 with sensitivity 95.0% and specificity 80.0% with a P: value < 0.001. LUS score is good predictors for weaning failure with a cut-off point ≥ 15.5 with sensitivity 70.0% and specificity 82.5 % with a P: value < 0.001. RSBI also is good predictors for weaning failure, with a cut-off point ≥ 71.5 with sensitivity 80.0% and specificity 70% with a p-value < 0.001.

Discussion

Weaning from MV is one of the most frequently encountered challenges in modern ICUs.

Tools available for determining the optimal timing of weaning and prediction of its outcome are limited. Subjective decisions are usually wrong. Stroetz and Hubmayr found that clinical prediction of extubation success or failure was often incorrect with the decision to extubate biased toward ventilator dependency [29]. The US is well established as a noninvasive widely available, and easy to use can be performed by the intensivist for evaluation and management of mechanically ventilated patients and guide weaning from it. Chest US should include the examinations of the lungs and diaphragm [14]. LUS can accurately detect extravascular lung water and also quantify the degree of regional lung aeration loss. Studies illustrate that LUS can detect extravascular lung water accurately since they compared LUS result with the result of pulse-induced contour cardiac output (PICCO) and pulmonary artery catheter and it was closely similar [30], [31]. An LUS score has been validated to provide quantifiable comparable measures of progressive changes in aeration [18]. This prospective observational study was conducted on 0ne hundred patients underwent SBT weaning from MV shows several findings: SBT is correlated with significant lung derecruitment as assessed by LUS score that was significantly higher in the failed weaning group (P: 0.001), with cut-off value ≥ 15.5 predicted weaning failure with a sensitivity 70% and specificity 82.5%, AUC (0. 836) and P < 0.001. Soummer et al., in their study, concluded that, the lung derecruitment during SBT (assessed by lung u/s) is greater in patients who develop post-extubation distress (irrespective of its primary cause) than in patients who are definitively weaned (p < 0.001), they also concluded that LUS score > 17 (at the end of the SBT) is highly predictive of weaning failure and that derecruitment was made of partial loss of lung aeration rather than appearance of new consolidation [15].

Up till now, all indices that were used to predict extubation failure are indirect measurements of disorders of lung aeration, oxygen saturation defect, conscious level disturbances, hemodynamic instability, and physiological compensatory mechanisms, RSBI, and tachycardia that are patient-dependent [17]. The possibility of quantifying lung aeration directly at the end of an SBT offers an advantage for predicting weaning failure because decreased aeration is one of the critical pathophysiological factors [17].

DTF and weaning

DTF was significantly higher in the successfully weaned group (p 0.001), and with a cut-

off value ≥ 29.5, it can predict weaning success with a sensitivity 88.0% and specificity 80.0%, AUC (0.933).

Since its first production [13], diaphragmatic thickening evaluation by the US has been correlated with diaphragm strength and muscle shortening [19], [20], [32]. The volume of diaphragm muscle mass is constant as it contracts. Therefore, as it shortens, it thickens, and measures of its thickening fraction are inversely related to changes in diaphragm length. In support of this concept, the absence of diaphragm thickening has been noted in patients with diaphragm paralysis [19]. Since the diaphragm is the major muscle of inspiration, the presence of diaphragm contraction and shortening should be a prerequisite for successful extubation. Diaphragm thickness may be estimated by B or M-mode [27], [32].

Similar to our results, Ayman I Baess et al., conducted a study on Thirty patients who were planned for weaning after MV, they concluded that diaphragmatic thickening was better than displacement in prediction of weaning outcome, and a cutoff value ≥ 30 can predict successful extubation with sensitivity (69%), specificity (71%) and AUC (0.65) [36]. One of the potential explanations that diaphragm thickening would be a more accurate index of diaphragm contractile activity than excursion during pressure-support ventilation, that thickening can only be influenced by active contraction [34].

DiNino et al. studied 63 intubated patients during the SBT and determined the DTF, They found that a DTF cutoff ≥ 30% was a good predictor for successful weaning with sensitivity and specificity, 88% and 71%, respectively and AUC (0.79) [21].

Limitations: The study is a single centre trial.US is an operator-dependent technique; however, in the current study, the only one trained intensive care physician performed the examination. We only assessed the right hemidiaphragm as it can be easily visualised compared to the left side where imaging is commonly impeded by gastrointestinal gases. However, this limitation is common in other studies on US assessment of diaphragmatic contractile function [33]. For the diaphragmatic us, we did not compare it with the gold standard methods (e.g. Trans-diaphragmatic pressure-time) because of the invasive nature of the procedure and relatively comparable results in previous studies [33], [35]. LUS couldn't confirm the causes of lung derecruitment.

Physiology, practice, and causes of weaning failure support the use of integrated US to predict weaning failure. Chest ultrasound is an integrated bedside examination performed by the intensivist to assess the lung and diaphragm and help to understand the pathophysiological effects of weaning and help to optimise the clinical condition to improve the chances of successful weaning from ventilatory support.

References

1. Pinsky, M.R. and J.-F.o.A. Dhainaut, Pathophysiologic foundations of critical care. Williams & Wilkins, 1993.

2. MacIntyre NR, Cook DJ, Ely EW, Epstein SK, Fink JB, Heffner JE. Evidence-based guidelines for weaning and discontinuing ventilatory support. Chest. 2001; 120(6):375S-95S. https://doi.org/10.1378/chest.120.6_suppl.375S PMid:11742959

3. Esteban A, Alia I, Gordo F, Fernandez R, Solsona JF, Vallverdu I, Macias S, Allegue JM, Blanco J, Carriedo D, Leon M. Extubation outcome after spontaneous breathing trials with T-tube or pressure support ventilation. American journal of respiratory and critical care medicine. 1997; 156(2):459-65. https://doi.org/10.1164/ajrccm.156.2.9610109 PMid:9279224

4. Spicher JE, White DP. Outcome and function following prolonged mechanical ventilation. Archives of internal medicine. 1987; 147(3):421-5. https://doi.org/10.1001/archinte.1987.00370030025005

5. Frazier SK, Stone KS, Moser D, Schlanger R, Carle C, Pender L, Widener J, Brom H. Hemodynamic changes during discontinuation of mechanical ventilation in medical intensive care unit patients. American Journal of Critical Care. 2006; 15(6):580-93. PMid:17053265

6. Papaioannou VE, Stakos DA, Dragoumanis CK, Pneumatikos IA. Relation of tricuspid annular displacement and tissue Doppler imaging velocities with the duration of weaning in mechanically ventilated patients with acute pulmonary edema. BMC cardiovascular disorders. 2010; 10(1):20. https://doi.org/10.1186/1471-2261-10-20 PMid:20478065 PMCid:PMC2880285

7. Vassilakopoulos T, Zakynthinos S, Roussos CH. Respiratory muscles and weaning failure. European Respiratory Journal. 1996; 9(11):2383-400. https://doi.org/10.1183/09031936.96.09112383 PMid:8947090

8. Heunks LM, van der Hoeven JG. Clinical review: The ABC of weaning failure-a structured approach. Critical Care. 2010; 14(6):245. https://doi.org/10.1186/cc9296 PMid:21143773 PMCid:PMC3220047

9. McCool FD, Tzelepis GE. Dysfunction of the diaphragm. New England Journal of Medicine. 2012; 366(10):932-42. https://doi.org/10.1056/NEJMra1007236 PMid:22397655

10. Jaber S, Petrof BJ, Jung B, Chanques G, Berthet JP, Rabuel C, Bouyabrine H, Courouble P, Koechlin-Ramonatxo C, Sebbane M, Similowski T. Rapidly progressive diaphragmatic weakness and injury during mechanical ventilation in humans. Am J Respir Crit Care Med. 2011; 183(3):364-71. https://doi.org/10.1164/rccm.201004-0670OC PMid:20813887

11. Boles JM, Bion J, Connors A, Herridge M, Marsh B, Melot C, Pearl R, Silverman H, Stanchina M, Vieillard-Baron A, Welte T. Weaning from mechanical ventilation. European Respiratory Journal. 2007; 29(5):1033-56. https://doi.org/10.1183/09031936.00010206 PMid:17470624

12. Tobin MJ. Principles and practice of mechanical ventilation, LWW, 2006.

13. Grant BJ, Lieber BB. Compliance of the main pulmonary artery during the ventilatory cycle. Journal of Applied Physiology. 1992; 72(2):535-42. https://doi.org/10.1152/jappl.1992.72.2.535 PMid:1559929

14. Lichtenstein DA. Lung ultrasound in the critically ill. Annals of intensive care. 2014; 4(1):1. https://doi.org/10.1186/2110-5820-4-1 PMid:24401163 PMCid:PMC3895677

15. Soummer A, Perbet S, Brisson H, Arbelot C, Constantin JM, Lu Q, Rouby JJ, Bouberima M, Roszyk L, Bouhemad B, Monsel A. Ultrasound assessment of lung aeration loss during a successful weaning trial predicts postextubation distress. Critical care medicine. 2012; 40(7):2064-72. https://doi.org/10.1097/CCM.0b013e31824e68ae PMid:22584759

16. Bouhemad B, Zhang M, Lu Q, Rouby JJ. Clinical review: bedside lung ultrasound in critical care practice. Critical care. 2007; 11(1):205. https://doi.org/10.1186/cc5668 PMid:17316468 PMCid:PMC2151891

17. Bouhemad B, Brisson H, Le-Guen M, Arbelot C, Lu Q, Rouby JJ. Bedside ultrasound assessment of positive end-expiratory pressure-induced lung recruitment. American journal of respiratory and critical care medicine. 2011;183(3):341-7. https://doi.org/10.1164/rccm.201003-0369OC PMid:20851923

18. Bouhemad B, Liu ZH, Arbelot C, Zhang M, Ferarri F, Le-Guen M, Girard M, Lu Q, Rouby JJ. Ultrasound assessment of antibiotic-induced pulmonary reaeration in ventilator-associated pneumonia. Critical care medicine. 2010; 38(1):84-92. https://doi.org/10.1097/CCM.0b013e3181b08cdb PMid:19633538

19. Gottesman E, McCool FD. Ultrasound evaluation of the paralyzed diaphragm. American journal of respiratory and critical care medicine. 1997; 155(5):1570-4. https://doi.org/10.1164/ajrccm.155.5.9154859 PMid:9154859

20. Matamis D, Soilemezi E, Tsagourias M, Akoumianaki E, Dimassi S, Boroli F, Richard JC, Brochard L. Sonographic evaluation of the diaphragm in critically ill patients. Technique and clinical applications. Intensive care medicine. 2013; 39(5):801-10. https://doi.org/10.1007/s00134-013-2823-1 PMid:23344830

21. DiNino E, Gartman EJ, Sethi JM, et al. Diaphragm ultrasound as a predictor of successful extubation from mechanical ventilation. Thorax. 2013; 2013:204111.

22. Lindenfeld J, Albert NM, Boehmer JP, Collins SP, Ezekowitz JA, Givertz MM, Katz SD, Klapholz M, Moser DK, Rogers JG, Starling RC. HFSA 2010 comprehensive heart failure practice guideline. Journal of cardiac failure. 2010; 16(6):e1-194. https://doi.org/10.1016/j.cardfail.2010.04.004 PMid:20610207

23. Kydonaki K. Decision-making processes of weaning from mechanical ventilation: a comparative ethnographic insight into the dynamics of the decision-making environment. 2011.

24. McConville JF, Kress JP. Weaning patients from the ventilator. New England Journal of Medicine. 2012; 367(23):2233-9. https://doi.org/10.1056/NEJMra1203367 PMid:23215559

25. Yang KL, Tobin MJ. A prospective study of indexes predicting the outcome of trials of weaning from mechanical ventilation. New England Journal of Medicine. 1991; 324(21):1445-50. https://doi.org/10.1056/NEJM199105233242101 PMid:2023603

26. Ueki J, De Bruin PF, Pride NB. In vivo assessment of diaphragm contraction by ultrasound in normal subjects. Thorax. 1995; 50(11):1157-61. https://doi.org/10.1136/thx.50.11.1157 PMid:8553271 PMCid:PMC475087

27. Wait JL, Johnson RL. Patterns of shortening and thickening of the human diaphragm. Journal of Applied Physiology. 1997; 83(4):1123-32. https://doi.org/10.1152/jappl.1997.83.4.1123 PMid:9338420

28. Boon AJ, Harper CJ, Ghahfarokhi LS, Strommen JA, Watson JC, Sorenson EJ. Two-dimensional ultrasound imaging of the diaphragm: Quantitative values in normal subjects. Muscle & nerve. 2013; 47(6):884-9. https://doi.org/10.1002/mus.23702 PMid:23625789

29. Stroetz RW, Hubmayr RD. Tidal volume maintenance during weaning with pressure support. American journal of respiratory and critical care medicine. 1995; 152(3):1034-40. https://doi.org/10.1164/ajrccm.152.3.7663780 PMid:7663780

30. Lichtenstein DA, Mezière GA, Lagoueyte JF, Biderman P, Goldstein I, Gepner A. A-lines and B-lines: lung ultrasound as a bedside tool for predicting pulmonary artery occlusion pressure in the critically ill. Chest. 2009; 136(4):1014-20. https://doi.org/10.1378/chest.09-0001 PMid:19809049

31. Enghard P, Rademacher S, Nee J, Hasper D, Engert U, Jörres A, Kruse JM. Simplified lung ultrasound protocol shows excellent prediction of extravascular lung water in ventilated intensive care patients. Critical Care. 2015; 19(1):36. https://doi.org/10.1186/s13054-015-0756-5 PMid:25656060 PMCid:PMC4335373

32. Cohn D, Benditt JO, Eveloff S, McCool FD. Diaphragm thickening during inspiration. Journal of Applied Physiology. 1997; 83(1):291-6. https://doi.org/10.1152/jappl.1997.83.1.291 PMid:9216975

33. Vivier E, Dessap AM, Dimassi S, Vargas F, Lyazidi A, Thille AW, Brochard L. Diaphragm ultrasonography to estimate the work of breathing during non-invasive ventilation. Intensive care medicine. 2012; 38(5):796-803. https://doi.org/10.1007/s00134-012-2547-7 PMid:22476448

34. Umbrello M, Formenti P, Longhi D, Galimberti A, Piva I, Pezzi A, Mistraletti G, Marini JJ, Iapichino G. Diaphragm ultrasound as indicator of respiratory effort in critically ill patients undergoing assisted mechanical ventilation: a pilot clinical study. Critical Care. 2015; 19(1):161. https://doi.org/10.1186/s13054-015-0894-9 PMid:25886857 PMCid:PMC4403842

35. Summerhill EM, El-Sameed YA, Glidden TJ, McCool FD. Monitoring recovery from diaphragm paralysis with ultrasound. Chest. 2008; 133(3):737-43. https://doi.org/10.1378/chest.07-2200 PMid:18198248

36. Baess AI, Abdallah TH, Emara DM, Hassan M. Diaphragmatic ultrasound as a predictor of successful extubation from mechanical ventilation: thickness, displacement, or both? Egyptian Journal of Bronchology. 2016 May 1;10(2):162. https://doi.org/10.4103/1687-8426.184370

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Apr 15; 7(7):1148-1152.
https://doi.org/10.3889/oamjms.2019.201
eISSN: 1857-9655
Clinical Science

Evaluation of Paclitaxel, Ifosfamide, and Cisplatin (TIP) Regimen on Penile Cancer in Adam Malik Medan: A Single Center 2 Years of Experience

Ardiansyah Periadi Sitompul, Fauriski Febrian Prapiska*, Syah Mirsya Warli

Urology Division of Surgery Department, Adam Malik General Hospital, Sumatera Utara, Indonesia

Citation: Sitompul AP, Prapiska FF, Warli SM. Evaluation of Paclitaxel, Ifosfamide, and Cisplatin (TIP) Regimen on Penile Cancer in Adam Malik Medan: A Single Center 2 Years of Experience. Open Access Maced J Med Sci. 2019 Apr 15; 7(7):1148-1152. https://doi.org/10.3889/oamjms.2019.201

Keywords: Penile cancer; Chemotherapy; TIP

***Correspondence:** Fauriski Febrian Prapiska. Urology Division of Surgery Department, Adam Malik General Hospital, Sumatera Utara, Indonesia. E-mail: fauriski@gmail.com

Received: 27-Jan-2019; **Revised:** 05-Apr-2019; **Accepted:** 06-Apr-2019; **Online first:** 14-Apr-2019

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Penile tumour is a rare tumour in the genitourinary system, account for 0.4-0.6%. Although rare, patients are often unaware and come in late stage, so the use of chemotherapy agents is becoming crucial.

AIM: This study was conducted to evaluate responses and overall survival rate of Paclitaxel, Ifosfamide, and Cisplatin (TIP) regimen in penile cancer with nodal involvement.

METHODS: We included all medical records of penile squamous cell carcinoma patients associated with nodal involvement who acquired TIP regimen in Adam Malik Hospital between 2014 and 2016. We administered 175 mg/m^2 of Paclitaxel on day 1, 1200 mg/m^2 of Ifosfamide on days 1 to 3, and 25 mg/m^2 of Cisplatin on days 1 to 3 as our standard TIP regimen. The regimen was re-administered every 21-28 days. Characteristics of the patient including age, history of circumcision, races, primary lesion of the tumour and TNM staging were noted. Adverse event, clinical responses, and overall survival were assessed and evaluated.

RESULTS: We extracted data from 17 patients of penile cancer with nodal involvement who acquired TIP regimen with a mean age of 44.18 ± 11.13 years old from our medical records. Only 10 patients completed the full 4 cycles of the regimen. Four patients died before completion, two patients refused to continue the regimen, and 1 patient is still on the second cycle. Total penectomy was the most frequent procedure had taken, and clinical stage T4 and N3 was the most findings at initial diagnosis. There was no complete response noted. Six patients were noted as partial response, and 1 patient was noted as progressive disease. The Kaplan-Meier curve shows an overall 6 months (95% CI: 4.4-7.6 months) of survival with a median of follow-up time was 7 (1-11) months. In subgroup analysis, we found that the responder group has significantly better overall survival than the non-responder group (log-rank test, p = 0.004).

CONCLUSION: Paclitaxel, Ifosfamide, and Cisplatin (TIP) regimen give significant clinical benefit in penile cancer with nodal involvement.

Introduction

The penile tumour is a rare tumour of the genitourinary system, found only 0.4-0.6 % from all incidence of malignancy among men in Europe and the United States of America. However, it made up 10 % of all incidence of malignancy among men in Asian, South America, and Africa countries [1], [2]. In Indonesia, there is no clear data yet regarding the incidence of penile cancer. In eleven years (1994-2005), there were 69 patients of penile cancer that recorded in Dr Cipto Mangunkusumo General Hospital and Dharmais National Cancer Center Hospital Jakarta [3]. In Sanglah Hospital Bali, there were 46 patients of penile cancer during 8 years (1993-2001) [4]. Meanwhile, at Haji Adam Malik General Hospital Medan, the incidence of penile cancer during the last four years (2012-2015) was 34 patients [5]. Squamous cell carcinoma (SCC) of the penis was the most frequently found type of penile cancer [2], [5], [6].

The principle of management for penile cancer is to remove the tumour with as much organ preserved as possible, by either partial or total

penectomy, as an effort to reduce recurrence. Aside from treating the primary tumour, the involvement of lymph nodes is also an important factor to improve the patient's prognosis. Penile cancer is an aggressive disease, and the success of local lesion treatment is only when it is treated during the early stages. Meanwhile, penile cancer in late stages, accompanied by the involvement of regional lymph nodes or distant metastases, is still considered as a challenging problem for euro-oncologists [7].

Patients with unilateral lymph nodes metastasis, without extranodal involvement or involvement of pelvic lymph nodes, had a low recurrence rate of 10% to 20% after surgical management only. The recurrence rate was higher in patients with metastasis of bilateral lymph nodes, extranodal involvement, or the involvement of pelvic lymph nodes; which was 80% to 90% [8], [9]. The multi-modality approach, an approach combining chemotherapy and aggressive surgical management, might be effective, even though the local recurrence rate and progressivity were still high. Zou et al. reported that the administration of chemotherapy before lymphadenectomy on penile cancer patients with the involvement of regional lymph nodes (N3) showed great benefits. The administration of combination chemotherapy of bleomycin, methoprene, and cisplatin before lymphadenectomy resulted in 5-year overall survival rate 45.8%. This result statistically was greater in patients who were responsive to chemotherapy compared to those who were unresponsive (73.3% vs 0%, $p < 0.001$) [10]. Sarma et al. provided chemotherapy as adjuvant chemotherapy for patients with penile cancer after the removal of pelvic lymph nodes. The research showed an improvement in overall survival [11]. In cases where there were systemic metastases, chemotherapy was the choice of treatment [7], [12].

Various chemotherapy regiments were used as part of the management for penile cancer, either as neoadjuvant or adjuvant therapy. Cisplatin, bleomycin, and methotrexate were first used as single anti-tumour agents for penile cancer in the year 1975. However, the response rate was still low, with severe adverse effects; especially for bleomycin which was toxic for lungs [13].

Therefore, to improve the response rate of chemotherapy in penile SCC, various combinations of chemotherapy regimens were tested, the combinations usually consisted of two to three drugs [14]. The use of combined chemotherapy regiments was first reported by Shmmas et al., using 1000 mg/m^2 5-fluorouracil (5-FU) and 100 mg/m^2 cisplatin with three weeks intervals. Results from the research showed partial responses in two out of a total of eight cases treated, with adverse effects including septicemia, decreased renal function, nausea, and vomiting [15].

Regiment of taxane-based chemotherapy has been successfully used to treat SCC from two different locations. Combination chemotherapy using paclitaxel has been used to treat penile SCC since early 2000. Bermejo et al., used a combination of paclitaxel as neoadjuvant chemotherapy on two patients, resulting in prolonged post-chemotherapy survival rate followed by the removal of lymph nodes [16].

Joerger et al. reported significant remission in one of the patients with advanced disease after three cycles of paclitaxel/carboplatin [17]. The two types of research reported that the regiments were well tolerated [16], [17]. Pagliaro et al. conducted a phase two clinical trial of taxane-based chemotherapy regiment as neoadjuvant chemotherapy for penile SCC with the involvement of lymph nodes; the results showed that taxane-based chemotherapy had good response towards penile cancer with metastases to lymph nodes [12].

Reports regarding the use of taxane-based regiment for the management of penile SCC, either as neoadjuvant or adjuvant chemotherapy, were still limited so far. This condition might be due to the small number of penile SCC cases. At Haji Adam Malik General Hospital, the use of taxane as part of the chemotherapy regimen for penile SCC has been started since 2014. This study aims to evaluate the response for the administration of chemotherapy paclitaxel, ifosfamide, and cisplatin (TIP) regiments as well as to evaluate the overall survival (OS) rate.

Methods

This research is a retrospective, descriptive analytic study. Samples were collected from the year 2014 to 2016. Samples were patients with penile SCC which has been confirmed with histopathological examination results: enlargement of either unilateral or bilateral lymph nodes > 4 cm, with no signs of distant metastases. Patients with enlargement of pelvic lymph nodes found through computed tomography (CT) scan imaging were also included in the study, with or without biopsy results.

We used chemotherapy regimen TIP consisting of four cycles of paclitaxel 175 mg/m^2 on day 1, ifosfamide 1200 mg/m^2 on day 1 to 3, and cisplatin 25 mg/m^2 on day 1 to 3 with duration of 21-28 days. Patient's characteristics including age, ethnic, primary lesion, and TNM staging were noted. Occurring adverse effects, clinical response rate post-chemotherapy based on Response Evaluation Criteria in Solid Tumors (RECIST) criteria [18] and overall survival rate were also noted.

Results

There were 17 patients of penile cancer with the involvement of lymph node, who received chemotherapy regiment consisting of paclitaxel, ifosfamide, and cisplatin from June 2014 to December 2016 at Haji Adam Malik General Hospital Medan. Out of a total of 17 patients, only 10 patients who managed to receive the full regimen of chemotherapy completely. Meanwhile, four patients passed away before completing chemotherapy, two patients refused to continue receiving chemotherapy, and one patient was still on the second cycle of chemotherapy in December 2016. Patients' characteristics were shown in table 1.

In this research, we found that the mean age of patients was 44.18 ± 11.13 years old. All samples were Bataknese, and 16 patients (94.1%) were uncircumcised. Primary tumour lesion was found on the penile shaft (58.8%) and penile base (35.3%), with pathological results showing that 35.3% tumour had invaded surrounding tissues (T4) and had fixated nodules (N3) in 10 patients (58.8%). There was no patient found with distant metastases when diagnosed. Three patients had tumour staging Tx because they underwent an operation at other hospitals and their histopathological assessment results did not describe the T staging of their tumour.

Table 1: Baseline characteristics of patients (N = 17)

Characteristics	Number of patients	Percentage
Age (year)		
Mean	44.18 ±11.13	
Circumcision		
Yes	1	5.9
No	16	94.1
Ethnic		
Batak	17	100
Location of the primary tumour		
Penile glans	1	5.9
Penile shaft	10	58.8
Penile base	6	35.3
Tumour Staging		
T1a	0	0
T1b	1	5.9
T2	3	5.9
T3	3	17.6
T4	7	35.3
Tx	3	35.3
Nodule Staging		
N0	0	0
N1	3	17.6
N2	4	23.5
N3	10	58.8
Metastasis	0	0
Type of operation		
Partial penectomy	6	35.3
Total penectomy	11	64.7
Histopathological type		
SCC	17	100

Eleven patients (64.7%) who received chemotherapy with paclitaxel, ifosfamide, and cisplatin underwent total penectomy while 6 patients (35.3%) underwent partial penectomy; the histopathological type of all tumour (100%) was squamous cell carcinoma (SCC).

Table 2: Response to chemotherapy after four cycles of TIP

Response towards chemotherapy	Frequency	Percentage
Complete response	0	0
Partial response	6	60
Stable	3	30
Progressive	1	10

There was no patient with a complete response. However, there were six patients with partial responses, three patients with a stable response, and one patient showed progressive disease.

Table 3: Adverse effects during chemotherapy of TIP

Adverse effects	Frequency (n)	Percentage (%)
Neutropenia	8	47.1
Thrombocytopenia	7	41.2
Anaemia	14	82.4
Mucositis	6	35.3
Nausea	12	70.6
Diarrhoea	2	11.8
Constipation	0	0
Alopecia	15	88.2
Allergy	0	0

Eleven out of 17 patients receiving chemotherapy passed away with median overall survival (OS) of 6 months (95% CI: 4.4-7.6 months). The median time for follow up was seven months (ranging from 1 to 11 months).

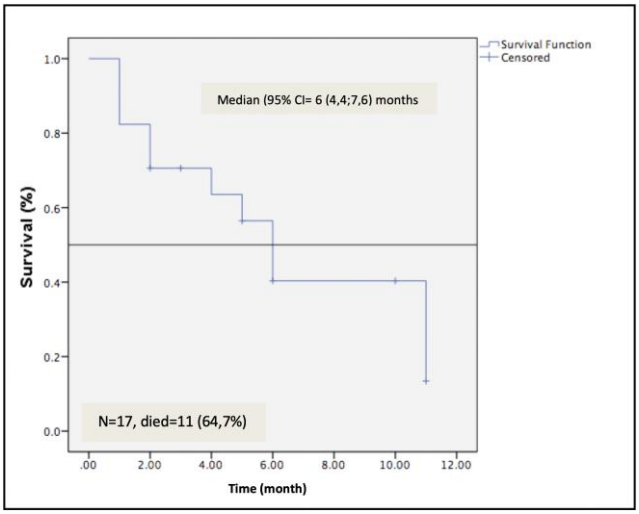


Figure 1: Kaplan-Meier graph overall survival rate (OS)

We conducted subanalysis towards the responsive and unresponsive sub-groups regarding the administration of paclitaxel, ifosfamide, and cisplatin-based chemotherapy. Eight patients passed away in the unresponsive group, while only two patients passed away in the responsive group. The overall survival rate was significantly higher in responsive patients compared to unresponsive patients (log-rank test, p = 0.004).

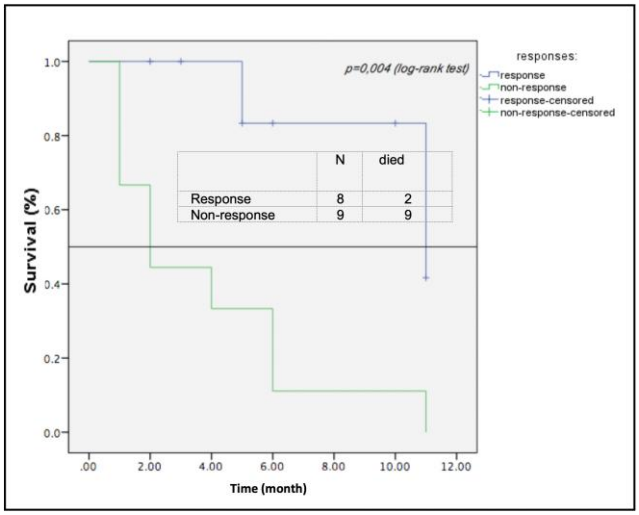


Figure 2: Kaplan-Meier graph overall survival rate (OS) of responsive and unresponsive sub-groups

Discussion

Based on our experiences in using paclitaxel, ifosfamide, and cisplatin-based chemotherapy as part of penile cancer management in Haji Adam Malik General Hospital, Medan, we found 17 patients who received the regiment from June 2014 to December 2016. Penile cancer is an aggressive disease, in which success for local lesion management could only be achieved during the early stages. The success of penile cancer management with regional and systemic metastases is still a challenge for urologists. In situations where there were metastases on regional lymph nodes, administration of chemotherapy combined with aggressive surgical management might be effective, even though the recurrence rate and progressivity were still high. In cases where systemic metastasis was found, chemotherapy becomes the only choice of treatment [7].

Most penile cancers are squamous cell carcinoma (SCC). In this research, the histopathological type of all samples was SCC (100%) with T4 (35.3%) and N3 (58.8%) as the most common staging results. Thus, it can be concluded that most patients who came to Haji Adam Malik General Hospital Medan were already in the late stages of the disease. This condition is commonly found in patients with penile SCC, as also found in the research by Lynch and Krush, which reported 15-50% patients delayed up to one year before they are finally looking for treatment [19].

There were six patients with partial responses after four cycles of chemotherapy in our research; however, there was no sample which showed a complete response. In the research by Pagliaro, there were found three patients with complete response and twelve patients showing the partial response from total research samples of 30 patients; 13.6% of all patients showing a complete pathological response (pCRs) [12]. Other researches which used a taxane-based combination as neoadjuvant chemotherapy in advance stage penile cancer resulted in 60% responsive patients, with 4% showing complete pathological response [20]. Kubota et al. reported that the TIP combination was effective for penile cancers with the involvement of lymph nodes [21].

The administration of chemotherapy for penile cancer with lymph nodes metastases is quite common. Both adjuvant and neoadjuvant chemotherapy could increase overall survival [12], [16], [22], [23]. The largest prospective research by the Southwest Oncology Group (SWOG) reported of patients receiving a combination of bleomycin, methotrexate, and cisplatin. In the research, the overall survival rate was 32.5% with median overall survival of 28 weeks. Even though the overall survival rate for the regimen was quite high, it was not well tolerated since there was also a high mortality rate due to chemotherapy (13.9%) [23]. Our study showed an overall survival of 6 months. This result was lower when compared to the results from the study by Pagliaro, which was OS 17.1 months [12]. This difference might be caused by the fact that in this research, the intervention was only chemotherapy; there was no patient who underwent inguinal lymphadenectomy (ILND).

Sub-group analysis of patients who were responsive and unresponsive to chemotherapy showed a statistically significantly higher overall survival in patients who were responsive to chemotherapy (11 months vs 2 months, $p = 0.004$). The response towards chemotherapy might be used as predictors for chemotherapy success as part of management for penile cancer.

There were a few adverse effects occurring during the administration of TIP. In this study, the most commonly found adverse effects included alopecia, nausea, and anaemia. The alopecia was temporary and will subside once the chemotherapy was stopped. Djajadiningrat et al. discovered a few adverse effects during the administration of taxane-based combination chemotherapy in each patient and concluded that the adverse effects of taxane-based combination chemotherapy were less well-tolerated [20].

In conclusion, the administration of paclitaxel, ifosfamide, and cisplatin-based chemotherapy is effective, showing improvement in overall survival of patients with penile cancer, with the involvement of regional lymph nodes. Patients who were responsive to chemotherapy had longer overall survival, although, a combination of chemotherapy and surgical management will show better results.

References

1. Mottet N, Avances C, Bastide C, Culine S, Ibora F, Kouri G, et al. Penile Tumors. Prog Urol. 2004; 14(903):905-11.

2. Pettaway CA, Lance RS, Davis JW. Tumors of the Penis. In: Wein AJ, editor. Campbell-Walsh Urology. 10 ed., 2012:901-1000. https://doi.org/10.1016/B978-1-4160-6911-9.00034-7

3. Tranggono U, Umbas R. Karakteristik dan terapi penderita keganasan penis di RS Cipto Mangunkusumo dan RS Kanker Dharmais. Indonesian Journal of Cancer. 2008:45-50.

4. Kusmawan E, Bowolaksono, Widiana R. The Clinical Features of Penile Cancer Patients at Sanglah General Hospital Bali-Indonesia. Bali Medical Journal. 2012; 1(1):1-5.

5. Irawan W, Warli SM. Karakteristik Penderita Kanker Penis di RSUP H Adam Malik Medan. 2015.

6. Cubilla AL, Lloveras B, Alemany L, Alejo M, Vidal A, Kasamatsu E, et al. Basaloid squamous cell carcinoma of the penis with papillary features: a clinicopathologic study of 12 cases. Am J Surg Pathol. 2012; 6:869-75. https://doi.org/10.1097/PAS.0b013e318249c6f3 PMid:22367299

7. Hakenberg OW, Protzel C. Chemotherapy in penile cancer. Therapeutic Advances in Urology. 2012; 4(3):133-8. https://doi.org/10.1177/1756287212441235 PMid:22654965 PMCid:PMC3361747

8. Novara G, Galfano A, Marco VD. Prognostic factors in squamous cell carcinoma of the penis. Nat Clin Pract Urol. 2007; 4:140-6. https://doi.org/10.1038/ncpuro0751 PMid:17347658

9. Pandey D, Mahajan V, Kannan RR. Prognostic factors in node-positive carcinoma of the penis. J Surg Oncol. 2006; 93:133-8. https://doi.org/10.1002/jso.20414 PMid:16425300

10. Zou B, Han Z, Wang Z, Bian J, Xu J, Wang H, et al. Neoadjuvant therapy combined with a BMP regimen for treating penile cancer patients with lymph node metastasis: a retrospective study in China. Journal of Cancer Research and Clinical Oncology. 2014; 140(10):1733-8. https://doi.org/10.1007/s00432-014-1720-5 PMid:24906876

11. Sharma P, Djajadiningrat R, Zargar-Shoshtari K, Catanzaro M, Zhu Y, Nicolai N, et al. Adjuvant chemotherapy is associated with improved overall survival in pelvic node-positive penile cancer after lymph node dissection: a multi-institutional study. urologic oncology. 2015; 33(11):17-23. https://doi.org/10.1016/j.urolonc.2015.05.008 PMid:26072110

12. Pagliaro LC, Williams DL, Daliani D, Williams MB, Osai W, Kincaid M, et al. Neoadjuvant Paclitaxel, Ifosfamide, and Cisplatin Chemotherapy for Metastatic Penile Cancer: A Phase II Study. Journal of Clinical Oncology. 2010; 28(24):3851-7. https://doi.org/10.1200/JCO.2010.29.5477 PMid:20625118 PMCid:PMC2940402

13. Protzel C, Ruppin S, Milerski S, Klebingat KJ, Hakenberg OW. The current state of the art of chemotherapy of penile cancer: results of a nationwide survey of German clinics. Urologe A. 2009; 48:1495-8. https://doi.org/10.1007/s00120-009-2108-z PMid:19774356

14. Protzel C, Hakenberg OW. Chemotherapy in patients with penile carcinoma. Urol Int. 2009; 82:1-7. https://doi.org/10.1159/000176016 PMid:19172088

15. Shammas FV, Ous S, Fossa SD. Cisplatin and 5-fluorouracil in advanced cancer of the penis. J Urol. 1992; 147:630-2. https://doi.org/10.1016/S0022-5347(17)37327-5

16. Bermejo C, Busby JE, Spiess PE, Heller L, Pagliaro LC, Pettaway CA. Neoadjuvant chemotherapy followed by aggressive surgical consolidation for metastatic penile squamous cell carcinoma. J Urol. 2007; 177(4):1335-8. https://doi.org/10.1016/j.juro.2006.11.038 PMid:17382727

17. Joerger M, Warzinek T, Klaeser B, J. T. Kluckert, Schmid HP, Gillessen S. Major tumor regression after paclitaxel and carboplatin polychemotherapy in a patient with advanced penile cancer. Urology. 2004; 63:778-80. https://doi.org/10.1016/j.urology.2003.12.026 PMid:15072904

18. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). European Journal of Cancer. 2009; 45:228-47. https://doi.org/10.1016/j.ejca.2008.10.026 PMid:19097774

19. Lynch HT, Krush AJ. Delay factors in detection of cancer of the penis. Nebr State Med J. 1969; 54(6):360-7. PMid:4238912

20. Djajadiningrat RS, Bergman AM, Werkhoven Ev, Vegt E, Horenblas S. Neoadjuvant taxane-based combination chemotherapy in patients with advanced penile cancer. cLINICal Genitourinary Cancer. 2015; 13(1):44-9. https://doi.org/10.1016/j.clgc.2014.06.005 PMid:25009098

21. Kubota Y, Nakano M, Nagai S, Matsuoka K, Arakawa H, Horie K, et al. Dramatic response of penile cancer with inguinal lymph node metastases to neoadjuvant chemotherapy with paclitaxel, ifosfamide and cisplatin: a case report. Acta urologica Japonica. 2015; 61(1):33-7.

22. Sharma P, Djajadiningrat R, Zargar-Shoshtari K, Catanzaro M, Zhu Y, Nicolai N, et al. Adjuvant chemotherapy is associated with improved overall survival in pelvic node-positive penile cancer after lymph node dissection: a multi-institutional study. Urol Oncol. 2015; 33(11):17-23. https://doi.org/10.1016/j.urolonc.2015.05.008 PMid:26072110

23. Haas GP, Blumenstein BA, Gagliano RG, Russell CA, Rivkin SE, Culkin DJ, et al. Cisplatin, methotrexate and bleomycin for the treatment of carcinoma of the penis: A Southwest Oncology Group study. Journal of Urology. 1999; 161(6):1823-5. https://doi.org/10.1016/S0022-5347(05)68815-5

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Apr 15; 7(7):1153-1159.
https://doi.org/10.3889/oamjms.2019.154
eISSN: 1857-9655
Clinical Science

The Role of Placental Growth Factor, Soluble Endoglin, and Uterine Artery Diastolic Notch to Predict the Early Onset of Preeclampsia

Muara Panusunan Lubis[1*], Herman Hariman[2], Sarma N. Lumbanraja[1], Adang Bachtiar[3]

[1]Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia; [2]Department of Clinical Pathology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia; [3]Department of Health Policy and Administration, Faculty of Public Health, Universitas Indonesia, Jakarta, Indonesia

Citation: Lubis MP, Hariman H, Lumbanraja SN, Bachtiar A. The Role of Placental Growth Factor, Soluble Endoglin, and Uterine Artery Diastolic Notch to Predict the Early Onset of Preeclampsia. Open Access Maced J Med Sci. 2019 Apr 15; 7(7):1153-1159. https://doi.org/10.3889/oamjms.2019.154

Keywords: Placental Growth Factor; sEng; Diastolic Notch; Preeclampsia

***Correspondence:** Muara Panusunan Lubis. Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia. E-mail: muaraplubis@gmail.com

Received: 05-Feb-2019; **Revised:** 09-Apr-2019; **Accepted:** 10-Apr-2019; **Online first:** 14-Apr-2019

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Reducing maternal mortality is one of the targets in the Millennium Development Goals (MDGs). In a systematic review, 4.6 per cent (95% CI 2.7-8.2) of pregnancies were complicated by preeclampsia worldwide. Preeclampsia occurs in around 10% of pregnancies in the world whereas developing countries contribute more than developed countries. In developing countries, there are 13 cases of preeclampsia in every 1,000 births, whereas in developed countries only 2-3 cases of preeclampsia are found in every 10,000 deliveries. Variations in prevalence among countries reflect, at least in part, differences in the distribution of maternal age and the proportion of nulliparous pregnant women in the population.

AIM: We aimed to investigate the role of placental growth factor, soluble endoglin, and uterine artery diastolic notch to predict the early onset of preeclampsia.

METHODS: This study used an analytical study with a nested case-control design. The study was conducted at Bunda Thamrin Hospital, Tanjung Mulia Mitra Medika Hospital, Sundari Hospital and a private clinic, from March to November 2018 with a total sample of 70 research subjects.

RESULTS: Uterine artery diastolic notch was not found in 50% of subjects. A total of 27 subjects (38.6%) had a unilateral diastolic notch, and 8 subjects (11.4%) had a bilateral diastolic notch. Cut-off point PlGF levels was 441 pg/ml, and Area Under Curve (AUC) 82.5% (95% CI 61.5%-100%), with sensitivity 80% and specificity 87.7%. The levels sEng in this study could not predict the incidence of early-onset preeclampsia (p = 0.113). Combined PlGF and pulsatile index of uterine arteries may predict early onset preeclampsia with sensitivity 40% and specificity 90.77%. From these results, pregnant women o 22-24 weeks of pregnancy, the levels of PlGF and the uterine artery pulsatility index can be a predictor of early-onset preeclampsia. Examination of PlGF levels alone is sufficient as a predictor of early-onset preeclampsia.

CONCLUSION: From these results, it can be concluded that in pregnant women of 22-24 weeks, the diastolic notches in uterine arteries cannot predict the incidence of early-onset preeclampsia. PlGF levels and pulsatile index of uterine arteries can be used as predictors of early-onset preeclampsia although examination of PlGF levels alone is sufficient as a predictor of early-onset preeclampsia.

Introduction

According to the American Family Association dividing the number of risk factors for Preeclampsia in 3 (three) groups, namely pregnancy-related factors, maternal factors and paternal factors. Maternal factors are more biologically maternal, including age, parity, race, history of preeclampsia, history of hypertension and so on. Also, many theories suggest that the pathogenesis of preeclampsia is related to the placentation process, but to date, the pathogenesis of preeclampsia is still unclear. Because the multifactorial pathogenesis of the pre-eclampsia phenotype is unexplained, prevention and prediction are still unknown, treatment of clinical symptoms must be the main thing in preventing maternal morbidity and mortality [1].

In preeclampsia, trophoblast invasion and velocimetry doppler can detect quantitative and qualitative changes in uterine arteries in waveform. In Doppler velocimetry, it can be seen the blood flow in the uterine artery, arcuate, radial and spiral around the trophoblast tissue, so that measurements can be made on the various indices needed [2], [3]. The

uterine artery wave appearance in the first trimester of pregnancy has a diastolic notch that disappears after 24 weeks of pregnancy. If the picture of this curve persists and the PI and RI values remain high after a pregnancy of 20-24 weeks, it means that there is high pressure in the uterine arteries which will usually result in preeclampsia or stunted fetal growth [4]. Endothelial dysfunction leads to progressive tissue and multiorgan damage to the mother and fetus. song is a dissolved form of a surface co-receptor transforming growth factor (TGF-β1 and TGF-β3) expressed on endothelial cells and syncytiotrophoblasts. It modulates the work of TGF-β1 and TGF-β3 which play an important role in vascular homeostasis. Concentration increases when placental perfusion is poo so that that serum levels can act as markers of changes in the impedance of the uteroplacental circulation [1], [5].

We aimed to investigate the role of placental growth factor, soluble endoglin, and uterine artery diastolic notch to predict the early onset of preeclampsia.

Methods

This study used an analytical study with a nested case-control design. The study was conducted at Bunda Thamrin Hospital, Tanjung Mulia Mitra Medika Hospital, Sundari Hospital and a private clinic, from March to November 2018 with a total sample of 70 research subjects.

Results

The research followed by 70 pregnant women with gestational age 22-24 The week came to Bunda Thamrin Hospital, Tanjung Mulia Mitra Medika Hospital, Sundari Hospital and private practice that had fulfilled the inclusion and exclusion criteria.

Table 1: Demographic Characteristics of Research Subjects

Characteristics of Subjects	n = 70
Age of Pregnancy, n (%)	
22 weeks	22 (31.4)
23 weeks	13 (18.6)
24 weeks	35 (50)
BMI, mean (SD), kg/m^2	24.47 (4.02)
Parity, n (%)	
Primigravida	31 (44.3)
Second Gravida	23 (32.9)
Multigravidas	16 (22.9)

Subjects with 24 weeks' gestation were the most subjects with a total of 35 people (50%). A total of 31 subjects (44.3%) were primigravida.

Table 2: Diastolic Notch Examination Results on Right and Left Uterine artery

Uterine Diastolic Notch, n (%)	n = 70
Without Diastolic Notch	35 (50)
Unilateral Diastolic Notch	27 (38.6)
Bilateral Diastolic Notch	8 (11.4)

Using ultrasound, it is known that as many as 50% of subjects have the one without the dichotomy. A total of 27 subjects (38.6%) had a unilateral diastolic notch, and 8 subjects (11.4%) had a bilateral diastolic notch.

Table 3: Results of Uterine artery Examination, Placental Growth Factor (PlGF) Level, and soluble Endoglin (sEng) level

	Uterine A.	PlGF	sEng
Mean	1.14	834.21	5.37
SD	0.36	413.75	1.99
Minimum	0.44	165	2.66
Maximum	2 , 08	2097	12.68
CI 95%	1.05-1.22	735.56 - 932.87	4.90 - 5.85

The results of the mean examination A. Female is 1.14 with SD = 0.36 with the lowest level was 0,44 and highest 2.08. PlGF is 834.21 with SD = 413.75, with the lowest level of 165 and the highest of 2097. The mean level of the sEng is 5.37 with SD = 1.99 with the lowest level of 2.66 and the highest 12.68.

Table 4: The incidence of Early Onset Preeclampsia

Preeclampsia	n = 70
Normal	65 (92.9)
Preeclampsia (proteinuria + 3)	2 (2.9)
Preeclampsia (proteinuria + 4)	3 (4.3)

From the results of monitoring of all subjects during the study, it was found that there were 65 subjects (92.9%) did not experience preeclampsia, 2 subjects (2.9%) had preeclampsia (proteinuria +3) and 3 subjects (4.3%) with preeclampsia (proteinuria +4).

Table 5: The difference of PlGF and sEng level between Subjects with Diastolic Notch and Without Diastolic Notch

	Uterine Diastolic Notch		P
	+ (n = 35)	(n = 35)	
IP a. Uterina, mean (SD)	1.42 (0.26)	0.85 (0.17)	< 0.001[a]
PlGF, mean (SD)	695.6 (385.69)	972.83 (398.88)	0.004[a]
sEng , mean (SD)	5.44 (2.39)	5.30 (1.51	0.533[b]

[a]*T Independent;* [b]*Mann Whitney.*

Mean A. uterina levels in subjects with diastolic notch group was 1.42 (SD = 0.26) while the mean A. uterina in the group without diastolic notch was 0.85 (SD = 0.17). Using the Independent T-test showed that there was a significant difference in uterine artery levels between subjects with diastolic notch and without diastolic notch ($p < 0.001$). The mean PlGF level in the group of subjects with a diastolic notch was 695.6 (SD = 385.69) while the mean of PlGF in the group without diastolic notch subjects was 972.83 (SD = 398.88). Using the Independent T-test showed that there was a significant difference in PLGF between subjects with diastolic notch and without diastolic notch ($p = 0.004$). The mean level in subjects in the diastolic notch group

was 5.44 (SD = 2.39) while the mean level in the group without diastolic notch subjects was 5.30 (SD = 1.51) using the Mann Whitney test indicating that there were differences in the mean sEng significant between subjects with diastolic notch and without diastolic notch (p = 0.533).

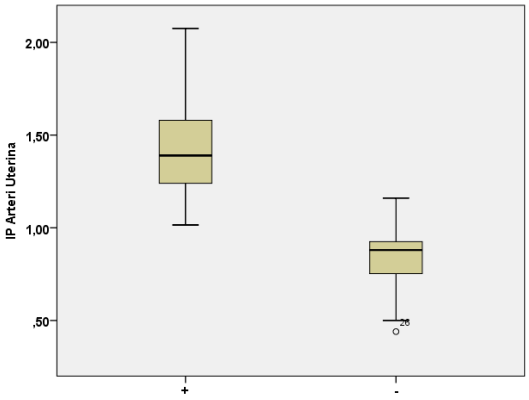


Figure 1: Boxplot Graph of PI Differences a. uterina in subjects with diastolic notch and without diastolic notch

There were no subjects over the age of 35 who had early onset preeclampsia, while there were 5 people (8.1%) subjects aged ≤ 35 years had preeclampsia. The results of the analysis using Fischer's exact test showed that no significant association was found between age and the incidence of early-onset preeclampsia (p = 1.000).

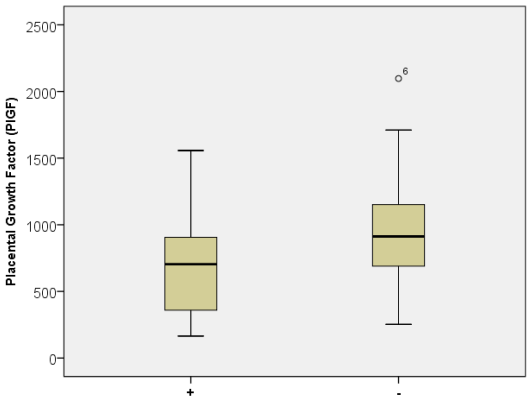


Figure 2: Boxplot graph differences in uterine arterial PlGF levels in subjects with diastolic notch and without diastolic notch

From the 31 subjects with primigravida pregnancies, there were only 3 subjects (9.6%) who had early onset preeclampsia, while there were 2 more samples with early-onset preeclampsia found in women with multigravida pregnancy. The results of the analysis using Fischer's exact test showed that there was no significant relationship between maternal parity and the incidence of early-onset preeclampsia (p = 0.251).

From the 32 subjects who had overweight and obesity BMI, there was only 1 subject (3.1%) who had early onset preeclampsia, while there were 4 people (14.8%) subjects with underweight and normoweight who had preeclampsia. The results of the analysis using Fischer's exact test showed that there was no significant relationship between BMI and the incidence of early-onset preeclampsia (p = 0.169).

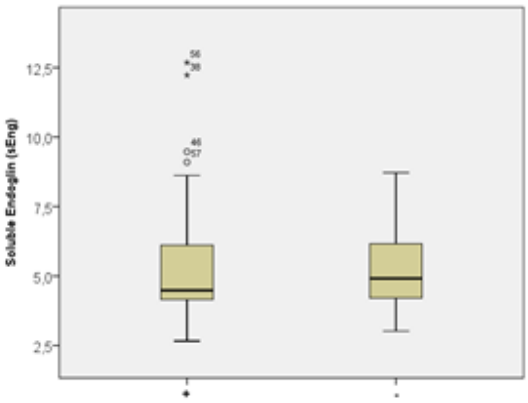


Figure 3: Boxplot graph of levels of difference in subjects with diastolic notch and without diastolic notch

From the 35 subjects who had uterine artery diastolic notch, there were 5 subjects (14.3%) who had early onset preeclampsia, while no preeclampsia was found in a subject that did not have a diastolic notch. The results of the analysis using Fischer's exact test showed that no significant association was found between uterine artery diastolic notch and the incidence of early-onset preeclampsia (p = 0.054).

Table 6: Relationship of Age, Parity, BMI, Diastolic Notch, PlGF level and sEng level early onset of preeclampsia

	Preeclampsia		p
	+ (n = 5)	- (n = 65)	
Age,n (%)			
> 35 years old	0	8 (100)	1.000[a]
≤ 35 years old	5 (8.1)	57 (91.9)	
Parity			
Primigravida	3 (9.6)	28 (90.4)	
Secundi Gravida	0	23 (100)	0.251[a]
Multigravida	2 (12.5)	14 (87.5)	
BMI, n (%)			
Overweight dan Obese	1 (3.1)	31 (96.9)	0.169[a]
Underweight dan Normoweight	4 (14.8)	23 (85.2)	
Diastolic Notch			
Found	5 (14.3)	30 (85.7)	0.054[a]
Not found	0	35 (100)	
PI mean (SD)	1.44 (0.30)	1.11 (0.35)	0.045[b]
PlGF, mean (SD)	411 (301.67)	866.77 (404.73)	0.016[c]
sEng, mean (SD)	8 (4.11)	5.17 (1.62)	0.113[c]

[a]Fischer's Exact; [b]T Independent; [c]Mann Whitney.

The mean PI uterine artery in early-onset preeclampsia subjects was lower with a mean of 1.44 (SD = 0.30) compared to subjects who did not experience preeclampsia with a mean of 1.11 (SD = 0.35). Using the Mann Whitney test showed that there were differences in the mean PI uterine artery between subjects with preeclampsia and those without preeclampsia (p = 0.045).

The mean PlGF in early-onset preeclampsia subjects was lower with a mean of 411 (SD = 301.67) than subjects who did not experience preeclampsia with a mean of 866.77 (404.73). Using the Mann Whitney test showed that there were differences in the

mean PLGF levels between subjects with preeclampsia and those without preeclampsia (p = 0.016).

The mean score in subjects with early-onset preeclampsia was seen to be higher with a mean of 8 (SD = 4.11) than subjects without preeclampsia with a mean of 5.17 (1.62). Using the Mann Whitney test showed that there was no difference in mean levels between subjects with preeclampsia and those without preeclampsia (p = 0.113).

Pulsality Index of Uterine Artery

Analysis showed that the ROC curve obtained a value of p = 0.037 which means that uterine artery IP in this study can predict the incidence of early-onset preeclampsia Area Under Curve (AUC) of 78.2% (95% IK 59.3% - 97%).

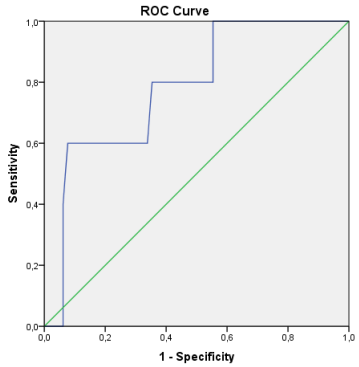


Figure 4: ROC curve of uterine artery IP against preeclampsia

Based on sensitivity and specificity curves in Figure 5, the Cut Off value for uterine arterial IP is obtained as 1,228. By using *cut-off points* 1,228, the uterine artery IP sensitivity value was 80%, and the specificity was 64.6%.

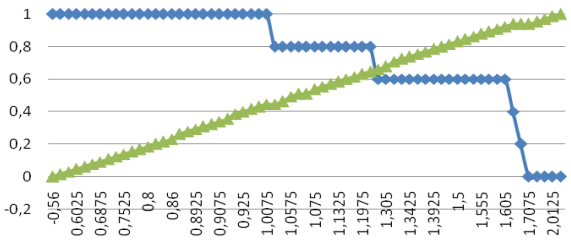


Figure 5: Uterine Arterial IP sensitivity (blue) and specificity (green) curve for the incidence of early-onset preeclampsia

PIGF

From the analysis using the ROC curve the value p = 0.016 means that PlGF in this study can predict the incidence of early onset preeclampsia with a value of Area Under Curve (AUC) 82.5% (95% IK 61.5% - 100%).

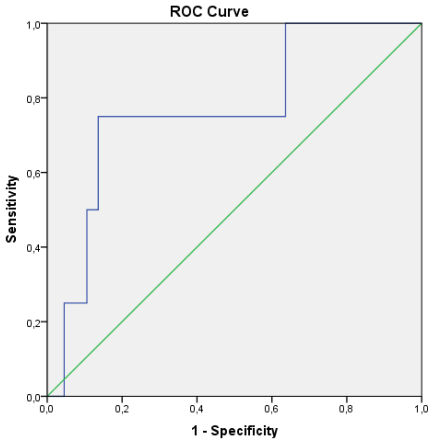


Figure 6: ROC Curve of PlGF against Preeclampsia

Based on the sensitivity and specificity curves in Figure 7, the Cut Off value for PlGF levels is obtained by 441. By using the cutoff point 441, the sensitivity value of PlGF is 80%, and the specificity is 87.7%.

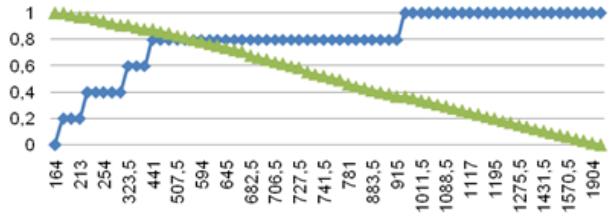


Figure 7: Curve sensitivity (blue) and specificity (green) of PlGF of genesis early-onset preeclampsia

sEng

From the results of analysis using ROC curves obtained by value p = 0,113 which means that sEng in this study could not predict the incidence of early-onset preeclampsia.

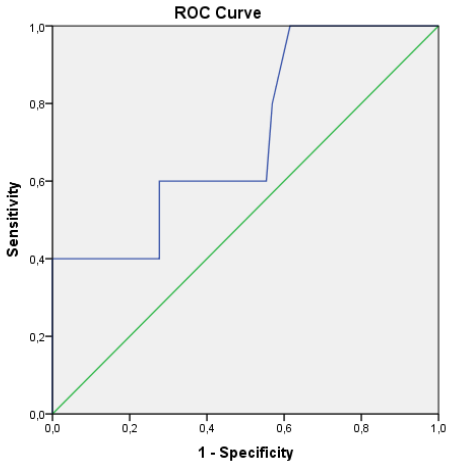


Figure 8. ROC Curves of Preeclampsia

Discussion

The research followed by 70 pregnant women aged 22-24 weeks who came to the Bunda Thamrin Hospital, Tanjung Mulia Mitra Medika Hospital, Sundari Hospital and private clinic that had met the inclusion criteria and exclusion. Pregnant women who collected a mean age of < 35 years (88.5%) dominated this study, with a mean Body Mass Index of 24.47 kg/m^2 (overweight). Subjects with 24 weeks' gestation were the most subjects with a total of 35 people (50%). A total of 31 subjects (44.3%) were primigravida who is one risk factor for early-onset preeclampsia. While the age of pregnant women > 35 years and nutritional conditions of women in overweight or obese conditions are a risk factor for the occurrence of late-onset preeclampsia, although this study still records the characteristics of the sample state [29].

Table 7: Sensitivity, specificity, the positive and negative predictive value of PI Uterine artery and PlGF level of Early-onset Preeclampsia

		Preeclampsia		Sensitivity	Specificity	NPP	NPN
		Positive	Negative				
IP.	1,228	4	2223	80%	64.6%	14., 8	97.7%
Uterina A.	<1,228	1	42				
PlGF	≤ 441	4	8	80%	87.7%	33.3%	98.3%
	> 441	1	57				
PlGF + IP Uterina A.				40%	90.77%	25%	95.16%

A total of 27 subjects (38.6%) had a unilateral diastolic notch, and 8 subjects (11.4%) had a bilateral diastolic notch. But this does not make the reference value a predictor of the incidence of early-onset preeclampsia. Different analytical methods are used to measure the reference value. Several other studies also included ultrasound examinations with the presence of diastolic notch, Kushtagi & Emani, 2016 found 38 patients with diastolic notch diagnosed with preeclampsia from a total of 48 patients with preeclampsia, Vartun, Flo, Widnes, & Acharya, 2016 found 23 samples with diastolic notch from 27 samples [6], [7].

Different studies have found that the mean PLGF level in preeclampsia is following 125 pg/ml at gestational age 20-37 weeks [8]: 95 pg/ml at midtrimester gestational age [9]; 38.7 pg/ml at 32-37 weeks gestation [10]; 54 pg/ml at 20-34 weeks gestation [11]; 83.75 pg/ml at 20-30 weeks gestation [12]; 239.75 pg/ml at 25-40 weeks' gestation [13].

The mean level sEng in this study was 8.0 ng/ml at 22-24 weeks' gestation. In other studies it can be found that the mean serum level in preeclampsia is 53.3 ng/ml at gestational age less than 34 weeks [14]; 11.08 ng/ml at gestational age 19-24 weeks [15]; 60.9 ng/ml during the first and second trimester of pregnancy [16]; 69.2 ng/ml at the third trimester of pregnancy [17] 11.58 ng/ml at gestational age less than 37 weeks [18].

Diastolic Notch and the incidence of Early Onset Preeclampsia

From the 35 samples of pregnant women who had a diastolic notch, it was found that there were 5 samples experiencing early-onset preeclampsia, with a p-value of 0.054 (> 0.05) that did not have a significant relationship, this was probably due to the number of samples there are still a few who have preeclampsia.

Other studies also found that there were significant differences between patients with normal pregnancies and patients with pregnancy who experienced preeclampsia, namely the study of Vartun, Flo, Widnes, & Acharya, 2016 with a p-value < 0.0001 (p < 0.05) very meaningful by finding 23 patients with diastolic notch who had preeclampsia compared to 4 patients with diastolic notch as well but not preeclampsia [19].

Mean Pulsatility Index of Uterine Artery for the Incidence of Early Onset Preeclampsia

Mean of IP uterine artery in subjects with early-onset preeclampsia seen higher with a mean of 1.44 (SD = 0.30) than subjects who did not experience preeclampsia with a mean of 1.11 (0.35) with a p-value of 0.045 which means that it has a significant value to distinguish cases preeclampsia. From the previous research studies, it was also dominated by significant results that were in line with this study, which made the first step to conduct a non-invasive screening. Other studies by (Vartun, Flo, Widnes, & Acharya, 2016), (Yu, Cui, Chen, & Chang, 2017), and (Narang, Agarwal, Das, Pandey, Agrawal, & Ali, 2016) with p-value very significant < 0,0001 was performed in early trimester pregnant women who ended preeclampsia [19], [20], [21].

PlGF and incidence of Early Preeclampsia

Previous studies have also been conducted by Bian, Shixia, & Duan, 2015 which showed a significant difference in the decrease in PLGF levels in preeclampsia performed in first trimester pregnant women with an mean of 115.72 pg/ml compared to control 217.30 pg/ml (p-value < 0.001) [22]; and by Gannoun, et al., in the following year (2016) which conducted a more in-depth study which divided based on gestational intervals with all the results of research on PlGF levels found to be significant at 24-29 weeks the PlGF mean levels were 58.62 pg./ml which ends early-onset preeclampsia (p-value 0.007) [21].

sEng on the incidence of Early Awakening Preeclampsia

From the results of this study, the p-value of 0.113 was found to be not significantly different between the levels of Sickness in early onset

preeclampsia with a mean value of 8 ng/ml in preeclampsia which was slightly higher than the mean in control, ie 5.17 ng/ml. In various studies, there are still various levels of sEng which have not yet become standard, as according to EL-Said, Mohammed, EL-Ashrawi, & Saad, 2013 the mean sEng levels in preeclampsia also increased (11.06 ng/ml) compared to the mean control (5, 92 ng/ml) but with p-value < 0.01 which means significant differences were found [22]. Equally with Perucci's research, 2014 which found that there was an increase in serum levels in preeclampsia, both early and late onset with a p-value of 0.001 [23]. Research by Masuyama, Nakatsukasa, Takamoto, & Hiramatsu, 2007 for very different levels of pre-eclampsia, ie 60.9 ng/ml with controls 11.2 ng/ml (p-value < 0.01) [24]. Although there are significant results from various studies above, there are other studies that are also not significantly different as significant as in this study, according to KEDuhig, 2015 with a p-value of 0.058 (p-value > 0.05) which was carried out examination in women the second trimester of pregnancy was found to have a mean level of 2.78 ng/ml which was lower than the control of 3.52 ng/ml but in the sample of other pregnant women in the third trimester of pregnancy who were also carried out in this study found significant results (p-value 0.001) [25].

The results of the study showed that the mean serum soluble endoglin (sEng) of mothers with Early Onset Preeclampsia was 41.47 ± 13.88 ng/ml while lower in Late-Onset Preeclampsia 33.19 ± 15.99 ng/ml.

Predictors of Early Onset preeclampsia

Based on bivariate data obtained significant results from a variety of predictor variables early-onset preeclampsia, which are detailed in the previous chapter with the following conclusion:

Table 8: Predictors of Early Onset Preeclampsia

	Area Under the Curve	p	cut off point
IP uterine artery	78.2%	0.037	1.228
PGIF	82.5%	0.016	441
sEng	71.4%	0.113	*

Various other studies also conducted bivariate statistics to look for cutoff values to help clinicians diagnose preeclampsia, with various cutoff values. Research by Chen (2009) has conducted an analysis of various previous studies to record the cut-off point score, although in this study it was found to be insignificant because it did not search for cut-off points, the study collected by Chen was Levine, 2006 gestational age 13-20 weeks 7.9 ng/ml, 21-32 weeks 7.2 ng/ml, 33-42 weeks 13.6 ng/ml; Salahuddin, 2007 24.8 ng/ml; Bauman, 2008 5 ng/ml; and Stepan 2008 4.14 ng/ml [27], [17], [28], [29].

Eremina et al., 2003 showed that glomerular capillary function was under VEGF control. That is when the level of VEGF in renal podocytes falls by 50%, glomerular endothelial cells swell, the capillary loop collapses, and proteinuria develops as occurs in patients with preeclampsia [30].

In pregnant women of 22-24 weeks of gestation, diastolic notch findings in uterine arteries cannot predict the incidence of early-onset preeclampsia. PlGF levels can be a predictor of early onset preeclampsia.

From this study an evaluation of the relationship between the variables on the incidence of early onset preeclampsia with the following results: a) the variables included in this study were maternal age, maternal body mass index, uterine arterial diastolic notch, pulsatility value of uterine artery index, PlGF levels, sEng; b) there were no significant differences between maternal age on the incidence of early onset preeclampsia; c) there were no significant differences between parity on the incidence of early onset preeclampsia; d) there was no significant difference between the maternal body mass index and the incidence of early onset preeclampsia; e) no significant differences were found between the presence or absence of uterine artery diastolic notch against the incidence of early onset preeclampsia; f) it was found a significant difference between the pulsatility value of the uterine artery index and the incidence of early onset preeclampsia; g) it was found that there were significant differences between PlGF levels and the incidence of early onset preeclampsia; h) no significant differences were found between sEng levels of the incidence of early onset preeclampsia.

From this study we can conclude: a) value of the variable cut-off point was significant, namely pulsatility levels of the uterine artery index with 1.228 cut-off points, Under Curve Area (AUC) 78.2% (95% IK 59.3%-97%), sensitivity 80%, specificity 64.6%; b) the value of the variable cut-off point is significant, namely PIGF levels with a 441 pg/ml cut-off point, Area Under Curve (AUC) of 82.5% (95% CI 61.5%-100%), sensitivity 80%, specificity 87.7%; and c) pulsatility index of uterine artery and PlGF combined have 40% sensitivity and specificity 90.77%.

References

1. Barton JR, Sibai BM. Prediction and Prevention of Recurrent Preeclampsia. Obstet Gynecol. 2008; 112 (2):359-72. https://doi.org/10.1097/AOG.0b013e3181801d56 PMid:18669736

2. Chaiworapongsa et al., 2010. Plasma Soluble Endoglin Concentration in Preeclampsia is Associated with an Increased Impedance to Flow in the Maternal and Fetal Circulations. Ultrasound Obstet Gynecol. 35(2):155-162. https://doi.org/10.1002/uog.7491 PMid:20101637 PMCid:PMC2944768

3. Nicolaides K, Rizzo G, Hecher K, Ximenes R. Doppler in Obstetrics-The Fetal Medicine Foundation; 2002.

4. Alves et al. Reference Range of Uterine Artery Doppler parameters between the 11th and 14th pregnancy weeks in a population sample from North East Brazil. Rev Bras Ginecol Obstet. 2013; 32:128-132.

5. Jido TA, Yakasai IA. Preeclampsia: A review of the evidence. Annals of African Medicine. 2013; 12(2):3. https://doi.org/10.4103/1596-3519.112395 PMid:23713013

6. Kushtagi P, Emani A. Arterial Resistance in Late First Trimester as a Predictor of Subsequent Pregnancy-Related Hypertension. Sultan Qaboos University Medical Journal. 2016:451-457. https://doi.org/10.18295/squmj.2016.16.04.008 PMid:28003891 PMCid:PMC5135456

7. Vårtun Å, Flo K, Widnes C, Acharya G. Static and functional hemodynamic profiles of women with abnormal uterine artery Doppler at 22-24 weeks of gestation. PloS one. 2016; 11(6):e0157916. https://doi.org/10.1371/journal.pone.0157916 PMid:27308858 PMCid:PMC4911143

8. Tardif C, Dumontet E, Caillon H, Misbert E, Dochez V, Masson D, et al. Angiogenic factors sFlt-1 and PLGF in preeclampsia: Prediction of risk and prognosis in a high-risk obstetric population. J Gynecol Obstet Hum Reprod. 2017.

9. Hassan MF, Rund NM, Salama AH. An elevated maternal plasma soluble fms-like tyrosine kinase-1 to placental growth factor ratio at midtrimester is a useful predictor for preeclampsia. Obstetrics and gynecology international. 2013; 2013.

10. Birdir C, Droste L, Fox L, Frank M, Fryze J, Enekwe A, Köninger A, Kimmig R, Schmidt B, Gellhaus A. Predictive value of sFlt-1, PlGF, sFlt-1/PlGF ratio and PAPP-A for late-onset preeclampsia and IUGR between 32 and 37 weeks of pregnancy. Pregnancy hypertension. 2018; 12:124-8. https://doi.org/10.1016/j.preghy.2018.04.010 PMid:29674192

11. Doherty A, Carvalho JC, Drewlo S, Afif EK, Downey K, Dodds M, Kingdom J. Altered hemodynamics and hyperuricemia accompany an elevated sFlt-1/PlGF ratio before the onset of early severe preeclampsia. Journal of Obstetrics and Gynaecology Canada. 2014; 36(8):692-700. https://doi.org/10.1016/S1701-2163(15)30511-9

12. Andersen LB, Frederiksen-Møller B, Havelund KW, Dechend R, Jørgensen JS, Jensen BL, Nielsen J, Lykkedegn S, Barington T, Christesen HT. Diagnosis of preeclampsia with soluble Fms-like tyrosine kinase 1/placental growth factor ratio: an inter-assay comparison. Journal of the American Society of Hypertension. 2015:1-11. https://doi.org/10.1016/j.jash.2014.11.008

13. Charkiewicz K, Jasinska E, Goscik J, Koc-Zorawska E, Zorawski M, Kuc P, et al. Angiogenic factor screening in women mild preeclampsia - New and significant proteins in plasma. Cytokine . 2017. PMid:29111087

14. Rios DR, Alpoim PN, Godoi LC, Perucci LO, Sousa LP, Gomes KB, et al. Increased Levels of sEng and sVCAM-1 and Decreased Levels of VEGF in Severe Preeclampsia. American Journal of Hypertension. 2016. https://doi.org/10.1093/ajh/hpv170 PMid:26476083

15. El-Said MH, El-Ghaffar A, Eldin ELashmawi HS, Saad GR. Role of serum soluble endoglin in patients with preeclampsia. J Appl Sci Res. 2013; 9:1249-55.

16. Masuyama H, Nakatsukasa H, Takamoto N, Hiramatsu Y. Correlation between soluble endoglin, vascular endothelial growth factor receptor-1, and adipocytokines in preeclampsia. The Journal of Clinical Endocrinology & Metabolism. 2007; 92(7):2672-9. https://doi.org/10.1210/jc.2006-2349 PMid:17426083

17. Salahuddin S, Lee Y, Vadnais M, Sachs BP, Karumanchi SA, Lim KH. Diagnostic utility of soluble fms-like tyrosine kinase 1 and soluble endoglin in hypertensive diseases of pregnancy. American journal of obstetrics and gynecology. 2007;197(1):28-e1. https://doi.org/10.1016/j.ajog.2007.04.010 PMid:17618745

18. Cui L, Shu C, Liu Z, Tong W, Cui M, Wei C, et al. The expression of serum sEGFR, sFlt-1, sEndoglin and PLGF in preeclampsia. Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health. 2018.

19. Respondek M, Woch A, Kaczmarek P, Borowski D. Reversal of diastolic flow in the middle cerebral artery of the fetus during the second half of pregnancy. Ultrasound in Obstetrics & Gynecology. 1997; 9(5):324-9. https://doi.org/10.1046/j.1469-0705.1997.09050324.x PMid:9201876

20. Bian Z, Shixia C, Duan T. First-trimester maternal serum levels of sFLT1, PGF and ADMA predict preeclampsia. PloS one. 2015; 10(4):e0124684. https://doi.org/10.1371/journal.pone.0124684 PMid:25906026 PMCid:PMC4408038

21. Gannoun MB, Bourrelly S, Raguema N, Zitouni H, Nouvellon E, Maleh W, Chemili AB, Elfeleh R, Almawi W, Mahjoub T, Gris JC. Placental growth factor and vascular endothelial growth factor serum levels in Tunisian Arab women with suspected preeclampsia. Cytokine. 2016; 79:1-6. https://doi.org/10.1016/j.cyto.2015.12.005 PMid:26702929

22. El-Said MH, El-Ghaffar A, Eldin ELashmawi HS, Saad GR. Role of serum soluble endoglin in patients with preeclampsia. J Appl Sci Res. 2013; 9:1249-55.

23. Perucci LO, Gomes KB, Freitas LG, Godoi LC, Alpoim PN, Pinheiro MB, Miranda AS, Teixeira AL, Dusse LM, Sousa LP. Soluble endoglin, transforming growth factor-Beta 1 and soluble tumor necrosis factor alpha receptors in different clinical manifestations of preeclampsia. PloS one. 2014; 9(5):e97632. https://doi.org/10.1371/journal.pone.0097632 PMid:24851923 PMCid:PMC4031102

24. Duhig KE, Shennan AH. Recent advances in the diagnosis and management of pre-eclampsia. F1000Prime Reports. 2015; 7:24.

25. Chen Y. Novel angiogenic factors for predicting preeclampsia: sFlt-1, PlGF, and soluble endoglin. Open Clin Chem J. 2009; 2:1-6. https://doi.org/10.2174/1874241600902010001

26. Baumann MU, Bersinger NA, Mohaupt MG, Raio L, Gerber S, Surbek DV. First-trimester serum levels of soluble endoglin and soluble fms-like tyrosine kinase-1 as first-trimester markers for late-onset preeclampsia. American journal of obstetrics and gynecology. 2008 Sep 1;199(3):266-e1. https://doi.org/10.1016/j.ajog.2008.06.069 PMid:18771978

27. Stepan H, Krämer T, Faber R. Maternal plasma concentrations of soluble endoglin in pregnancies with intrauterine growth restriction. The Journal of Clinical Endocrinology & Metabolism. 2007; 92(7):2831-4. https://doi.org/10.1210/jc.2006-2774 PMid:17426082

28. Eremina V, Sood M, Haigh J, Nagy A, Lajoie G, Ferrara N, et al. Glomerular-specific alterations of VEGF-A expression lead to distinct congenital and acquired renal diseases. Journal of Clinical Investigation. 2003; 111:707-16. https://doi.org/10.1172/JCI17423 PMid:12618525 PMCid:PMC151905

29. Lumbanraja SN. Determining the maternal characteristics that predicts the adverse outcomes for patients with preeclampsia. Journal of Health and Translational Medicine. 2013 Dec 30;16(1):5-10. https://doi.org/10.22452/jummec.vol16no1.2

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Apr 15; 7(7):1160-1165.
https://doi.org/10.3889/oamjms.2019.271
eISSN: 1857-9655
Clinical Science

Effect of Exercises on Quality of Life in Patients with Postmenopausal Osteoporosis – Randomized Trial

Valentina Koevska*, Erieta Nikolikj-Dimitrova, Biljana Mitrevska, Cvetanka Gjeracaroska-Savevska, Marija Gocevska, Biljana Kalcovska

Institute of Physical Medicine and Rehabilitation, Faculty of Medicine, Ss. Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia

Citation: Koevska V, Nikolikj-Dimitrova E, Mitrevska B, Gjeracaroska-Savevska C, Gocevska M, Kalcovska B. Effect of Exercises on Quality of Life in Patients with Postmenopausal Osteoporosis – Randomized Trial. Open Access Maced J Med Sci. 2019 Apr 15; 7(7):1160-1165. https://doi.org/10.3889/oamjms.2019.271

Keywords: Postmenopausal osteoporosis; Exercises; Quality of life

***Correspondence:** Valentina Koevska. Institute of Physical Medicine and Rehabilitation, Faculty of Medicine, Ss. Cyril and Methodius University, Skopje, Republic of Macedonia. E-mail: valeskoevska@yahoo.com

Received: 18-Feb-2019; **Revised:** 03-Apr-2019; **Accepted:** 04-Apr-2019; **Online first:** 14-Apr-2019

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Osteoporosis is a systemic skeletal disease characterised by a low bone density and microarchitectural deterioration of bone tissue leading to decrease of its strength and increased risk of fracture. Drug therapy decreases the risk of fracture, thus influencing on the mechanism of bone remodelling. Non-pharmacological interventions include specific exercises for osteoporosis that improve muscle strength and balance, decrease pain and improve quality of life.

AIM: To compare the quality of life in patients with postmenopausal osteoporosis who practice exercises with those who do not practice on the beginning and after a year.

MATERIJAL AND METHODS: A randomised Single-blind randomised controlled prospective trial study, which included 92 women with postmenopausal osteoporosis diagnosed and treated at the Institute of Physical Medicine and Rehabilitation in Skopje, Republic of Macedonia. Patients were randomly assigned to three groups: the first group of patients with exercises and physical modalities (gr. I), the second group with exercises (gr. II), and the third control group of patients who did not practice exercises (gr. III). Exercises were practised 3 times a week; each exercise was repeated for 5-8 times. Patients regularly took bisphosphonates, calcium and vitamin D. The follow-up period lasted for one year. Quality of life was determined with a specific questionnaire Qualeffo-41.

RESULTS: The results showed, significant statistical difference in terms of pain, physical activity, social life, the perception of own health were shown between the groups ($p < 0.0001$), only in term of mental function were no significant ($p < 0.3$).

CONCLUSION: Patients who practice exercises for osteoporosis have a significantly better quality of life than patients who do not perform exercises.

Introduction

Osteoporosis is a systemic skeletal disease characterised by a low bone mineral density and microarchitectural deterioration of bone with a consequent decrease of its density and increased risk of fractures [1]. According to the data of the World Health Organization (WHO), osteoporosis is on the fourth place after cardiovascular diseases, cancer and stroke. Approximately 30% of all postmenopausal women in the United States of America and Europe have PO. Ageing of populations worldwide will be responsible for a major increase in the incidence of osteoporosis in postmenopausal women [2]. The first clinical manifestation very often is vertebral fractures, which can happen during normal activities such as stairs climbing, weight bearing etc. Age, deformity as kyphosis, disturbance of balance and non-specific back pain, increases the risk of a fall, for a new fracture, poor quality of life, disability and high mortality [3], [4].

Treatment of these patients has to be comprehensive and include both, pharmacological and non-pharmacological interventions. Drug therapy reduces the risk of fracture by influencing the mechanism of bone remodelling. These drugs decrease the risk of vertebral fracture by 30-70%, hip fractures by 20-40% and non-vertebral fractures by 15-20% [5], [6]. Non-pharmacological interventions

include specific physical exercises for osteoporosis to improve muscle strength and balance, decrease pain, and improve quality of life [7]. In general, exercises are simple, do not require large financial costs and specific expensive equipment, and can be done at home. Exercises for osteoporosis are designed so that they enable better spinal stability and posture [8], [9], [10]. In the literature, only a few previous randomised studies have been published about the effect of exercises and physical activity on quality of life in osteoporotic patients. These studies have applied diverse methodologies and have presented controversial results [11], [12]. In our country, no such study has been conducted so far. Also, the questionnaire about the quality of life in postmenopausal osteoporotic patients has been applied for the first time in our country.

Our study aimed to evaluate the effectiveness of exercises for osteoporosis on quality of life in patients with postmenopausal (PO).

Material and Method

This was a randomised blind, one-sided trial, which included 92 patients with postmenopausal osteoporosis (PO) diagnosed and treated at the Institute of Physical Medicine and Rehabilitation in Skopje, R. Macedonia. The inclusion criterion was diagnosed osteoporosis. Diagnostic criterion was taken from total t score -1.5 SD to -2.5 SD, determined with x-ray DXA densitometry. The survey was approved by the Ethics Committee for people research at the Faculty of Medicine, Ss. Cyril and Methodius University, the Republic of Macedonia (Num.03-124/2, approved 22.03.2015year), and each of the patients was previously informed about the research from the researcher and signed informed consent to participate in it. Exclusion criteria were: secondary osteoporosis, fever, lumbar sciatica, pacemaker, cardiorespiratory instability, arrhythmia, malignant disease, neurological diseases.

Patients were randomly assigned to three groups: the first group of patients with exercises and physical modalities (gr. I), the second group with exercises (gr. II), and the third control group of patients who did not practice exercises (gr. III). At the beginning of the study, the first group (gr. I) received physical modalities (interferent currents and magnetic therapy) for 3 weeks, each day with a weekend break. Physical modalities were given for the treatment of pain. Exercise program consisted of: respiratory exercises, active exercises and exercises for strengthening the paravertebral muscles, active exercise for maintaining the range of motion of the joints of upper and lower extremities and spine, exercises for strengthening the muscles of the upper and lower extremities, isometric exercises for strengthening the abdominal muscles and exercises for balance. For weight-bearing exercises, the weight was determined by the functional abilities of the patients, 1 (one) kilogram at the most. Exercises were performed 3 times per week; each exercise was repeated for 5 to 8 times. Patients regularly took bisphosphonates, calcium and vitamin D. The follow-up period lasted for one year. Quality of life was evaluated with the specifically designed questionnaire for quality of life-Qualeffo-41 at the beginning and the end of the investigation.

Qualeffo-41 is a specially designed questionnaire approved by the International Association for Osteoporosis for measuring the quality of life in patients with postmenopausal osteoporosis [13]. The questionnaire consists of 41 items and includes 5 domains: pain, physical function, social activities, general health perception and mental function. Total score (TS) and domain scores (DS) are calculated by the following formulas:

$$TS = \frac{\text{(actual score –lowerest possible score) x 100}}{\text{score range}}$$

$$DS = \frac{\text{(average score- lp score*)x100}}{\text{score range}}$$

* lowest possible score

Total score along with domain scores was standardised to a 100-point scale; score 0 shows that the patient has no problems, that is, the quality of life is excellent, whereas score 100 shows maximum problems, that is, poor quality of life. All patients independently filled in the questionnaire at the beginning and the end of the investigation.

The statistical analysis of the obtained data was made in the statistical program SPSS for Windows 17.0. Qualitative data were presented with absolute and relative numbers; the quantitative data were shown by the measures of descriptive statistics (mean ± SD, median with IQR). Cronbach's alpha was used to determine the degree of internal consistency of the questions in the five areas of the Quality of Life Questionnaire in postmenopausal women (QUALEFFO-41). For comparing the three analysed groups of patients, nonparametric and parametric methods for independent samples were used (Chi-square test, and Kruskal-Wallis test. The values of $p < 0.05$ were statistically significant.

Results

Patients were with a mean age of 60.64±6.7 years; the youngest patient was 43 years old, and the oldest 73 years. The largest number/percentage of examined patients was at the age of 60 to 69 years-53 (57.61%). According to the level of education, patients with completed high school predominated (48.91%)

(Table 1).

Table 1: Distribution of patients according to age and level of education

Characteristics of patients	N (%)
Age groups, n (%)	
40 – 49	4 (4.35)
50 – 59	28 (30.43)
60 – 69	53 (57.61)
70 – 75	7 (7.61)
Mean ± SD	(60.64 ± 6.7) min – max (43 – 73)
Education, n (%)	
Primary	22 (23.91)
High	45 (48.91)
University	25 (27.17)

Patients of the three groups did not differ significantly in terms of menopausal status (p = 0.3). Patients without physical agents and exercises (gr.I), insignificantly rarely from patients in the other two groups provided anamnestic data for early menopause (36.67%, 46.88%, 56.67% respectively). The presence of risk factors for osteoporosis was 65.63% of patients treated with physical agents and exercises (gr.I), 80% of patients treated with exercise (gr.II), and 73.33% of patients without physical therapy and exercises (gr.III),. Statistical analysis was non-significant, between the three groups, for the frequency of risk factors for PO (p = 0.44). The difference was also non-significant in terms of the number of risk factors present (p = 0.7). The analysis of the three groups compared to the present comorbidity showed that 71.88% of patients treated with physical agents and exercises (gr.I), 56.67% of patients treated with exercise (gr.II), and 76.67% of patients without physical therapy and exercises (gr.III), had accompanying chronic conditions. The tested difference between subjects with and without co-morbidity, and depending on how the disease was treated, was statistically non-significant (p = 0.22) (Table 2).

Table 2: Statistical difference between groups for early menopause, risk factors, number of risk factors, and comorbidity

Variable	I groups	II groups	III groups	P value
Early menopause n (%)				
No	17 (53.13)	13 (43.33)	19 (63.33)	χ =2.41
Yes	15 (46.88)	17 (56.67)	11 (36.67)	P = 0.299
Risk factors n (%)				
No	11 (34.38)	6 (20)	8 (26.67)	χ = 1.62
Yes	21 (65.63)	24 (80)	22 (73.33)	P = 0.444
Number of risk factors n (%)				
0	11 (34.38)	6 (20)	8 (26.67)	χ = 0.67
1	7 (21.88)	11 (36.67)	10 (33.33)	P = 0.7
2	12 (37.5)	9 (30)	10 (33.33)	
3	2 (6.25)	4 (13.33)	2 (6.67)	
Comorbidity n (%)				
No	9 (28.13)	13 (43.33)	7 (23.33)	χ = 3.04
Yes	23 (71.88)	17 (56.67)	23 (76.67)	P = 0.22

X (Chi-square test); H(Kruskal-Wallis).

When comparing the results of QUALEFFO-41 obtained at the beginning and at the end of our study for each group longitudinally, we noticed a significant improvement in all domains in the first and in the second group of patients, whereas in the third group, who did not practice exercises, there were no significant changes in the domains, except in the social life that showed a substantial impairment.

Table 3. Results from the questionnaire on quality of life Qualeffo-41

Domain	Reception / control	Group	All groups		P value	
			Mean ±SD	Min - Max		
Domain 1 /pain/	Reception	I	59.09 ± 17.2	25 – 90	F=5.3, p = 0.006** 1 vs 2, p = 0.023* 2 vs 3 p = 0.011*	Post-hoc
		II	44.4 ± 25.7	0 - 85		
		III	60.77 ± 20.7	0 - 100		
	Control	I	40.87 ± 20.6	0 - 100	F=13.2, p = 0.000** 1 vs 3, p = 0.004** 2 vs 3, p < 0.001*	Bonfe-rroni
		II	31.0 ± 23.2	0 – 90		
		III	59.3 ± 21.3	0 – 80		
Domain 2 / Physical function	Reception	I	36.59 ± 17.9	0 – 80	F=2.84, p = 0.06	Post-hoc
		II	28.47 ± 19.8	0.2 – 63		
		III	39.42 ± 17.8	10 – 86		
	Control	I	19.95 ± 13.3	5 – 100	F=18.55, p < 0.001** 1 vs 3, p < 0.001** 2 vs 3, p < 0.001**	Dunnett T3
		II	19.99 ± 15.4	0 – 93		
		III	41.8 ± 19.3	13 – 100		
Domain 3 /social function/	Reception	I	48.66 ± 24.5	5 – 100	F=1.2, p = 0.32	Post-hoc
		II	43.29 ± 28.8	0 – 93		
		III	53.48 ± 24.0	13 – 100		
	Control	I	34.58 ± 19.9	5 – 91	F=24.5, p < 0.001** 1 vs 3, p < 0.001** 2 vs 3, p < 0.001**	Bonfe-rroni
		II	27.65 ± 21.64	0 – 90		
		III	67.06 ± 27.9	0.8 – 100		
Domain 4 /health perception	Reception	I	62.76 ± 23.1	1 – 92	F=2.87, p < 0.06	Post-hoc
		II	54.8 ± 26.9	16 – 100		
		III	69.9 ± 23.1	25 – 100		
	Control	I	45.88 ± 22.1	8 – 92	F=25.71, p< 0.000** 1 vs 3, p < 0.000** 2 vs 3, p < 0.000**	Bonfe-rroni
		II	41.5 ± 21.9	16 – 100		
		III	78.2 ± 21.2	33 – 100		
Domain 5 /mental function	Reception	I	44.42 ± 11.7	25 – 78	F=1.21, p = 0.3	Post-hoc
		II	41.17 ± 11.1	22 – 66		
		III	40.13 ± 11.3	16 – 64		
	Control	I	41.16 ± 11.5	25 – 70	F=0.36, p = 0.3	Bonfe-rroni
		II	39.37 ± 7.8	25 – 61		
		III	39.3 ± 9.5	22 – 55		

We found a statistically significant difference among the three groups of patients in the average score for the domain pain at the end of the investigation (p < 0.000). However, this significant difference was due to the significantly higher average score in the control group compared to the group treated with physical agents and exercises (59.3 ± 21.3 *vs* 40.87 ± 20.6 p=0.004), and the group treated with exercises alone (59.3 ± 21.3 *vs* 31.0 ± 23.2 p < 0.0001). This was mainly a result of the regular practising of exercises by patients in the first and the second group. We can conclude that at the end of the follow-up period, patients who did not perform exercises had substantially diminished the quality of life regarding presence and severity of pain in comparison with the other two groups of patients (Table 3).

On the final check-up, a significantly different average score for the domain physical activities among the three groups was observed (p < 0.0001). Post hoc analysis confirmed a significant difference between the control and the first group (41.8 ± 19.3 *vs* 19.95 ± 13.3; p < 0.0001), and between the control and the second group (41.8 ± 19.3 *vs* 19.99 ± 15.4; p < 0.0001). After one-year follow-up, patients from the control group, who did not practice exercises for osteoporosis, showed substantially diminished the quality of life regarding physical activities when compared to the other two groups of patients (Table 3). On the final check-up, the difference was statistically significant (p < 0.0001) for the domain social life, which was due to the significantly higher average score for this domain between the control and the first group (67.06 ± 27.9 *vs* 34.58 ± 19.9, p < 0.0001), and between the control and the second

group (67.06 ± 27.9 *vs* 27.65 ± 21.64, p < 0.0001). The comparison of the quality of life among the three groups from the aspect of social functioning at the end of the investigation, showed that patients from the control group, who did not practice exercises, had a significantly larger number of problems in performing social activities when compared to the other two groups, (Table 3). Significant differences were observed (p < 0.000) in the domain of perception of their general health at the end of the investigation. On the last check-up, the average score for the general health perception in the group treated with interferential currents, magnet and exercises was 45.88 ± 22.1, in the group performing exercises 41.5 ± 21.9, and in the control group, who did not practice exercises, the score was significantly higher (78.2 ± 21.2). At the end of the follow-up period, patients who did not practice exercises rated their general health condition as significantly poorer compared to the patients from the other two groups (Table 3).

The three groups of patients had non-significantly different quality of life regarding their mental functioning, both on admission (p = 0.3) and at the end of the follow-up (p = 0.3), indicating that the method of treatment of primary osteoporosis had no significant impact on their mental functions (Table 3).

Table: 4 Results from the statistically significant difference in total Qualeffo-41 score among groups

Total Qualiffo-41	(mean ± SD)	All groups min - max	p value
On admission			
Group 1	41.32 ± 12.2	20 – 67	F = 2.36, p = 0.1
Group 2	36.57 ± 15.2	12 – 71	
Group 3	43.92 ± 12.3	16 – 75	
Control			
Group 1	28.86 ± 8.4	15 – 50	F = 33.5, p < 0.0001
Group 2	26.98 ± 11.8	12 – 65	1 vs 3, p = 0.00011
Group 3	47.43 ± 11.75	20 – 65	2 vs 3, p = 0.00011

The comparative analysis of total average scores showed that, on admission, there were no significant differences in quality of life among the three groups of patients (p = 0.1), whereas, at the end of the follow-up, significant differences were registered (p < 0.0001). Post hoc analysis showed a significant difference in total average score between the control and the first group and between the control and the second group (p = 0.00011). The average total score on the last check-up was 28.86 ± 8.4 for the first group, 26.98 ± 11.8 for the second group, and significantly higher for the third group (47.43 ± 11.75) (Figure 1) (Table 4).

Discussion

There are many general questionnaires for measuring the quality of life to evaluate the general health condition; however, none of them is as specific for PO as is the Qualeffo-4. Over the last decade, its application in assessing the quality of life in patients with OP has been emphasised due to its specificity, coherence and extensiveness. Osteoporosis (OP) can negatively influence the quality of life, thus limiting and restricting the performance of everyday activities. Chronic pain as a result of PO might lead to depression, anxiety, frustration and social isolation. Practising exercises have become an important intervention in increasing the self-confidence in women in performing their activities and tasks. Regular practising of exercises in women with PO has a positive effect on the general health condition, social inclusion, self-respect, better mood and conscience for better body shape and has decreased depression, anxiety and fear from falls.

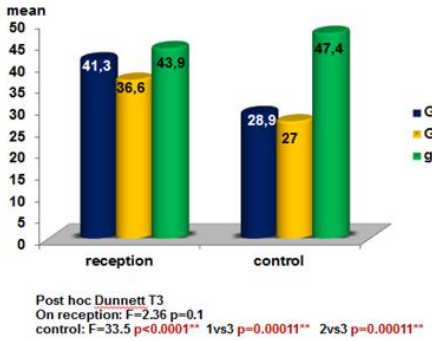


Figure 1: Results from the statistically significant difference in total Qualeffo-41 score among Groups

Our study has shown that all patients with PO demonstrated a significant improvement in the quality of life in all domains of the questionnaire for one year. This was supported by the results obtained for the average scores in all domains when we compared them at the beginning and the end of the study. It means that patients with back pain have a poor quality of life. Also, the other domains such as physical functioning, social life, general health perception and mental function showed a substantial improvement at the end of the study. This is also supported by the result in the total Qualeffo-41 score, which at the end of the study was significantly increased. In the last 10 years, a small number of studies has been conducted that examined the influence of exercises on quality of life in patients with OP for one year by applying the Qualeffo-41 questionnaire.

Similar to our study is the investigation of Evstigneeva *et al*., which demonstrated that after 12 months of performing exercises for PO the questionnaire about the quality of life showed a significant improvement in the quality of life in patients. Total Qualeffo-41 score after 12 months was significantly better in the group of patients who practised exercises (44.2 ± 7.5) compared to the control group (56.6 ± 9.4), p < 0.0001.

In another study, a general questionnaire about the quality of life in patients with PO was applied. Patients were followed for 13 months and were divided into three groups: the first group

practised exercises 3 times per week, the second 2 times per week and the third, control group, did not perform exercises. Their results were similar to our results. The total score about the quality of life in both groups who practiced exercises showed a significant improvement ($p < 0.0001$); gr. 1 (at the beginning 330.2 ± 22.02 and at the end 369.05 ± 1.5) and gr. 2 (at the beginning 313.3 ± 22.01 and at the end 348.8 ± 22.6), but not in the control group (at the beginning 312.3 ± 35.09 and at the end 311.4 ± 35.7) [14].

The largest number of investigations is with a shorter follow-up period, and usually, the type of the exercises and their influence on the quality of life in patients with PO has been compared. For example, one study compared the effect of the three different types of exercises on the back pain and quality of life in an adult population with low bone density. A total of 98 women participated in the study, divided into three groups. The investigation lasted for 6 months. The results showed no significant difference among the groups in the total score of Qualeffo-41 questionnaire [15].

The study conducted by Schröder et al. comprised 45 patients with PO who were assigned to two groups: the first group had the usual exercise program for PO, and the second group had exercises similar to our program. The total score for the quality of life in both groups showed improvement; in the first group (at the beginning 26.0 ± 11.2 and the end 23.9 ± 10.0; $p = 0.766$) and in the second group (at the beginning 29.7 ± 9.8 and the end 21.8 ± 8.1; $p < 0.001$), but the changes in the second group were statistically significant. In three months, there was an improvement in all domains on the Qualeffo-41 questionnaire in both groups, with higher significance in the second group [16].

The study of Schröder *et al*. covering three months and the study of Liu-Ambrose TY *et al.,* covering six months have shown that even for a shorter period the exercises for PO improved the quality of life in patients with osteoporosis.

Similar results have been demonstrated in the study of Bennell *et al*., presenting a significant pain decrease and a significant improvement in the domain of physical functioning even though patients practised the exercises only for three months [17], which was not the case in our study.

One of the advantages of exercises is that they do not represent a burden to the home budget and they can be performed at home. They can yield good results as it was presented in the investigation of Papaioannou A *et al*., where the questionnaire about the quality of life showed a significant improvement after a 12-month follow-up period [18]. It has to be emphasised that practising exercises should be a continuous process in which patients are actively involved.

Exercises should be practised regularly because with advancing age the muscle tissue is decreased as well as the strength of the muscles and physical abilities. Therefore, exercises maintain physical condition, mobility and social life and hence contribute to a better quality of life.

In conclusion, the exercise program for osteoporosis has significantly improved the quality of life in patients after one year of practising in all four domains: pain, physical activities and mobility, social activities and perception about general health condition ($p < 0.0001$). The role of exercises in the treatment of patients with postmenopausal osteoporosis is undisputable.

References

1. Black DM, Rosen CJ,Clinical Practice. Postmenopausal Osteoporosis. N Engl J Med. 2016; 374:254-62. https://doi.org/10.1056/NEJMcp1513724 PMid:26789873

2. Reginster JY. Burlet N. Osteoporosis: A still increasing prevalence. Bone. 2006; 38 (2 suppl.1):4-9. https://doi.org/10.1016/j.bone.2005.11.024 PMid:16455317

3. Kanis JA, Johansson H, Odén A, Johnell O, De LAet C, Eisman JA, et al. A familiy history of fracture and fracture risk: a meta-analysis. Bone. 2004; 35:1029-37. https://doi.org/10.1016/j.bone.2004.06.017 PMid:15542027

4. Johnell O, Kanis JA. An estimate of the worldwide prevalence and disability associated with osteoporotic fractures. Osteoporos Int 2006; 17(12):1726-33. https://doi.org/10.1007/s00198-006-0172-4 PMid:16983459

5. Body JJ, Bergmann P, Boonen S, Boutsen Y. Devogelaer JP, Goemaere S et al. Evidence-based guidelines for the pharmacological treatment of postmenopausal osteoporosis: a consensus document by the Belgian Bone Club. Osteoporos Int. 2010; 21:1657-80. https://doi.org/10.1007/s00198-010-1223-4 PMid:20480148 PMCid:PMC2931762

6. MacLean C, Newberry S, Maglione M, McMahon M, Ranganath V, Suttorp M et al. Systematic review: comparative effectiveness of treatments to prevent fractures in men and women with low bone density or osteoporosis. Ann Intern Med. 2008; 148:197-213. https://doi.org/10.7326/0003-4819-148-3-200802050-00198 PMid:18087050

7. Pfeifer M, Sinaki M, Geusens P, Boonen S, Preisinger E, Minne HW; ASBMR Working Group on Musculoskeletal Rehabilitation. Musculoskeletal rehabilitation in osteoporosis: a review. J Bone Miner Res. 2004; 19(8):1208-14. https://doi.org/10.1359/JBMR.040507 PMid:15231006

8. Burr J, Shephard R, Cornish S, Vatanparast H, Chilibeck P. Arthritis, osteoporosis, and low back pain: evidence-based clinical risk assessment for physical activity and exercise clearance. Can Fam Physician. 2012; 58(1):59-62. PMid:22267624 PMCid:PMC3264014

9. Sinaki M. Exercise for patients with osteoporosis: management of vertebral compression fractures and trunk strengthening for fall prevention. PMR. 2012; 4(11):882-8. https://doi.org/10.1016/j.pmrj.2012.10.008 PMid:23174554

10. Caputo EL, Costa MZ, Influence of physical activity on quality of life in postmenopausal women with osteoporosis, (Rev). Bras reumatol. 2014; 54(6):467-73.

11. Korpelainen R, Keinänen-Kiukaanniemi S, Nieminen P, Heikkinen J, Väänänen K, Korpelainen J. Long-term outcomes of exercise: follow-up of a randomized trial in older women with

osteopenia. Arch Intern Med. 2010; 170(17):1548-56. https://doi.org/10.1001/archinternmed.2010.311 PMid:20876406

12. Evstigneeva L, Lesnyak O, Bultink IE, Lems WF, Kozhemyakina E, Negodaeva E et al. Effect of twelve-month physical exercise program on patients with osteoporotic vertebral fractures: a randomized, controlled trial, Osteoporos Int. 2016; 27:8: 2515-24. https://doi.org/10.1007/s00198-016-3560-4 PMid:26984569

13. Van Schoor NM, Knol DL, Glas CA, Ostelo RW, Leplège A, Cooper Cet al. Development of the Qualeffo-31, an osteoporosis-specific quality-of-life questionnaire. Osteoporos Int. 2006; 17:543-51. https://doi.org/10.1007/s00198-005-0024-7 PMid:16362146

14. Borba-Pinheiroa CJ. Resistance training programs on bone related variables and functional independence of postmenopausal women in pharmacological treatment: A randomized controlled trial. Archives of Gerontology and Geriatrics. 2016; 65:36-44. https://doi.org/10.1016/j.archger.2016.02.010 PMid:26956618

15. Liu-Ambrose TY, Khan KM, Eng JJ, Lord SR, Lentle B, McKay HA. Both resistance and agility training reduce back pain and improve helth-related quality of life in older women with low bone mass, (Original article). Osteoporosis Int. 2005; 16(11):1321-29. https://doi.org/10.1007/s00198-005-1842-3 PMid:15702262

16. Schröder G, Knauerhase A, Kund G, Schober HC et al. Effects of physical therapy on quality of life in osteoporosis patients - a randomized clinical trial. Health and Quality of Life Outcomes. 2012; 10:101. https://doi.org/10.1186/1477-7525-10-101 PMid:22920839 PMCid:PMC3511275

17. Bennell LK, Matthews B, Greig A, Briggs A, Kelly A, Sherburn M, et al. Effects of an exercise and manual therapy program on physical impairments, function and quality-of-life in people with osteoporotic vertebral fracture: a randomised, single-blind controlled (pilot trial). BMC Musculoskeletal Disorders. 2010; 11:36. https://doi.org/10.1186/1471-2474-11-36 PMid:20163739 PMCid:PMC2830179

18. Papaioannou A, Adachi JD, Winegard K, Ferko N, Parkinson W, Cook RJ et al: Efficacy of home-based exercise for improving quality of life among elderly women with symptomatic osteoporosis related vertebral fractures. Osteoporos Int. 2003; 14(8):677-82. https://doi.org/10.1007/s00198-003-1423-2 PMid:12879220

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Apr 15; 7(7):1166-1169.
https://doi.org/10.3889/oamjms.2019.240
eISSN: 1857-9655
Clinical Science

Association Between Bispectral Index (BIS) Value and Postoperative Shivering in Patients Undergoing Orthopedic Surgery

Manijeh Yousefi Moghadam[1], Mohammad Nemat-Shahi[1*], Bardia Dowlat-Abadi[1], Seyed Ehsan Safari[2], Saeed Yajan[1]

[1]Department of Anesthesiology, School of Medicine, Sabzevar University of Medical Sciences, Sabzevar, Iran; [2]Centre for Quantitative Medicine, Duke - NUS Medical School, Singapore, Asia

Citation: Yousefi Moghadam M, Nemat-Shahi M, Dowlat-Abadi B, Safari SE, Yajan S. Association Between Bispectral Index (BIS) Value and Postoperative Shivering in Patients Undergoing Orthopedic Surgery. Open Access Maced J Med Sci. 2019 Apr 15; 7(7):1166-1169. https://doi.org/10.3889/oamjms.2019.240

Keywords: Anesthesia; Consciousness Monitors; Bispectral index (BIS); Shivering; Orthopedics

***Correspondence:** Mohammad Nemat-Shahi. Department of Anesthesiology, School of Medicine, Sabzevar University of Medical Sciences, Sabzevar, Iran. E-mail: mahneamatshahi@yahoo.com

Received: 14-Feb-2019; **Revised:** 21-Mar-2019; **Accepted:** 22-Mar-2019; **Online first:** 14-Apr-2019

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Postoperative shivering is one of the most common adverse effects after general anaesthesia.

AIM: This study aimed to evaluate the association between the Bispectral index (BIS) monitoring value and postoperative shivering in patients undergoing orthopaedic surgery.

MATERIAL AND METHODS: This cross-sectional study was conducted in Shahid Beheshti Hospital in Sabzevar city, from August 2017 to September 2018. Patients who underwent orthopaedic surgery, using general anaesthesia, were enrolled. Recording of the depth of anaesthesia using BIS monitoring was started exactly 5 minutes after intubating the patient and continued until the discharge from post-anesthesia care unit (PACU). The incidence of postoperative shivering was evaluated using a scale proposed by Crossley and Mahajan.

RESULTS: A total number of 80 patients were evaluated. 32.5% of patients experience postoperative shivering grade 2, with mean BIS score 41.85. The univariate and multivariate linear regression analysis indicated a statistically significant relationship between shivering score and patients' heart rate, blood pressure, BIS score, temperature, age, height, gender and blood cell distribution width (RDW) ($p < 0.05$).

CONCLUSION: The results of this study indicate a significant positive association between BIS value and postoperative shivering in patients undergoing orthopaedic surgery, so that, patients with higher BIS score experienced significantly more postoperative shivering. It seems that BIS-guided anaesthesia can reduce the risk and incidence of postoperative shivering in patients undergoing orthopaedic surgery.

Introduction

Postoperative shivering is one of the most common adverse effects after general anaesthesia, with an incidence ranging from 20 to 70% [1], [2], [3]. Postoperative shivering which has been defined as an involuntary movement of the muscles can lead to an increase in patients' metabolism two times more than the normal and cannot be controlled by them. Also, it can cause discomfort and dissatisfaction for patients after surgery. Postoperative shivering has different adverse effects such as 400 times increase in oxygen consumption, increase in plasma catecholamine concentrations, carbon dioxide production, increase in blood pressure and heart rate, increase in intracranial and intraocular pressure [3], [4]. Additionally, it can aggravate postoperative pain, which can consequently prolong the recovery from anaesthesia and delay discharge after surgery [5]. There are different approaches for managing post-operative shivering. Postoperative skin surface is rewarming, keep the room warm and administration of oxygen are among the non-pharmacologic interventions for prevention of shivering after surgery.

Nonetheless, they are less efficient than certain drugs such as tramadol, dexamethasone, pethidine, clonidine and physostigmine [6], [7], [8]. Pethidine is the most prevalent drug for managing postoperative shivering [9]. This drug may cause respiratory depression. Using pethidine may cause a

delay in patients' discharge from post-anesthesia care unit (PACU), increase the incidence of postoperative nausea and vomiting, decrease the peristaltic movement of the gastrointestinal system and delay in normal enteral nutrition in the postsurgical period [10]. The exact mechanism of postoperative shivering is not well elucidated [11]. Although perioperative hypothermia has been considered as one of the possible mechanisms of postoperative shivering [7], [11], this has not been confirmed by more recent studies [12]. Recently, the depth of anaesthesia has been suggested as a probable risk factor for developing postoperative shivering [3], [13], [14]. One method in monitoring the depth of anaesthesia is using electroencephalogram [bispectral index system (BIS)] [15]. BIS score is clinically used to monitor patients' responsiveness and anaesthesia depth during general anaesthesia. BIS scores ranging from 0 to 100 that has a vice versa relationship with the depth of anaesthesia [16]. For instance, once a BIS score is more than 60, the anaesthesia is light, and once it is less than 40, it is too deep. Anaesthesiologists can use BIS monitoring for better managing of anesthetised patients and prevent the associated complications [16], [17].

Considering the probable effect of depth of anaesthesia in the occurrence of shivering after surgery and also the adverse effects of current remedies for managing this complication, as well as the relatively high prevalence of postoperative shivering in patients undergoing orthopaedic surgeries [18].

This study aimed to investigate the relationship between BIS value and postoperative shivering in patients undergoing orthopaedic surgery.

Material and Methods

In a cross-sectional study, all patients with age between 16-60 years, who were a candidate for any orthopaedic surgeries under general anaesthesia were included in this study. The study was done from August 2017 to September 2018, in an orthopaedic unit of a teaching hospital affiliated with Sabzevar University of Medical Sciences, Sabzevar, Iran. Exclusion criteria were postoperative bleeding greater than one litre, needing to blood transfusion, surgery lasting more than three hours, premedication administration, corticosteroid's administration during surgery, administration of any drug which might change the result (such as pethidine, ondansetron, hydrocortisone) and opium addiction. Approval from the Sabzevar University of Medical Sciences ethics committee, as well as informed consents from patients, was obtained. Patients' demographic characteristics were recorded. Also, venous blood samples for complete blood count (CBC) test was taken from all patients, preoperatively.

For anaesthesia, all patients received midazolam with a dosage of 0.01 to 0.02 mg/kg of body weight. Then 2 microgram/kg of fentanyl, thiopental sodium 5 mg/kg and 0.5 mg/kg Atracurium was bloused. For maintenance of anaesthesia, 50 per cent nitrous oxide and 50 per cent oxygen at a flow of 3-6 litre/minute were used as an adjacent to the main drugs. Isoflurane was administered at MAC of 1.15. Atracurium with a 0.2 mg/kg of body weight every 30 minutes was administered. Fentanyl with one mg/kg dosage every 45 minutes were also given. The muscle relaxant was reversed with a combination of neostigmine and atropine (with a dosage of 0.04 and 0.02 mg/kg in order). The standard monitoring, including pulse oximetry, noninvasive blood pressure cuff and electrocardiogram leads were attached to the patients. BIS monitoring sensors (BIS Vista, Aspect Medical System, USA) were also attached to the patients for monitoring anaesthesia depth. BIS score classified from 0 to 100 indicating unconscious to fully awake. A typical BIS score in patients who are awake is 90 to 100. Lower values indicate a higher hypnotic effect [16], [17]. Recording of the depth of anaesthesia was started exactly 5 minutes after intubating the patient and was continued on the recovery stage of anaesthesia (stopping the maintenance drugs). Patients' vital signs, as well as BIS values, were recorded every 5 minutes before and after transfer to the post-anaesthesia care unit (PACU). In the PACU and until PACU discharge, patients' shivering was graded using the scale described by Crossley and Mahajan which is 0 = no shivering, 1 = cyanosis and piloerection, 2 = visible tremors only in one muscle group, 3 = visible tremors in more than one group of muscles, and 4 = intense shivering, tremors of the head, arm, by an anaesthesia nurse who was blinded to the study groups [19].

The data were analysed using Statistical Analysis Software (SAS; edition 9.2). The demographic and clinical data were tabulated into two groups; shivered and not-shivered for normally distributed data, independent T-test and for non-normal distributed data the Mann-Whitney test was used. For determination of the association between anaesthesia depth and shivering incidence a logistic regression model was used. A p-value of 0.05 and less was considered as significant.

Results

In total 80 patients completed the study; of them 14 (17.5%) were female, and 66 (82.5%) were male. The mean age was 34.07 years, with a minimum of 16 and a maximum of 65 years. The average weighs for the patients was 72.52 (50-120) kg. Average haemoglobin level was 15.69 g/dL. Other

demographic and preoperative laboratory profiles are listed in Table 1.

Table 1: Patients' demographic and preoperative laboratory profile

Variables	Mean	Standard Deviation	Minimum	Maximum
Age	34.07	15.44	16.00	65.00
Weight	72.52	13.22	50.00	120.00
Height	173.83	7.11	148.00	190.00
Hematocrit	36.18	6.78	14.70	47.00
RDW	14.13	0.82	12.70	16.60
MCV	83.74	4.97	76.00	97.00
MCH	96.18	439.88	25.20	28.79
Hemoglobin	15.69	20.56	7.00	14.50
MCHC	195.71	724.11	30.00	33.75

RDW: Red cell Distribution Width; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration

The mean values of patients' vital signs in PACU and BIS scores during surgery are presented in Table 2.

Table 2: Mean values of patients' vital signs and BIS scores in PACU

Variables	Mean	Standard Deviation	Minimum	Maximum
Heart Rate	84.23	16.18	58.80	128.00
Blood Pressure	120.09	13.78	95.01	155.51
BIS	41.85	12.06	20.40	70.73
Temperature (°F)	97.30	1.01	91.87	99.64

Regarding the shivering incidence and severity, most of our patients did not experience shivering (42.50%); however, a large majority of them had the last stage (grade 4) of shivering (32.50%); the data for Crossley's classification are shown in Table 3.

Table 3: Shivering Stages of the patients

Shivering grade	Frequency	Percentage
0	34	42.50
1	5	6.25
2	1	1.25
3	14	17.50
4	26	32.50

The mean value of the BIS score was 41.85. The univariate linear regression model indicated a statistically significant relationship between Crossley shivering score and heart rate ($p = 0.0009$), BIS score ($p < 0.0001$), temperature ($p = 0.01$), age ($p < 0.0001$), height ($p = 0012$), and female gender ($p = 0.0018$). Multiple linear regression models, adjusted for age and gender, showed a significant p-value for blood pressure, BIS score, temperature, red blood cell distribution width (RDW), and age (Table 4).

Table 4: Univariate and multivariate linear regression model, adjusted for age and gender

Parameter	Univariate		Multiple	
	Beta Coefficient (95% CI)	P value	Beta Coefficient (95% CI)	P value
Heart Rate	0.04 (0.02, 0.06)	0.0009	0.01 (-0.02, 0.04)	0.4172
BP	-0.00 (-0.04, 0.02)	0.7063	-0.02 (-0.05-0.00)	0.0472
BIS	-0.90 (-0.11, -0.63)	<0.0001	-0.08 (-0.10, -0.06)	<0.0001
T	-0.05 (-0.88, -0.11)	0.0122	-0.54 (-0.88, -0.20)	0.0021
Weight	-0.01 (-0.05, 0.02)	0.3078	-0.03 (-0.06, 0.00)	0.0906
Height	0.10 (0.04, 0.14)	0.0012	0.00 (-0.07, 0.07)	0.9794
HTC	-0.01 (-0.09, 0.07)	0.6873	-0.01 (-0.09, 0.06)	0.7482
RWD	-0.30 (-1.02, 0.30)	0.3196	-0.40 (-1.03, 0.30)	0.0240
MCV	-0.07 (-0.18, 0.04)	0.2144	-0.04 (-0.15, 0.07)	0.4240
MCH	0.00 (-0.0004, 0.002)	0.1849	0.00 (-0.0005, 0.002)	0.2801
HB	-0.01 (-0.04, 0.02)	0.4068	-0.02 (-0.04, 0.01)	0.1963
MCHC	-0.00 (-0.001, 0.0002	0.1856	-0.00 (-0.001, 0.0002)	0.1625
Gender	-1.62 (-2.63, -2.62)	0.0018	-0.88 (-1.92, 0.17)	0.0986
Age	-0.06 (-0.09, -0.04)	<0.0001	-0.06 (-0.08, -0.03)	<0.0001

Discussion

Our data indicated a positive relationship between BIS score and occurrence of postoperative shivering; this suggests that an awake state (higher BIS value during anaesthesia) would result in a higher stage of postoperative shivering. Recent studies on BIS and its benefits stated many advantages for BIS-guided anaesthesia [16]; however, there was a controversy for the clinicians about the effect of BIS on their decision for drug utilisation, many declared no change for their choice-of-drug [20]. Our study may introduce a new insight into BIS; that is the effect of anaesthesia depth on postoperative shivering. We have found that a BIS value around 40, which is the maximum allowable patients' anaesthesia stage, would be beneficial for reducing the incidence of shivering in the recovery period. In the literature, researchers suggested different cause for shivering; mainly, the thermoregulation was discussed. In the current study, we tried to maintain normothermia throughout the perioperative period; however, the lower body temperature, the higher grade of shivering existed. Among other reasons for shivering was gender; some studies reported no relationship and some found males more prone to shivering [3], [13], [21]. Contradictory to these results, in our study, a higher stage of shivering happened for the female; this may have happened because of the type of surgeries in this study. A few researchers have found that less use of opiates can cause more shivering, as well as we know that pain threshold is higher in women and indeed BIS monitor would show a less score for a woman suffering from a pain compare to a man in the same situation [7], [9]. It has been previously shown that patients who underwent anaesthesia with Pentothal experience a higher incidence of postoperative shivering compare to propofol [2], [22].

Furthermore, using halogenated agents seemed to cause more post-op shivering. Lewis et al. did one study on the effects of alpha2-adrenergic agonists on inhibition of post-op shivering [23]. In their study has been shown that clonidine and dexmedetomidine had a positive effect on decreasing post-op shivering. Our protocol for anaesthetising patients was a standard and normal balanced approach which was equal for all of our cases. We used thiopental sodium for induction to avoid any biases about propofol and its probable anti-shaking effects. Also, administrating of the opiates and benzodiazepines for patients was according to their BIS level. Some clinicians prefer to use drugs according to heart rate, blood pressure, lacrimation and other motor sensory signs. However, in our study to avoid any bias and diminish individual decisions, the BIS score was the guide for drugs, and it should have been maintained between 40 and 60. The result of a study aiming to evaluate different pharmacological classes to decrease post-operative

shivering indicated among the drugs which affected shivering, pethidine was the best [23]. Our study was limited to one centre; further studies with different surgeries and a more population are suggested.

In conclusion, the results of this study indicate a significant inverse association between BIS value and postoperative shivering in patients undergoing orthopaedic surgery, so that, patients with higher BIS score experienced significantly more postoperative shivering. It seems that BIS-guided anaesthesia can reduce the risk and incidence of postoperative shivering in patients undergoing orthopaedic surgery.

References

1. Golembiewski J. Pharmacological Management of Perioperative Shivering. J Perianesth Nurs. 2015; 30(4):357-9. https://doi.org/10.1016/j.jopan.2015.05.002 PMid:26210569

2. Alfonsi P. Postanaesthetic shivering: epidemiology, pathophysiology, and approaches to prevention and management. Drugs. 2001; 61(15):2193-205. https://doi.org/10.2165/00003495-200161150-00004 PMid:11772130

3. Lopez MB. Postanaesthetic shivering - from pathophysiology to prevention. Rom J Anaesth Intensive Care. 2018; 25(1):73-81. PMid:29756066 PMCid:PMC5931188

4. Weant KA, Martin JE, Humphries RL, Cook AM. Pharmacologic options for reducing the shivering response to therapeutic hypothermia. Pharmacotherapy. 2010; 30(8):830-41. https://doi.org/10.1592/phco.30.8.830 PMid:20653360

5. Caruselli M. Postoperative shivering: a common phenomenon with multiple causes. Minerva Anestesiol. 2018; 84(12):1340-1342. https://doi.org/10.23736/S0375-9393.18.13138-5 PMid:30328332

6. Yousuf B, Samad K, Ullah H, Hoda MQ. Efficacy of tramadol in preventing postoperative shivering using thiopentone or propofol as induction agent: A randomized controlled trial. J Anaesthesiol Clin Pharmacol. 2013; 29(4):521-5. https://doi.org/10.4103/0970-9185.119166 PMid:24249991 PMCid:PMC3819848

7. Lotfi Fatemi SN, Armat MR, Emami Zeydi A, Soleimani A, Hasanzadeh Kiabi F. Inadvertent Perioperative Hypothermia: A Literature Review of an Old Overlooked Problem. Acta Facultatis Medicae Naissensis. 2016; 33(1):5-11. https://doi.org/10.1515/afmnai-2016-0001

8. Song Y-K, Lee C. Effects of ramosetron and dexamethasone on postoperative nausea, vomiting, pain, and shivering in female patients undergoing thyroid surgery. J Anesth. 2013; 27(1):29-34. https://doi.org/10.1007/s00540-012-1473-8 PMid:22965329

9. Chiang MH, Chung KC, Syue YJ, Chia-Shen Yang J, Chien CY, Kuo YR. The role of meperidine in reduction of postanesthetic shivering and its possible impact on flap outcomes. Microsurgery. 2014; 34(2):106-11. https://doi.org/10.1002/micr.22133 PMid:23843309

10. Bhukal I, Solanki SL, Kumar S, Jain A. Pre-induction low dose pethidine does not decrease incidence of postoperative shivering in laparoscopic gynecological surgeries. J Anaesthesiol Clin Pharmacol. 2011; 27(3):349-53. https://doi.org/10.4103/0970-9185.83680 PMid:21897506 PMCid:PMC3161460

11. Alfonsi P. Postanaesthetic shivering. Epidemiology, pathophysiology and approaches to prevention and management. Minerva Anestesiol. 2003; 69(5):438-42. PMid:12768180

12. Hoshijima H, Takeuchi R, Kuratani N, Nishizawa S, Denawa Y, Shiga T, et al. Incidence of postoperative shivering comparing remifentanil with other opioids: A meta-analysis. J Clin Anesth. 2016; 32:300-12. https://doi.org/10.1016/j.jclinane.2015.08.017 PMid:26432635

13. Díaz M, Becker DE. Thermoregulation: physiological and clinical considerations during sedation and general anesthesia. Anesth Prog. 2010; 57(1):25-32. https://doi.org/10.2344/0003-3006-57.1.25 PMid:20331336 PMCid:PMC2844235

14. Crowley LJ, Buggy DJ. Shivering and neuraxial anesthesia. Reg Anesth Pain Med. 2008; 33(3):241-52. https://doi.org/10.1097/00115550-200805000-00009

15. Ekman A, Lindholm ML, Lennmarken C, Sandin R. Reduction in the incidence of awareness using BIS monitoring. Acta Anaesthesiol Scand. 2004; 48(1):20-6. https://doi.org/10.1111/j.1399-6576.2004.00260.x PMid:14674969

16. Chan MT, Cheng BC, Lee TM, Gin T, Group CT. BIS-guided anesthesia decreases postoperative delirium and cognitive decline. J Neurosurg Anesthesiol. 2013; 25(1):33-42. https://doi.org/10.1097/ANA.0b013e3182712fba PMid:23027226

17. Medical Advisory S. Bispectral index monitor: an evidence-based analysis. Ont Health Technol Assess Ser. 2004; 4(9):1-70.

18. Kiekkas P, Poulopoulou M, Papahatzi A, Souleles P. Effects of hypothermia and shivering on standard PACU monitoring of patients. AANA J. 2005; 73(1):47-53. PMid:15727284

19. Crossley AW, Mahajan RP. The intensity of postoperative shivering is unrelated to axillary temperature. Anaesthesia. 1994; 49(3):205-7. https://doi.org/10.1111/j.1365-2044.1994.tb03422.x PMid:8147511

20. Oliveira CRD, Bernardo WM, Nunes VM. Benefit of general anesthesia monitored by bispectral index compared with monitoring guided only by clinical parameters. Systematic review and meta-analysis. Braz J Anesthesiol. 2017; 67(1):72-84. https://doi.org/10.1016/j.bjan.2016.10.002

21. De Witte J, Sessler DI. Perioperative ShiveringPhysiology and Pharmacology. Anesthesiology. 2002; 96(2):467-84. https://doi.org/10.1097/00000542-200202000-00036 PMid:11818783

22. Habibi MR, Baradari AG, Soleimani A, Emami Zeydi A, Nia HS, Habibi A, et al. Hemodynamic responses to etomidate versus ketamine-thiopental sodium combination for anesthetic induction in coronary artery bypass graft surgery patients with low ejection fraction: a double-blind, randomized, clinical trial. J Clin Diagn Res. 2014; 8(10):GC01-5. https://doi.org/10.7860/JCDR/2014/10237.5006

23. Lewis SR, Nicholson A, Smith AF, Alderson P. Alpha-2 adrenergic agonists for the prevention of shivering following general anaesthesia. Cochrane Database Syst Rev. 2015; (8):CD011107. https://doi.org/10.1002/14651858.CD011107.pub2

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Apr 15; 7(7):1170-1173.
https://doi.org/10.3889/oamjms.2019.219
eISSN: 1857-9655
Clinical Science

P53 and Survival Rate in Penile Cancer

Fauriski Febrian Prapiska*, Syah Mirsya Warli

Department of Urology, University of Sumatera Utara Hospital, Medan, Indonesia

Citation: Prapiska FF, Warli SM. P53 and Survival Rate in Penile Cancer. Open Access Maced J Med Sci. 2019 Apr 15; 7(7):1170-1173. https://doi.org/10.3889/oamjms.2019.219

Keywords: P53 expression; Survival rate; Penile cancer; Gene overexpression; Mortality

***Correspondence:** Fauriski Febrian Prapiska. Department of Urology, University of Sumatera Utara Hospital, Medan, Indonesia. E-mail: fauriski@gmail.com

Received: 15-Jan-2019; **Revised:** 17-Mar-2019; **Accepted:** 18-Mar-2019; **Online first:** 14-Apr-2019

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Penile cancer accounts for 0.4-0.6% of all malignancy in men in Europe and the United States of America. It also accounts for 10% of all malignancy in men in some Asian, South American, and African countries. P53 protein has the function to regulate apoptosis in the cell cycle. Therefore, the presence of p53 in cells may indicate higher proliferative activity of the cells as a feedback mechanism, indicating disease progression.

AIM: This study aims to identify the association between p53 expression and survival rate in penile cancer patients.

METHODS: This study was a retrospective observational analytic study. This study was conducted in Pathology Anatomy Laboratory Faculty of the Medicine University of Sumatera Utara/Haji Adam Malik Hospital/University of Sumatera Utara Hospital to assess p53 expression. This study was conducted from January 2018 to December 2018.

RESULTS: The total subjects in this study were 33 with the mean age of 50.79 ± 10.62. Based on clinical stage, patients in this study are divided into 11 patients (33.3%) in stage T II and 22 patients (66.7%) in stage T III/T IV. P53 expression was positive in 13 patients (35.3%). There were 19 patients (57.6) alive and 14 patients (42.4%) deceased. Statistical analysis using chi-square showed that there was an association between p53 expression and mortality ($p = 0.011$). In the Kaplan-Meier Curve for 3-year overall survival based on p53 expression, the survival rate in 36 months in the p53 positive group is 18%, while in p53 negative group, the survival rate was 60%. The survival rate based on p53 status was significantly different ($p = 0.025$).

CONCLUSION: There is a significant association between p53 expression and mortality in penile cancer patients. In conclusion, p53 expression in penile cancer cells examined by immunohistochemistry may show prognostic values in the disease progression.

Introduction

Penile cancer accounts for 0.4-0.6% of all malignancy in men in Europe and the United States of America. It also accounts for 10% of all malignancy in men in some Asian, South American, and African countries [1]. In Indonesia, there were 69 men diagnosed with penile malignancies in Dr Cipto Mangunkusumo Hospital and Dharmais Hospital Jakarta Cancer Center for 11 years period (1994-2005) [2]. Another study in Sanglah Hospital Bali showed that there were 46 penile cancer patients for 8 years period. Meanwhile, in Haji Adam Malik Medan Hospital, the incidence of penile cancer for the last 4 years (2012-2015) was 34 patients [3], [4].

The principal value in the management of penile cancer is the levitation of the tumour with good organ preservation along partial or total penectomy in regards to lowering the recurrence rate. Aside from the treatment of a primary tumour, the involvement of the lymph node remains to be an important factor in enhancing the patient's prognosis. Penile cancer is an aggressive disease. The success rate of local lesion treatment is only for early stage disease. Besides, the more-progressive and advanced disease with the involvement of regional lymph node or distant metastasis remains a problem in the field of neuro-oncology [4].

The incidence of penile cancer varies within circumcision status, hygiene standard, phimosis, sexual partner, Human papilloma virus (HPV) infection, tobacco exposure, and other factors [1]. Etiologic factors known were chronic irritation from smegma, a product from bacterial activity in desquamated cells that are accumulated in the

preputium. The most common histopathology found in penile cancer is Squamous Cell Carcinoma (SCC) [5].

P53 protein is a product from the TP53 gene in the body; this protein has the function to regulate apoptosis in the cell cycle. The presence of p53 in cells may indicate higher proliferative activity of the cells as a feedback mechanism, indicating disease progression. The study showed that the excessive protein expression of p53 was found in penile cancer cells [6]. Furthermore, mutation or deletion of p53 in the cell may precipitate cancer to further progress [7]. Based on this theoretical background, p53 could be used to see prognosis of the disease where ass more expression of the protein is associated with worse clinical parameters [6]. However, the use of any diagnostic or prognostic tool to measure the expressions is not widely implemented [8].

This rationale accelerates the urgency to do a study on the association between p53 expression and survival rate in penile cancer patients.

Material and Methods

This study was a retrospective observational analytic study. This study aimed to analyse the association between p53 expression and survival rate in penile cancer patients. This study was conducted in Pathology Anatomy Laboratory Faculty of Medicine University of Sumatera Utara/ Haji Adam Malik Hospital / University of Sumatera Utara Hospital. This study has the ethical approval issued by Research Ethics Committee Faculty of the Medicine University of Sumatera Utara. This study was conducted from January 2018 to December 2018.

Immunohistochemistry of p53

The protein expression of p53 was observed using Immunohistochemistry examination done on FFPE preparation. Specific antibodies for p53 (mouse monoclonal antibody) obtained from Sigma Aldrich (St Louis, Missouri). Preparation/micro slicing was done for each sample, and slides are provided for each sample for microscopic evaluation.

Microscopic evaluation was performed by an experienced pathologist. The semiquantitative examination was done on random fields per specimen containing a minimum of 500 cells using ImageJ (Research Service Branch, NIH.gov) in 40 times magnification. The expression of the protein would be positive if cells were stained brownish in the cytoplasm or the nuclei with a granular pattern. If the number of positive cells exceeds 60%, the sample is considered positive.

Results

The total subjects in this study were 33 with the mean age of 50.79 ± 10.62. Based on clinical T stage, patients in this study are divided into 11 patients (33.3%) in cT2 and 22 patients (66.7%) in cT3/cT4. There were 22 patients (66.7%) who had cancer invasion to the urethra.

Table 1: Characteristics of the subjects

Variable		p-Value
Total patients	33	
Mean Age ±SD	50.79 ± 10.62	0.71**
Clinical T stage (cT; %)		0.72*
cT2	11 (33.3)	
cT3/cT4	22 (66.7)	
Urethral invasion (%)	22 (66.7)	0.72*
Management (%)		
Total penectomy	18 (54.5)	
Partial penectomy	10 (30.3)	
No operation	5 (15.2)	
Chemotherapy (%)	8 (24.2)	0.416*
p53 expression (%)		
Positive	13 (39.4)	
Negative	20 (60.6)	
Mortality status (%)		
Alive	19 (57.6)	
Deceased	14 (42.4)	

*Fisher's Exact Test, **Mann-Whitney Test.

Based on the treatment type, there were 18 patients (54.4%) who had total penectomy, 10 patients (30.3%) who had partial penectomy, 5 patients (15.2%) who had no operation, and 8 patients (24.2%) who had completed chemotherapy cycles using TIP (Paclitaxel, ifosfamide, and cisplatin) regimen. P53 expression was positive in 13 patients (35.3%). There were 19 patients (57.6) alive and 14 patients (42.4%) deceased.

Table 2: Association between p53 expression and mortality

p53 Expression	Mortality		p-Value
	Alive	Deceased	
Positive	2	11	0.011
Negative	12	8	

Statistical analysis using chi-square showed that there was an association between p53 expression and mortality (p = 0.011).

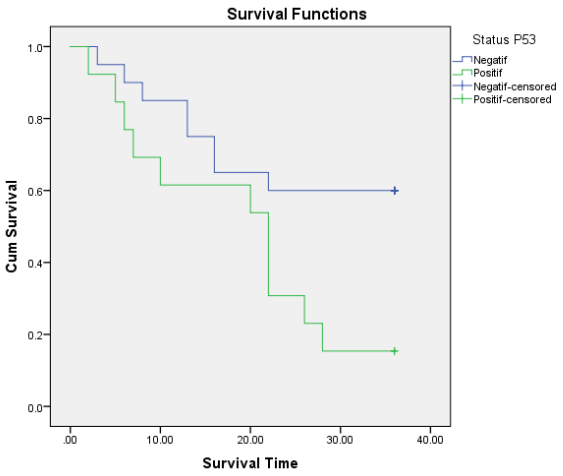


Figure 1: Kaplan-Meier Curve for 3-year overall survival in Penile Squamous Cell Carcinoma based on p53 status

In the Kaplan-Meier Curve for 3-year overall survival based on p53 expression, the survival rate in 36 months in the p53 positive group is 18%, while in p53 negative group, the survival rate was 60%. The survival rate based on p53 status was significantly different (p = 0.025).

Discussion

In this study, we assessed the role of p53 oncoprotein expression as a prognostic factor in penile cancer. P53 expression was analysed with the survival rate of penile cancer patients. In this study, we assessed the p53 expression as a predictor for mortality in penile cancer patients. Subjects were enrolled in one tertiary hospital which was comprised of the varied stage of cancer.

In this study, p53 positive was found in 13 of 33 patients (39.4%). By previous studies about p53 expression, this study used 20% cut-off point for the nucleus staining. This positive cut-off is higher than the study conducted by Levi et al. (26% or 15 of 58 cases) [9] and almost the same with the study conducted by Lam et al., (40% or 17 of 42 cases) [10]. The difference depends on the antibody reagent used.

Compared to the previous correlation study with other types of cancer, this study did not find a correlation between p53 expression and clinical or histopathological variables. Cordon Carlo et al. who conducted a study about p53 and its correlation with bladder cancer found a positive correlation between p53 expression and vascular invasion [11]. Cabelguenne et al., who studied head and neck tumour [12], and Maeda et al., who studied carcinoma of the stomach, showed that p53 immunoreactivity was not correlated with lymph node metastasis [13]. On the other hand, Unal et al. noted a higher cervical metastasis in tongue cancer case in p53 positive [14].

Lopes et al. showed that the immunoreactivity and staining stage of p53 was significantly associated with lymph node metastasis in N stage penile cancer [15]. Patient with p53 immunoreactivity had 4.8 times risk of having metastasis compared to a patient with the p53 negative. Lymphatic embolism by neoplasm cell and positive p53 was a single predictive factor for lymph node metastasis in multivariate analysis. In this study, metastasis was not assessed because of the lacking number of the patient who had metastasis, some of which could not be followed.

In the study conducted by Marinescu et al., it is found that all (100%) of the poorly differentiated penile cancer had a positive expression of p53 oncoprotein, regardless of the tumour stage. P53 was found in 91.2% of the moderately-differentiated tumour and 72.2% of the well-differentiated tumour [16]. Based on the tumour stage, Marinescu identified a positive association of p53 in all stage II and stage III tumour, and in 43 (84.3%) stage I tumour. In a well-differentiated tumour, the p53 marker is in the nuclei of the peripheral islands of tumour cell and seldom isolated inside the islands, with low or moderate intensity. For moderately- or poorly-differentiated squamous penile cancer, p53 immunohistochemistry staining was found in the nuclei, in the peripheral neoplastic tumour island, with moderate or high intensity. The association between squamous cell carcinoma and carcinoma in situ was statistically different. In addition to that, the association between poorly-differentiated carcinoma and well-differentiated carcinoma was statistically different [16]. In this study, we evaluated the clinical stage of the clinical T stage and found no association between p53 expression and clinical T stage cT2 or cT3 or cT4 penile cancer.

A prospective study conducted by Kamran-Shostari et al. about the clinical significance of p53 and p16 in penile cancer survival rate in North America revealed that p53 could be a predictor for penile cancer carcinoma metastasis to the lymph node. However, this association was only found in patients with the p16 negative. P16 was a predictor in penile carcinoma in association with HPV (Human Papilloma Virus) infection [17]. In another study about penile cancer survival, positive p53 was associated with bad prognosis. Ficarra et al. showed that the survival rate was only 34% in 47 patients with penile cancer. In addition to that, a patient with p53 negative gene expression had a 5-year- and 10-year-survival rate of 64.5% and 54.6%. Meanwhile, the p53 positive gene expression had 30.2% and 26.4%. These differences were significant between two groups (p = 0.009) [18]. This study is by our study which showed that p53 expression is significantly different with 3-year-survival-rate of a penile cancer patient (p = 0.025).

In this study, we found that p53 could be a predictive factor for mortality in penile cancer. However, this study has a limitation in the number of the sample and follow-up. The development of treatment modalities such as prophylaxis lymphadenectomy and chemotherapy have a role that should be considered for further study. Involvement of other than p53 gene, such as p16 should also be considered as a comparator.

In conclusion, there is a significant association (p = 0.025) between p53 expression and mortality in penile cancer patients. In conclusion, p53 expression in penile cancer cells examined by immunohistochemistry may show prognostic values in the disease progression.

Further research is needed to assess p53 expression and its association with treatment response in penile cancer patients. In addition to that, the involvement of other than p53 gene, such as p16 should also be considered as a comparator.

References

1. Pettaway CA, Lance RS, Davis JW. Tumors of the penis. In Campbell-WalshUrology, 2012:901-1000.

2. Tranggono U, Umbas R. Karakteristik dan terapi penderita keganasan penis di RS Cipto Mangunkusumo dan RS Kanker Dharmais. Indonesian Journal of Cancer. 2008:45-50.

3. Kusmawan E, Bowolaksono, Widiana R. The Clinical Features of Penile Cancer Patients at Sanglah General Hospital Bali-Indonesia. Bali Medical Journal. 2012; 1(1):1-5.

4. Hakenberg OW, Protzel C. Chemotherapy in penile cancer. Therapeutic Advances in Urology. 2012; 4(3):133-8. https://doi.org/10.1177/1756287212441235 PMid:22654965 PMCid:PMC3361747

5. Irawan W, Warli SM. Karakteristik Penderita Kanker Penis di RSUP H Adam Malik Medan, 2015.

6. Reed SI. Cellcycle in Cancer Principles & Practice of Oncology 7th Ed. Editor: DeVitaJr V, Hellman S, Rosenberg S A. Lippincott & Wilkins, Philadelphia, 2005; 1: 89-94.

7. Zargar-Shostari K, Spiess P E, Berglund A E et al. Clinical Significanceof p53 and p16ink4 Status in a Contemporary North American Penile Carcinoma Cohort. Clinicalgenitourinary cancer. 2016; (12):1-6.

8. Gunia S, Kakies C, Erbersdobler A, Hakenberg O W, Koch S, May M. Expressionof p53, p21 andcyclin D1 in penile cancer: p53 predictspoor prognosis. JounalofClinicalPathology. 2012; 65:232-236.

9. Levi JE, Rahal P, Sarkis ÁS, Villa LL. Human papillomavirus DNA and p53 status in penile carcinomas. International journal of cancer. 1998; 76(6):779. https://doi.org/10.1002/(SICI)1097-0215(19980610)76:6<779::AID-IJC1>3.0.CO;2-V

10. Lam KY, Chan AC, Chan KW, Leung ML, Srivastava G. Expression of p53 and its relationship with human papillomavirus in penile carcinomas. European Journal of Surgical Oncology (EJSO). 1995; 21(6):613-6. https://doi.org/10.1016/S0748-7983(95)95262-4

11. Cordon-Cardo C, Zhang ZF, Dalbagni G, Drobnjak M, Charytonowicz E, Hu SX, Xu HJ, Reuter VE, Benedict WF. Cooperative effects of p53 and pRB alterations in primary superficial bladder tumors. Cancer research. 1997; 57(7):1217. PMid:9102201

12. Cabelguenne A, Blons H, de Waziers I, Carnot F, Houllier AM, Soussi T, Brasnu D, Beaune P, Laccourreye O, Laurent-Puig P. p53 alterations predict tumor response to neoadjuvant chemotherapy in head and neck squamous cell carcinoma: a prospective series. J Clin Oncol. 2000; 18(7):1465-73. https://doi.org/10.1200/JCO.2000.18.7.1465 PMid:10735894

13. Maeda K, Kang SM, Onoda N, Ogawa M, Sawada T, Nakata B, et al. Expression of p53 and vascular endothelial growth factor associated with tumor angiogenesis and prognosis in gastric cancer. Oncology. 2008; 55:594. https://doi.org/10.1159/000011918 PMid:9778629

14. Unal OF, Ayhan A, Hosal AS. Prognostic value of p53 expression and histopathological parameters in squamous cell carcinoma of oral tongue. J Laryngol Otol. 2009; 113:446.

15. Lopes A, Bezerra AR, Pinto CA, Serrano SV et al. p53 as a new prognostic factor for lymph node metastasis in penile carcinoma: analysis of 82 patients treated with amputation and bilateral lymphadenectomy. AUA. 2002; 168:81-6.

16. Marinescu A, Stepan AE, Mărgăritescu C et al. P53, p16 and Ki67 immunoexpression in cutaneous squamous cell carcinoma and its precursor lesions. Rom J Morphol Embryol. 2016; 57(2):691-6. PMid:27833960

17. Zargar-Shoshtari K, Spiess PE, Berglund AE, Sharma P, Powsang JM, Giuliano A, Magliocco AM, Dhillon J. Clinical significance of p53 and p16ink4a status in a contemporary North American Penile Carcinoma Cohort. Clinical genitourinary cancer. 2016; 14(4):346-51. https://doi.org/10.1016/j.clgc.2015.12.019 PMid:26794389

18. Ficarra V, D'Amico A, Cavalleri S, Zanon G, Mofferdin A, Schiavone D et al. Surgical treatment of penile carcinoma: our experience from 1976 to 2007. Urol Int. 2009; 62:234. https://doi.org/10.1159/000030404 PMid:10567891

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Apr 15; 7(7):1174-1179.
https://doi.org/10.3889/oamjms.2019.251
eISSN: 1857-9655
Case Report

Management Comprehensive Multidisciplinary of Malignant Ovarian Germ Cell Tumors and Feto - Maternal Outcome: A Case Series Report and Literature Review

Lany Christina Prajawati Ni Luh[1], I Nyoman Bayu Mahendra[2*], Ketut Suwiyoga[2], ING Budiana[2], IGP Mayun Mayura[2], AAG Putra Wiradnyana[3], I Wayan Artana Putra[3], I Ketut Surya Negara[3], Ketut Ariawati[4], IGA Sri Mahendra Dewi[5], AAA Ngurah Susraini[5], Ni Putu Sriwidyani[5], Wahyudi Wirawan[1], Ida Ayu Vikananda[1]

[1]Obstetrics and Gynaecologic Registrar, Obstetrics and Gynecologic Department, Bali, Indonesia; [2]Oncology-Gynecology Division, Bali, Indonesia; [3]Maternal-Fetal Medicine Division, Bali, Indonesia; [4]Hematology-Oncology Pediatric Division, Bali, Indonesia; [5]Pathology Department, Sanglah General Hospital, Udayana University, Bali, Indonesia

Citation: Ni Luh ICP, Mahendra INB, Suwiyoga K, Budiana I, Mayura IGPM, Wiradnyana AAGP, Putra IWA, Negara IKS, Ariawati K, Dewi ISM, Susraini AAAN, Sriwidyani NP, Wirawan W, Vikananda IA. Management Comprehensive Multidisciplinary of Malignant Ovarian Germ Cell Tumors and Feto - Maternal Outcome: A Case Series Report and Literature Review. Open Access Maced J Med Sci. 2019 Apr 15; 7(7):1174-1179. https://doi.org/10.3889/oamjms.2019.251

Keywords: Ovarian malignant germ cell tumours; Pregnancy; Chemotherapy; Surgical staging

***Correspondence:** Bayu Mahendra, IN. Staff of Oncology-gynecology Division Sanglah General Hospital Denpasar, Bali, Indonesia. E-Mail: bayu.mahendra.nyoman@gmail.com

Received: 08-Mar-2019; **Revised:** 26-Mar-2019; **Accepted:** 27-Mar-2019; **Online first:** 03-Apr-2019

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Malignant Ovarian Germ Cell Tumors (MOGCT) most commonly occur in young women in the reproductive age group. Timely antenatal diagnosis and treatment of the tumour to enhance maternal and perinatal outcomes are the main challenges confronting the obstetrician and the gyne-oncologist.

CASE PRESENTATION: Here we present three cases of pregnancy complicated with MOGCTs. The first case (immature teratoma) was complicated by maternal psychological symptoms consistent with stress and histopathological examination confirmed the diagnosis of premature ovarian failure (POF). The second case (dysgerminoma) preterm labour occurred as an obstetric complication, but the baby was born in good condition without IUGR. The third case (yolk sac tumour) treated with docetaxel (brexel)-carboplatin chemotherapy administration there was no maternal or fetal complication. At the end of the pregnancy and delivery, complete surgical staging and cytoreduction were performed, and no metastases were found.

CONCLUSION: Optimal management strategies centre on a multi-disciplinary comprehensive team approach is critical resulting in better outcomes for the mother and the baby by avoiding complications.

Introduction

Germ cell tumours (GCTs) originate from ovarian primordial germinal cells [1]. Malignant Ovarian Germ cell tumours occur most commonly in young women, usually unilateral (84.3%) and diagnosed at stage I (76.4%) [2]. The most common is dysgerminoma, the second is immature teratoma, and the third is the Yolk sac tumour. These tumours can be pure or mixed. The worldwide cancer prevalence in 2012 according to the International Agency for Research on Cancer (IARC) was 6,657,518. The prevalence of ovarian cancer was 238,719 (3.6%) with overall mortality 151,917 (4.3%) [3]. Based on Dharmais Cancer Hospital data in 2010-2013, the incidence of ovarian cancer in Indonesia was 536, and 126 women died. There were 8 cases of MOGCTs recorded at Pathology Department of Sanglah Hospital in 2016 to 2017 which consisted of 1 case of dysgerminoma, 5 cases of immature teratoma, 1 case of yolk sac tumour, and 1 case of mixed GCTs. Three cases of MOGCTs in pregnancy will be described in this report. The incidence of MOGCTs in pregnancy is very rare which 1 in 12,500-25,000 pregnancies [4]. Prognosis is dependent on histopathological tumour grading [2]. Decision-making regarding timing, mode, and place of delivery should be made by a

comprehensive team approach which will reduce maternal and perinatal morbidity and mortality [3], [4]. The incidence of gynaecological cancer over the past four decades has been rising associated with the trend of delayed childbearing [5]. As a result, the average age of primigravidae increased thus raising the risk of ovarian cancer and ovarian cancer during pregnancy.

We present three cases of pregnancy complicated with MOGCTs. All cases were delivered at term after conservative primary surgery performed involving unilateral salpingo-oophorectomy in late mid-trimester followed by chemotherapy until delivery and then surgical staging and debulking at relaparotomy after delivery of the baby. Surgical staging was conducted according to the FIGO Staging classification of ovarian tumours.

Case 1

A 31 y.o. Multigravida (G2P1A0) at 8 weeks 1 day (8w1d) single/life gestation with solid ovarian tumour referred by the obstetrician to Obstetric and Gynecologic Policlinic of Sanglah Hospital, Denpasar. The pelvic examination found an adnexal tumour which increased progressively in size from 15 x 15 cm at 8w1d gestational age (GA) to 30 x 30cm at 19w5d GA, which was solid, with an irregular surface, mobile, and painful. Tumor marker levels were raised, AFP: 699.9 IU/mL (normal level at 2nd trimester: 22-93 IU/mL), CA-125: 285.4 U/mL (normal level at 2nd trimester: < 35 U/mL), LDH: 579 U/L (normal level at 2nd trimester: 240-480). The patient had primary surgery (left oophorectomy, cytological test, and omentectomy) at 19w5d GA with minimal manipulation of the uterus. A midline abdominal incision performed, and an operation, a unilateral solid ovarian tumour identified 40 x 40cm in size with an irregular surface, arising from the left ovary with omental adhesions requiring adhesiolysis despite rupture of the tumour, it was successfully removed (Figure 1).

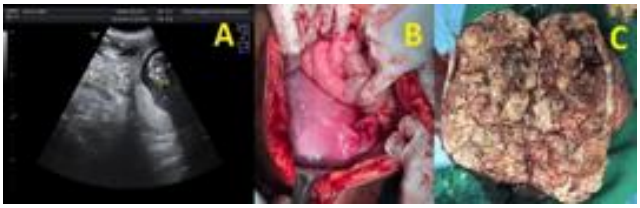


Figure 1: Immature Teratoma Ultrasound (US) and Macroscopic Finding Durante Operation. CASE 1: A. US showed hypohyperechoic left adnexal mass which cannot reach totally inside probe range at 19w5d GA, irregular border, unsmooth surface; B. uterine size appropriate with 18-20 weeks GA, left Fallopian tube was normal, post-oophorectomy; and C. Lobulated solid mass with internal multiseptal cystic mass

Internal abdominal and genitalia organ evaluation of uterus, size/consistency appropriate 18-20 weeks GA, right and left Fallopian tube to seem normal, right ovarium seems normal, no nodules on the peritoneum, omentum, and liver.

Histopathological assessment of left ovary was a grade II immature teratoma with immature components consisting of neuro-epithelial tubule and immature fat tissue; there was no evidence of malignant cell/immature component cell spreading on peritoneal fluid cytology. The patient's tumour was designated as a Stage IC1 grade II Ovarian immature teratoma (FIGO). An MRI conducted at 25w3d gestation without evidence of tumour spreading. After appropriate consultation of the multidisciplinary team, four courses of Bleomycin-Etoposide-cisplatin (BEP) chemotherapy regimen treatment was instituted (starting at 27w2d gestation), with two weeks follow up of fetal well-being with USG fetal scanning and fetal well-being every 2 weeks after chemotherapy was instituted along with routine NST after 34 weeks' gestation. Chemotherapy was paused 2 weeks before labour at 37w 6d gestation. The pregnancy continued until term and at gestation age 40w2d a female baby, 2700 gram, was delivered vaginally following the spontaneous onset of labour. At delivery, the baby had an Apgar score 7-8, with no evidence of congenital abnormality. Secondary surgery (relaparotomy complete surgical staging) was performed on day 58 postpartum. The surgeon performs a Total Abdominal Hysterectomy (TAH), left salpingectomy, right salpingo-oophorectomy, bilateral pelvic and paraaortic lymphadenectomy, omentectomy and peritoneal biopsy (bilateral paracolic- and prevesical peritoneum). Histopathological examination revealed nodules containing only mature glial components on the peritoneum (prevesical and cavum Douglas), with no evidence of malignant cells or metastases.

Case 2

A 24-year old pregnant woman was sent by Karangasem Hospital to Sanglah Hospital Denpasar with a chief complaint of dyspnea with a swollen abdomen for 11 days before admission. The patient was in her third pregnancy at 29w gestation with a solid ovarian tumour. Tumour marker levels were raised, AFP: 135.48 IU/mL, CA-125: 113.4 U/mL, RMI: 340.32, *Human Epididymis Protein 4* (HE4): 68.9, ROMA: 99.37%. Corticosteroids were administered before the planned surgical procedure to enhance fetal lung maturation, and tocolytics were also administered. Utrogestan orally 2 x 200 mg was given 24 hours before surgery and continued for 48 hours post-laparotomy.

The patient had primary surgery (Laparotomy

left salpingo-oophorectomy and omentectomy, frozen section and cytological test) at 30w gestation through a midline abdominal incision. The operation revealed a solid ovarian mass admixed with a cystic component 19 cm x 16 cm x 13 cm in size. Internal abdominal evaluation of the uterus was consistent with 30w gestation, the right and left Fallopian tubes were normal, the right ovary was normal, and there were no nodules on the peritoneum, omentum, and liver (Figure 2). The result of the histopathological assessment of left ovary was a dysgerminoma with a round to oval shape atypical cells separated with complex thin fibrous tissue septal network. Ascitic fluid cytology was positive for malignant cells, but no malignant cells were found in the momentum. The patient was staged as a FIGO Stage IC dysgerminoma ovarian cancer. No fetal anomalies were detected on feto-maternal ultrasound examination before treatment.

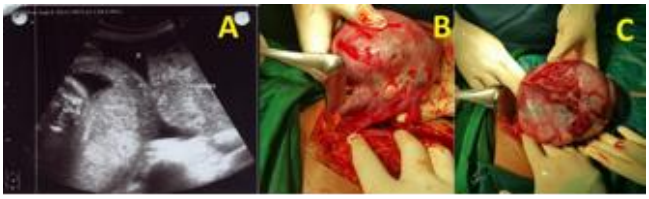


Figure 2: Dysgerminoma Ultrasound and Macroscopic Finding Durante Operation. Case 2. A. USG showed hypo-hyperechoic left adnexa mass 11.17x8.16 with papillae at 29w2d GA; B. and C. Durante operation: solid mass mixed with cystic 19 cm x 16 cm x 13 cm in size, arising from left ovarium

Following primary surgery, the patient received 4 courses of BEP regimen (starting at 33w1d gestation), and she was planned to have a re-laparotomy with surgical staging at the time of caesarean section at 38-weeks gestation age. However, the patient began in labour spontaneously at 35 weeks' gestation and delivered vaginally with the baby, a male, 2100 g with Apgar scores of 7 and 8, and Ballard scores of 28 equivalent to 35-36w gestation. The placenta appeared normal at the time of delivery, and histopathological examination was not performed. Postpartum follow up was uneventful. No signs of myelosuppression of the baby were found, with haemoglobin level being 15,9 g/dL. The baby was not breastfed during the period of maternal chemotherapy. Abdominal USG was performed after delivery which demonstrated no spread of the tumour. Re-laparotomy surgical staging was performed at 42 days postpartum, and chemotherapy continued until completion of the 4 courses.

It was decided to perform TAH and right salpingo-oophorectomy. Histopathological examination revealed no evidence of malignancy. The patient advised to have a routine physical check-up, ultrasonography, CA 125 and AFP serum tumour markers every 3 months in the first year which normal. The baby is now 2 y.o., in good condition and growing normally.

Case 3

A 27-year old pregnant woman referred to Sanglah Hospital by a provincial hospital from West Nusa Tenggara with a diagnosis of 17–18 weeks' gestation pregnancy and ovarian cyst suspicious of malignancy.

USG revealed a single fetus, fetal heart and movements were present, breech presentation, with 18w5d GA. At 19–20 weeks GA an ovarian cyst suspicious of malignancy (RMI: 400.80) confirmed and based on that finding the RMI was 400. A laparotomy was a right salpingo-oophorectomy, omentectomy and appendicectomy performed. Cytological examination of the ascitic fluid revealed seeding of mucinous adenocarcinoma, and the FZ result was mucinous adenocarcinoma. The result of histopathological examination of paraffin-embedded tissue was a Yolk Sac ovarian tumour (reticular and glandular pattern) (Figure 3). After pathological review, a Yolk Sac tumour of right ovary was confirmed with diagnosis G2P1001 21w3d gestation singleton pregnancy with an ovarian cancer FIGO stage IC post SOD, omentectomy, appendicectomy.

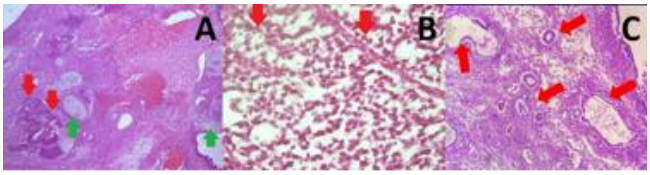


Figure 3: Microscopy of Immature Teratoma, Dysgerminoma, and Yolk Sac Tumor. A: Immature teratoma, consist of immature component/neuro-epithelial rosette and tubules (red arrow), mature glial tissue which pointed with green arrow, and another mature mesodermal component. B: Dysgerminoma, consist of round to oval atypical cell separated with complex thin fibrous tissue septal network which have rich lymphocyte infiltrate (red arrow). C: Yolk sac tumor, consist of cuboidal to columnar atypical cell forming gland-like structure, in part have clear cytoplasm forming glandular pattern (red arrow)

Docetaxel (Brexel)–carboplatin given for 6 courses. Follow up of fetal well being performed by the Feto-maternal Division every 2 weeks until chemotherapy was completed. Fetal scanning was performed at gestation age 26w 5d. No major congenital anomaly was found. Monitoring with NST was started at 35 weeks' gestation and remained normal. Chemotherapy was ceased 3 weeks before a planned caesarean section. This was done to allow time for recovery of the bone marrow of mother and baby.

The final diagnosis was FIGO ovarian cancer stage IC post TAH + BSO + omentectomy + appendicectomy, and post-chemotherapy. Patient advised to undergo routine physical examination, ultrasonography, CA 125 and serum AFP every month for the first 3 months and then 6 monthly for 2 years.

Discussion

In our case series, the first case was a 31 y.o pregnant woman with immature teratoma, the second case was a 24 y.o pregnant woman with a dysgerminoma, and the third case was a 27 y.o pregnant woman with a yolk sac tumour — the characteristics of the cases described in Table 1.

Table 1: Table of Case Illustration

Criteria	Cases		
	First	Second	Third
Maternal Age	31 y.o	24 y.o	27 y.o.
Patient finding	Abdomen enlarged at 8w 1d gestation	Abdomen enlarged & dyspneu at age 29w gestation	Abdomen enlarged at 19-20w gestation
Ultrasonography Finding	Hypo-hyperechoic mass, sircuscribed, rough surface, papillary, with septa	Hypo-hyperechoic mass, papillary	Hypo-hyperechoic mass, with septa
Tumor Marker	(-) (-) AFP: 699.9	CA 125: 113.4 RMI: 340.32 AFP: 135.48	CA 125: 400.8; RMI : 400.8 (-)
Antenatal Surgery time/type	19w 5d, left oophorectomy, omentectomy	30 w, Left salphingo-oophorectomy - FZ	21w, Right salphingo-oophorectomy-FZ, Omentectomy, Appendisectomy
Durante Operation Finding	Prominent solid component, 40x40 cm, lobulated, with cystic component, multisepta	Solid mass with cystic component, 19x16x13 cm	Solid mass with cystic component
FIGO Stage	IC1	IC	IC
Histophatology Type	Immature Teratoma grade II	Dysgerminoma	Yolk sac tumor
Metastase Evaluation	MRI (no metastase)	USG (no metastase)	(-)
Chemotherapy time/type	BEP 4 series at 27w2d gestation	BEP 4 series at 33w gestation	Docetaxel (Brexel) – Carboplatin 6 series at 21w 3d
Complication maternal after chemotherapy	POF	(-)	(-)
Complication fetal after chemotherapy	(-)	Preterm delivery	(-)
Mode of delivery/time	Vaginal delivery At 40w 2d gestation	Vaginal delivery At 35w gestation	CS At 38w gestation,
Placental metastasis	(-)	Not assesed	Not assesed
Fetal Outcome	2700 g (persentile 10-25), vigorous, no congenital abnormality	2105 g (persentile 25-75), vigorous, no congenital abnormality	3165 g (persentile 50-75), vigorous, no congenital abnormality
Surgical staging time/type	Complete surgical Staging at 58 d postpartum	Cytoreduction at 42 d postpartum,	Cytoreduction at the same with CS, at 39w
Breast feeding	(-)	(-)	(-)
Survival 5 years	62%	>90%	90%

Diagnosis of MOGCTs in the young female is challenging using tumour markers (AFP, LDH, hCG, CA125-RMI) and the US, with histopathological as the gold standard. Increased tumour markers level occur during pregnancy. Hence tumour markers level should be cautiously interpreted, and treatment strategies should not be based on these markers alone [8]. AFP produced initially by the fetal yolk sac and continued by the liver and digestive tract with a high AFP level associated with the suspicion of neural defect abnormalities such as spina bifida, anencephaly, oesophageal defect, and fetal abdominal separation failure. The AFP level decreased exponentially after primary ovarian tumour removal, and it concluded that the increased AFP level in this patient, originated from the primary tumour, and similarly with the other 2 cases where no major structural abnormality found.

The first case complicated by maternal psychological symptoms consistent with stress. The FSH level was high at 50.01 mU/L, but FSH has poor accuracy as a diagnostic marker in pregnancy for ovarian failure. The AMH is more reliable, and this plus the right ovarian atrophy (contralateral ovary) demonstrated on histopathological examination confirmed the diagnosis of premature ovarian failure (POF) as a possible contributor to her symptoms. Following immunohistochemistry (IHC) to detect estrogen receptors (ER) and progesterone receptors (PR) on affected left ovary (ovary with immature teratoma) it was concluded that hormone replacement therapy (HRT) could be safely given due to ER (-) and PR (+) [6]. With the second case, preterm labour occurred as an obstetric complication, but the baby was born in good condition without IUGR (percentile 25-50). The third case (yolk sac tumour) treated with docetaxel (brexel)-carboplatin chemotherapy administration there was no maternal or fetal complication. Carboplatin a second generation platinum-based drug was shown by Mir *et al*., to be a safer drug compared to cisplatin with no apparent fetal malformation or maternal toxicity [2], [7].

In our case series, all the babies had normal weight. Laboratory findings at first week after birth not found any evidence of transient myelosuppression. It was suggested that routinely follow up of the baby every 6 months needed for the first 2 years as a minimum. At the end of pregnancy and delivery, complete surgical staging and cytoreduction were performed, and no metastases were found.

Management of Patient

Conservative Surgery

The treatment of MOGCTs in pregnancy is by primary conservative surgery and chemotherapy followed by a complete surgical staging and cytoreduction post-delivery. Removal of an adnexal mass at pregnancy as conservative surgery is best done through a midline abdominal incision with a unilateral oophorectomy and cystectomy, *omentectomy,* and peritoneal fluid cytology to confirm the histopathological diagnosis. An adnexal mass detected in the first trimester of pregnancy in a patient with an ovarian lesion of suspected low malignant potential can be treated with conservatively. However in women with an ovarian mass with septae, solid areas, papillae, nodules, or persisting until 16 weeks gestation, surgery may be postponed until the second trimester (16-18 weeks gestation), as it is a ‘safe

period' for the fetus and the best period to perform surgical intervention of an adnexal mass as the spontaneous abortion risk is low (up to 10%), and an acceptable operative field is still available, allowing minimal uterine manipulation and lowering the risk of obstetric complications. The surgery can be performed ideally at 24-26 weeks gestation [9].

Adnexal mass surgery in the third trimester increases the incidence of preterm labour up to 22% and is associated with adverse obstetric outcome. Adnexal masses detected after 35 weeks gestational age can be removed at caesarean section [9]. Other indications for operative management include acute abdominal symptoms such as pain, rupture, and torsion. Routine tocolytic administration is still controversial but can be considered if there are signs of preterm labour [10, [11].

There is still a chance for spontaneous vaginal delivery with surgical staging completed 3 to 6 weeks after delivery. If operative delivery chosen then cytoreduction may be done at the same time as caesarean section. Re-laparotomy to complete surgical staging or cytoreduction is recommended as these may be limited during the pregnancy period, and there are reports of rapid growth and recurrence of OMGCTs during the puerperium.

Chemotherapy

The most challenging problem of chemotherapy during pregnancy is to obtain optimal chemotherapy without disturbing fetal growth and development with gestation age as a consideration [7]. Malignant ovarian GCTs are sensitive to chemotherapy and radiotherapy. Chemotherapy combination is usually used as adjuvant if the choice to continue the pregnancy. The choice of treatment determined by tumour type and its histological features to obtain the best response and improve prognosis. *Bleomycin-etoposide-cisplatin* is the first line chemotherapy for MOGCTs with treatment every 3 weeks for 3-4 courses. Patients have a good prognosis after *cisplatin-based* chemotherapy combination [13].

One review of the literature found that BEP chemotherapy was not associated with increasing congenital malformations [11]. Another study the use of cisplatin suggested an association with hearing loss (2,7%), ventriculomegaly (2%), IUGR and prematurity (8.3%), oligohydramnios (5.6%), anaemia (5.6%), micro-ophthalmia (2.8%) [6], [12]. Additionally, BEP may cause an imbalance of procoagulant-anticoagulant associated with disseminated intravascular coagulation, thrombosis, and bleeding (cisplatin-associated with liver vessel endothelial damage which causes ischemia and infarction; bleomycin associated with disturbance of blood clotting cascade; etoposide associated with thrombocytopenia).

Labour and Delivery

The optimal labour time is after 35-37 weeks and 3 weeks post-chemotherapy to avoid chemotherapy accumulation and to allow recovery from possible bone marrow suppression of mother and baby [13]. Mode of delivery preference was spontaneous vaginal rather than caesarean section. The benefits of spontaneous delivery are less blood loss, less operative risk and low infection risk with shorter length of stay at the hospital. Women having chemotherapy not recommended breastfeeding due to the risk of chemotherapy excreted through breast milk which could result in neonatal pancytopenia. Placental metastases often mentioned as a curiosity but occurred very rarely with only 80 cases reported since 1866. The incidence of fetal metastases is even more rare with only 11 cases reported in the literature [15].

Prognosis

The staging system used was the International Federation of Gynecology and Obstetrics (FIGO) 2014 with three ovarian cancers being stage IC. Malignant germ cell tumour has a better prognosis (> 95%) if detected at stage I. Advanced MOGCTs very aggressive, but prognosis may still be good if not delay chemotherapy. Prognosis of immature ovarian teratoma is governed by grade and stage. Five-year survival rate overall for all stages is 70-80%, and 90-95% for stage I patients. The five-year survival rate is 82%, 62%, and 30% respectively in patients with grades 1, 2, and 3 treated with optimum chemotherapy. Recurrence occurs on 36% during 5 years follow up, and the survival rates of early and advanced dysgerminomas are 95 and > 80%, respectively. Stage 1a dysgerminoma after unilateral salpingo-oophorectomy has a relapse rate of 10 to 20% with overall survival rate 90-100%. Recurrence patients who undergo repeat chemotherapy still have a survival rate greater than 90% [5].

In conclusion, malignant ovarian germ cell tumours in pregnancy are rare but challenging. Optimal management strategies centre on a multi-disciplinary comprehensive approach about diagnosis, primary surgery, chemotherapy, delivery, complete surgical staging, and neonatal care. Such an approach is critical resulting in better maternal and fetal outcomes by avoiding complications.

Acknowledgements

The authors wish to acknowledge the invaluable assistance given to them by the nursing and theatre staff and Pathology Department of Sanglah Hospital in the management of the patients and for the advice offered by Prof John Svigos,

Visiting Professor Department of Obstetrics and Gynecology, Sanglah Hospital and University of Udayana in the preparation of this paper for publication.

References

1. Akhtar K, Ahmad S S, Kumar A, Afshan N. Dysgerminoma with Pregnancy and Viable Baby: A Case Report. Oman Medical Journal. 2011; 26:198-200. https://doi.org/10.5001/omj.2011.48 PMid:22043416 PMCid:PMC3191700

2. Robert J K, Maria L C, Simon C H, Robert H Y. WHO Classification of Tumours of Female Reproductive Organs. 4th Edition. Lyon: International Agency for Research on Cancer, 2014.

3. Andrijono. Kanker Ovarium. Dalam: Sinopsis Kanker Ginekologi. Edisi Keempat. Jakarta: Balai Penerbit Fakultas Kedokteran Universitas Indonesia, 2013.

4. Peccatori et. al. Cancer, Pregnancy and Fertility: ESMO Clinical Practice Guidelines for Diagnosis, Treatment and Follow-Up. Annals of Oncology. 2013; 24(6):vi160-70. https://doi.org/10.1093/annonc/mdt199

5. Gupta M and Saini V. Germ Cell Tumors and Their Association with Pregnancy. New Delhi: Hindu Rao Hospital & NDMC Medical College, 2018. https://doi.org/10.5772/intechopen.72556

6. Morgan S. How Do Chemotherapeutic Agents Damage the Ovary? Edinburgh: The University of Edinburgh, 2014.

7. Berek. J.S, Friedlander. M.L, Hacker. N.F. Germ Cell and Nonepithelial Ovarian Cancer In: Berek and Hacker's Gynecologic Oncology. 6th ed. Wolters Kluwer. Philladelphia. 2015:541-2.

8. Voulgaris E, Pentheroudakis G, Pavlidis N. Cancer and Pregnancy: A Comprehensive Review. Surgical Oncology. 2011; 20:175–85. https://doi.org/10.1016/j.suronc.2011.06.002 PMid:21733678

9. Gezginc K, Karatayli R, Yazici F, Acar A, Celik C, Capar M. Ovarian Cancer during Pregnancy. International Journal of Gynecology and Obstetrics. 2011; 115:150-143. https://doi.org/10.1016/j.ijgo.2011.05.025 PMid:21872237

10. Grimm D. et al. Clinical management of epithelial ovarian cancer during pregnancy. European Journal of Cancer. 2014; 50:963–971. https://doi.org/10.1016/j.ejca.2013.12.020 PMid:24462638

11. Hoffman L B, Schorge J O, Schaffer J I, Halvorson L M, Bradshaw K D, Cunningham F G. Ovarian Germ Cell and Sex Cord-Stromal Tumors In: Williams Gynecology. 2nd Edition. New York: McGraw Hill Medical, 2012:879-97.

12. Robert J K, Lora H E, Brigitte M R. Blaustein's Pathology of the Female Genital Tract. Sixth Edition. New York: Springer, 2011.

13. Koren G, Carey N, Gagnon R, Maxwell C, Nulman I, Senikas V. Cancer Chemoterapy and Pregnancy. SOGC Clinical Practice Guideline. 2013; 288:263-78.

14. Amant. F et al. Gynecologic cancer in pregnancy: Guideline of an international consensus meeting. 2009; 19:51.

15. Sebire N J and Jauniaux E. Fetal and Placental Malignancies: Prenatal Diagnosis and Management. Ultrasound Obstet Gynecol. 2009; 33:235–4. https://doi.org/10.1002/uog.6246 PMid:19009536

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Apr 15; 7(7):1180-1183.
https://doi.org/10.3889/oamjms.2019.253
eISSN: 1857-9655
Case Report

Catheter Left Main Dissection Bailout Treatment with Stenting

Hajdin Çitaku[1*], Xhevdet Krasniqi[1,2]

[1]Clinic of Cardiology, University Clinical Center of Kosovo, Prishtina, Kosovo; [2]Faculty of Medicine, University of Prishtina "Hasan Prishtina", Prishtina, Kosovo

Citation: Çitaku H, Krasniqi X. Catheter Left Main Dissection Bailout Treatment with Stenting. Open Access Maced J Med Sci. 2019 Apr 15; 7(7):1180-1183. https://doi.org/10.3889/oamjms.2019.253

Keywords: Left main dissection; stenting

***Correspondence:** Hajdin Çitaku. Clinic of Cardiology, University Clinical Center of Kosovo, Prishtina, Kosovo. E-mail: dinicitaku@gmail.com

Received: 11-Feb-2019; **Revised:** 08-Apr-2019; **Accepted:** 10-Apr-2019; **Online first:** 13-Apr-2019

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Iatrogenic left main coronary artery dissection is a rare complication during invasive coronary procedures. Prompt action is needed avoiding acute pump failure followed with hemodynamic collapse and fatal results.

CASE REPORT: We report a 48-year-old woman who underwent bail-out stenting.

CONCLUSION: The best therapeutic strategy depends upon the prompt recognition of this complication, hemodynamic condition of the patient and operative skills. The therapeutic strategy by bail-out stenting should be performed in most cases of severe dissection toward good outcomes.

Introduction

Iatrogenic left main coronary artery dissection is a rare complication during coronary angiography or angioplasty. The incidence has been reported by about 0.07% [1]. Prompt action is needed because deterioration of antegrade coronary blood flow leads to acute pump failure resulting in hemodynamic collapse and fatal results [2]. Percutaneous coronary intervention (PCI) is considered as the best therapeutic option based on the type of dissection [3].

We report a case of a woman with iatrogenic left main coronary artery dissection treated by percutaneous coronary intervention (PCI).

Case Report

A 48-year-old woman was admitted to our hospital complaining of chest pain and palpitations. Her family history was positive for coronary artery disease. Physical examination was unremarkable while laboratory data showed no abnormal finding. There was no sign of cardiac ischemia on electrocardiography (ECG).

Transthoracic echocardiography demonstrated normal left ventricular function without wall motion abnormalities and no valvular disorder.

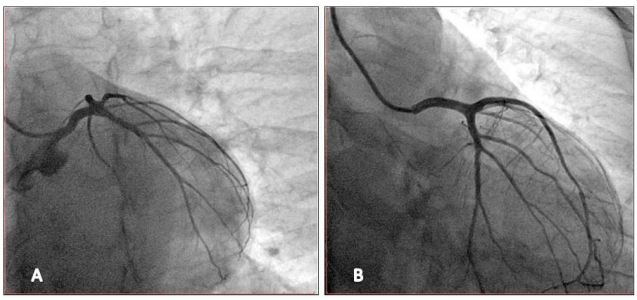


Figure 1: A) Normal left the coronary system in the left anterior oblique, caudal projection; B) Normal left the coronary system in the left anterior oblique, cranial projection

Coronary angiography was performed via the

right transradial approach. During angiography to engaged the left coronary artery was used a diagnostic 6 Fr left Judkins 4.0 catheter (Medtronic, Inc.) and a diagnostic 6 Fr right Judkins 3.5 catheter (Medtronic, Inc.) for engaging right coronary artery. Angiography of the left coronary artery was normal in the first and last view (LAO caudal and LAO cranial view) (Figure 1A and 1B).

Angiography of the right coronary artery was normal (LAO view) but in the level of the left main (LM) was showed the presence of contrast (Figure 2).

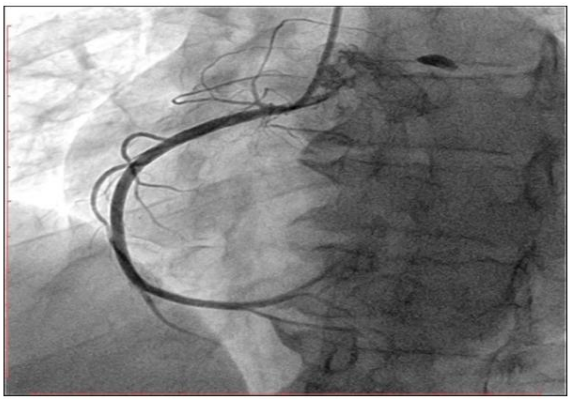


Figure 2: Normal right coronary artery with the presence of contrast in the level of the left main (LM).

Immediately, the patient complained of severe chest pain, accompanied by ST-segment elevation on the ECG and hemodynamic condition deteriorated.

A right femoral approach was chosen to perform the angiography of the left coronary artery with a 6 Fr 4 JL guiding catheter (Boston Scientific) showing an acute occlusion of the left anterior descending artery (LAD) (Type F) (LAO caudal view) (Figure 3A). A 0.014" choice floppy guidewire was advanced through LMCA into the true lumen of LAD, and a bare metal stent (BMS) 4.5 x 15 mm (Apolo 3, Balton) was placed in left main (LM), re-establishing TIMI 3 flow in LAD (Figure 3B, 3C and 3D).

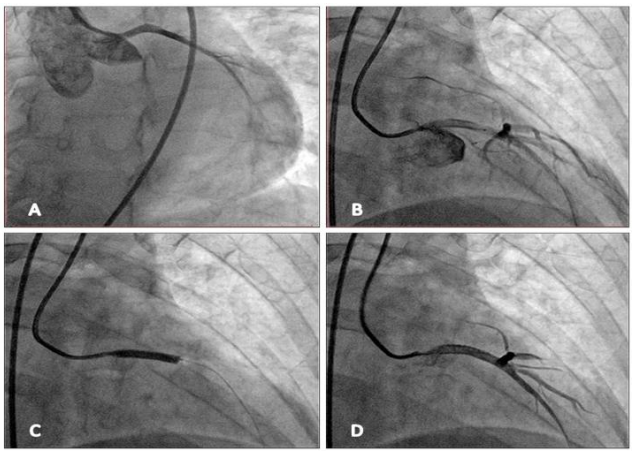


Figure 3: Left main dissection and stent placement; A) Left the main dissection followed with LAD occlusion; B) Stent position; C) Deploying stent; D) Left main stented

Post-dilatation was performed with a non-compliant balloon (NC-Quantum Apex, Boston Scientific) 4.5 x 15 mm (Figure 4A and 4B) (caudal view). Final angiography showed good results with TIMI 3 flow across the left coronary artery (Figure 4C, caudal view and 4D, RAO cranial view).

The patient was in a stable condition without symptoms after the procedure. She was discharged three days later with optimal medical treatment. At 4-month clinical follow-up, she did not have any symptoms with a good clinical condition.

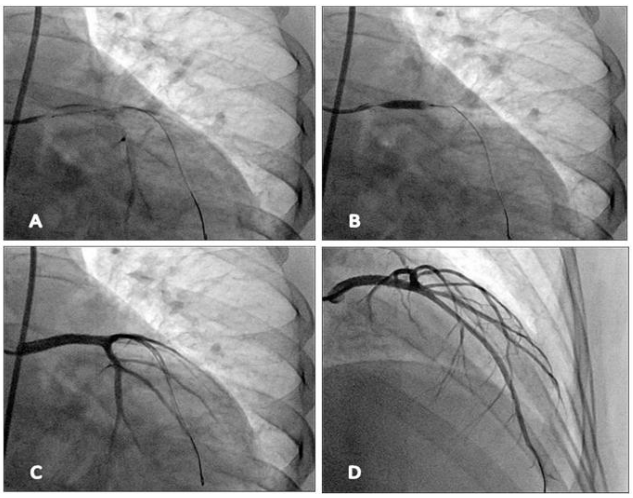


Figure 4: Balloon post-dilation; A) Balloon position; B) Inflation of balloon; C) Angiography results in caudal view; D) Angiography results in RAO cranial view

Discussion

Iatrogenic left main dissection as a life-threatening condition is a rare complication during coronary angiography or angioplasty. Risk factors for left main dissection include coronary artery anomalies, connective tissue disorders-Marfan syndrome, atherosclerotic changes-left main stenosis, arterial hypertension, bicuspid aortic valve, aortic root calcification and older age [4]. Iatrogenic left main dissection results from catheter manipulation, forceful injection of contrast medium, balloon dilatation and stenting [5], [6].

In our case, inappropriate position, deep intubation of the diagnostic catheter and vigorous hand-injection of contrast medium may have caused left the main dissection as a result of increased wall stress. The careful position of the catheter in co-axial alignment with the artery before any vigorous contrast injection cannot be stressed enough [2], [7].

Based on NHLBI classification (The National Heart, Lung and Blood Institute) dissection of the coronary artery is divided into six types (A-F)

according to an appearance on coronary angiography [8]. Clinically benign are types A and B, whereas types C through F portend significant morbidity and mortality if untreated [9]. Dunning classification of coronary artery dissection is based on the retrograde extension into the aortic root as a class I-focal dissection restricted to the coronary cusp, class II-involves the cusp and extends up the ascending aorta but is less than 40mm and class III-extends from the coronary cusp up the ascending aorta greater than 40 mm [10]. Eshtehardi's simplified classification according to the extension of left main (LM) ostial dissection includes three types of iatrogenic aortocoronary dissection (IACD): type I-a localized dissection in the LM ostium, type II-extension of the dissection from the LM into the left anterior descending artery (LAD) or left circumflex artery (LCx) and type III-extension of the dissection flap into the aortic root [1].

Left main coronary artery (LMCA) dissection may be followed with different Thrombolysis in Myocardial Infarction (TIMI) grade flow determining hemodynamic stability and clinical picture.

The alternative strategies for the treatment of iatrogenic LMCA dissection are a percutaneous coronary intervention (PCI), coronary artery bypass grafting (CABG) and conservative therapy.

Percutaneous coronary intervention (PCI) is preferred in patients hemodynamically unstable in terms of time and technique [11], [12], [13]. The foremost is the successful wiring of the true lumen of the LMCA and its branches considering that the false lumen is usually larger and inadvertent stenting of the false lumen will completely occlude the coronary artery [14].

In an observational study of patients with iatrogenic LM dissection from 38 patients, 1 (3%) patient died before any therapeutic attempt was performed, 6 (16%) patients were treated conservatively, 14 patients were treated by bail-out stenting, and 17 patients were treated by coronary artery bypass grafting (CABG). There was no difference during the 5-year follow-up between bailout stenting and CABG [1]. A review of the literature with 54 patients, 50 patients was treated by bailout stenting, 4 patients were treated by CABG, and there was only 1 cardiac death [2]. In patients with iatrogenic left main coronary artery dissection hemodynamically unstable prompt management by bailout stenting is crucial determining favourable outcomes.

In conclusion, in patients with iatrogenic left main coronary artery dissection to choice, the best treatment strategy is required prompt recognition of this complication, hemodynamic condition of the patient and operative skills. The therapeutic strategy by bail-out stenting should be performed as promptly as possible in hemodynamically unstable patients toward acceptable results.

Authors' Contributions

HÇ was contributed in the preparation of manuscript and literature analysis. XK was a major contributor in preparation of the manuscript, the design of manuscript and literature analysis. Authors read and approved the final manuscript.

Consent for publication

Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Ethics Approval and Consent to Participate

Approval from our local ethics committee was obtained for publication.

References

1. Eshtehardi P, Adorjan P, Togni M, Tevaearai H, Vogel R, Seiler C, Meier B, Windecker S, Carrel T, Wenaweser P, Cook S. Iatrogenic left main coronary artery dissection: incidence, classification, management, and long-term follow-up. American heart journal. 2010; 159(6):1147-1153. https://doi.org/10.1016/j.ahj.2010.03.012 PMid:20569732

2. Onsea K, Kayaert P, Desmet W, Dubois CL. Iatrogenic left main coronary artery dissection. Netherlands Heart Journal. 2011; 19(4):192-195. https://doi.org/10.1007/s12471-011-0089-1 PMid:22020998 PMCid:PMC3077877

3. Celik M, Yuksel UC, Yalcinkaya E, Gokoglan Y, Iyisoy A. Conservative treatment of iatrogenic left main coronary artery dissection: report of two cases. Cardiovascular diagnosis and therapy. 2013; 3(4):244. PMid:24400208 PMCid:PMC3878113

4. Awadalla H, Sabet S, El AS, Rosales O, Smalling R. Catheter-induced left main dissection incidence, predisposition and therapeutic strategies experience from two sides of the hemisphere. The Journal of invasive cardiology. 2005; 17(4):233-236. PMid:15831980

5. Kovac JD, De Bono DP. Cardiac catheter complications related to left main stem disease. Heart. 1996; 76(1):76-78. https://doi.org/10.1136/hrt.76.1.76

6. Boyle AJ, Chan M, Dib J, Resar J. Catheter-induced coronary artery dissection: risk factors, prevention and management. Journal of Invasive Cardiology. 2006; 18(10):500. PMid:17015916

7. Antoniades D, Apostolakis S, Tzoras S, Lazaridis K. Iatrogenic right coronary artery dissection distal to a total occlusion: a case

report. Cases journal. 2009; 2(1):6797. https://doi.org/10.4076/1757-1626-2-6797 PMid:19829863 PMCid:PMC2740282

8. Rogers JH, Lasala JM. Coronary artery dissection and perforation complicating percutaneous coronary intervention. The Journal of invasive cardiology. 2004; 16(9):493-499. PMid:15353832

9. Bittl JA, Ryan TJ, Keaney JF, Tcheng JE, Ellis SG, Isner JM, Sanborn TA. Coronary artery perforation during excimer laser coronary angioplasty. Journal of the American College of Cardiology. 1993; 21(5):1158-1165. https://doi.org/10.1016/0735-1097(93)90240-2

10. Dunning DW, Kahn JK, Hawkins ET, O'Neill WW. Iatrogenic coronary artery dissections extending into and involving the aortic root. Catheterization and Cardiovascular Interventions. 2000; 51(4):387-393. https://doi.org/10.1002/1522-726X(200012)51:4<387::AID-CCD3>3.0.CO;2-B

11. Vatrano M, Dattilo G, Mandraffino G, Gangemi S, Ciconte VA, Quartuccio S, Ceravolo R, Imbalzano E. A quick bailout ongoing of cardiogenic shock and iatrogenic dissection of the left main coronary artery. International journal of cardiology. 2015; 184:473-474. https://doi.org/10.1016/j.ijcard.2015.03.001 PMid:25756567

12. Cheng CI, Wu CJ, Hsieh YK, Chen YH, Chen CJ, Chen SM, Yang CH, Hung WC, Yip HK, Chen MC, Fu M. Percutaneous coronary intervention for iatrogenic left main coronary artery dissection. International journal of cardiology. 2008; 126(2):177-182. https://doi.org/10.1016/j.ijcard.2007.03.125 PMid:17490760

13. Boukhris M, Tomasello SD, Marzà F, Azzarelli S, Galassi AR. Iatrogenic aortic dissection complicating percutaneous coronary intervention for chronic total occlusion. Canadian Journal of Cardiology. 2015; 31(3):320-327. https://doi.org/10.1016/j.cjca.2014.11.030 PMid:25660151

14. Suarez-Mier MP, Merino JL. False lumen stent placement during iatrogenic coronary dissection. Cardiovascular Pathology. 2013; 22(2):176-177. https://doi.org/10.1016/j.carpath.2012.06.002 PMid:23153587

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Apr 15; 7(7):1184-1192.
https://doi.org/10.3889/oamjms.2019.256
eISSN: 1857-9655
Dental Science

Effect of Er,Cr:YSGG on Remineralization Using CPP - ACPF (MI - Paste Plus) after Enamel Erosion Caused by Carbonated Soft Drink in Primary Teeth: In-Vitro Study

Ghada Ezzeldin Elwardani[1*], Tarek Abdel Hamid Harhash[2], Ahmed Abbas Zaky[2]

[1]Pediatric Dentistry and Dental Public Health Department, Faculty of Dentistry, Alexandria University, Alexandria, Egypt; [2]Medical Applications of Laser Department, National Institute of Laser Enhanced Sciences (NILES), Cairo University, Cairo, Egypt

Citation: Elwardani GE, Harhash TAH, Zaky AA. Effect of Er,Cr:YSGG on Remineralization Using CPP - ACPF (MI - Paste Plus) after Enamel Erosion Caused by Carbonated Soft Drink in Primary Teeth: In-Vitro Study. Open Access Maced J Med Sci. 2019 Apr 15; 7(7):1184-1192. https://doi.org/10.3889/oamjms.2019.256

Keywords: Erosion; Primary teeth; DIAGNOdent; CPP-ACPF; Er,Cr:YSGG

***Correspondence:** Ghada Ezzeldin Elwardani. Pediatric Dentistry and Dental Public Health Department, Faculty of Dentistry, Alexandria University, Alexandria, Egypt. E-mail: gwardany@hotmail.com

Received: 01-Apr-2019; **Revised:** 09-Apr-2019; **Accepted:** 10-Apr-2019; **Online first:** 13-Apr-2019

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Erosion is a widespread phenomenon with higher predilection in primary dentition.

AIM: The aim of the present study is to assess the remineralising effect of Er,Cr:YSGG laser application combined with CPP-ACPF after erosive demineralisation by Coca-Cola in primary teeth.

METHODS: Fifty teeth (n = 10) were divided into; Group I: Artificial saliva, (Saliva natural, Medac, UK), Group II: CPP-ACPF (MI Paste Plus, GC Corp, USA), Group III: Er,Cr:YSGG (Waterlase iPlus, USA), Group IV: CPP-ACPF + Er,Cr:YSGG, Group V: Er,Cr:YSGG + CPP-ACPF. Teeth were immersed in Coca-Cola for 10 min, 5 times/day for 5 days. DIAGNOdent (DD) measurements were taken before and after the experiment.

RESULTS: There was a significant increase in DD readings after erosive-treatment cycles in all test groups. The highest reading was in samples immersed in artificial saliva, and the lowest was in those subjected to combined CPP-ACPF and Er,Cr:YSGG laser application, regardless of the sequence used. There was no significant difference between samples immersed in artificial saliva, and after CPP-ACPF application. Similarly, there was no significant difference between samples treated by combined treatment of CPP-ACPF and Er,Cr:YSGG application. However, there was a significant difference between samples immersed in artificial saliva or treated with CPP-ACPF application and those subjected to combined treatment CPP-ACPF along with Er,Cr:YSGG.

CONCLUSION: Combining Er,Cr:YSGG laser and CPP-ACPF paste significantly increased enamel remineralisation, regardless of the sequence implemented. Saliva naturally and CPP-ACPF application had a comparable effect on remineralisation.

Introduction

The two most common dental diseases are caries followed by erosion. There is strong evidence that the presence of dental erosion is widespread. Dental erosion is a surface phenomenon, defined as progressive, irreversible loss of tooth minerals caused by acid that are not produced by bacteria [1]. Primary teeth are more susceptible to erosion than permanent teeth [2]. Kazoullis et al. stated that the prevalence of erosion in the primary dentition is triple the prevalence in permanent dentition [3]. This can be explained by the thinner enamel, lower mineralisation, the inferior degree of crystalline arrangement and higher permeability of primary teeth. Hence, the erosive process appears shortly and more aggressively than in permanent teeth [2].

Epidemiological studies for dental erosion in children have reported a wide range of prevalence stretching between 0.6% to 95% [4], [5]. Such variation in epidemiological studies can be explained by the different evaluation standards used (examiner calibration and scoring systems) and the variation in population examined (sample size, age, gender and geographic location) [6]. The prevalence of erosion in primary dentition increases proportionally with children's age. Huang et al., tracked erosion in the

same group of children (2-4 years) annually. 7% had erosion detected for the first time at 3 years, while 28% at 4 years [7].

Common sites for erosion in primary teeth are the occlusal surface of molars and the palatal surfaces of maxillary incisors. A typical early sign of erosion appears as the smooth glazed surface. As the lesion progresses flattening of convexities, cupping of cusps and grooving of incisal edge occurs [2]. A meta-analysis by Li et al. concluded that erosion is strongly associated with carbonated soft drinks [8]. Moreover, advanced erosion (reaching dentin/pulp) was significantly correlated to the frequency of carbonated soft drinks consumption before bedtime and/or during the night [5].

Dental hard tissue is made up of hydroxyapatite crystals with the pure chemical formula; $Ca_{10}(PO_4)_6(OH)_2$. However, in reality, enamel is composed of a highly substituted hydroxyapatite-better described as a "calcium deficient" and "carbonated"-because some calcium ions can be substituted by sodium and some phosphate ions can be replaced by carbonates. Hence, making the tooth susceptible to dissolution. On the other hand, some hydroxyl groups can be replaced by fluoride ions to form fluoro-hydroxyapatite, $Ca_{10}(PO_4)_6(F)_2$, which has decreased susceptibility to dissolution, when compared to hydroxyapatite [2].

Demineralisation cannot be detected visually until it has progressed 200-300 μm into enamel, appearing chalky usually referred to as white spot lesions (WSL). Hence, diagnosis of early erosion may be overlooked, as it is accompanied by a few signs and fewer if any symptoms [9]. Various techniques have been utilised to evaluate dental erosion. Scanning electron microscope (SEM), atomic force microscopy and confocal laser scanning microscopy are examples of the qualitative assessment for surface topography. Generally, the problem with qualitative assessments is that the interpretation of the findings is subjective depending on the investigator evaluation. To get objective and measurable data, quantitative analyses should be implemented when possible. The most frequently adopted quantitative techniques include surface hardness and profilometry, as well as iodide permeability and chemical analysis of dissolved minerals. In addition to transverse microradiography, which is considered the gold standard for the quantification of enamel erosion. These techniques require sample preparation; therefore they are mainly used for in-vitro studies [2]. Both quantitative light-induced fluorescence (QLF) and ultrasonic measurement of enamel thickness are non-destructive and can be used in vivo [2], [10]. However, QLF correlated poorly with lesion depth, and ultrasonic measurement could not reliably detect early erosive lesion < 0.3 mm deep [10]. Moreover, the precise repositioning of the specimen for comparative readings is difficult. Also, the presence of organic pellicle may affect the accuracy of the reading [2].

Laser Fluorescence (LF), as in DIAGNOdent (DD), is the most recent technology for early diagnosis of demineralisation. It is a quantitative, non-invasive, chair side diagnostic tool that can be used both in vivo and in vitro. It can effectively monitor demineralisation, as well as, remineralisation for primary and permanent teeth [9], [11], [12]. Accordingly, DD provides a good means for patient education and motivation for treatment progress. It was explained that demineralisation stimulates more fluorescence [13]. There was a strong positive correlation between DD readings smooth-surface caries lesions in primary teeth and demineralisation depth measured by polarised light microscopy ($r = 0.78$) [14]. Mendes and Nicolau interpreted the best cut off reading to detect smooth surface demineralisation in primary teeth are at the value of 5. With this value; sensitivity, specificity, and accuracy were 0.78, 1, and 0.89, respectively [13].

Conventional strategies for preventing dental erosion involve restricting contact with the acidic intake. However, the success of such methods heavily relies on patient co-operation [1]. While fluoride is an effective anti-erosive agent, researches have geared towards finding a topical compound with synergistic benefits for remineralisation therapy. As a result, current recommendations for management of demineralised lesions include the use of casein containing products. MI paste plus is composed of casein phosphopeptide (CPP) that stabilises calcium and phosphate in a soluble form; known as amorphous calcium phosphate (ACP), with the addition of sodium fluoride (900 ppm) which approximates that of adult toothpaste. Calcium, phosphate and fluoride are essential remineralising components, which form highly insoluble complexes with tooth structure [9]. CPP-ACPF showed the best remineralisation potential after erosion by Coca-Cola on primary teeth when compared to CPP-ACP, fluoridated toothpaste and artificial saliva [15]. Moreover, CPP-ACPF resulted in favourable surface changes in primary teeth [11].

Ana et al., review confirmed the significant synergism of laser irradiation combined with topical remineralising agents in increasing enamel resistance to erosion [16]. A review by Ramalho et al. stated that Er,Cr:YSGG (2.78 μm) and Er:YAG (2.94 μm) lasers are most popular in dentistry. The advantage of Er,Cr:YSGG over Er:YAG was explained due to its higher absorption by hydroxyapatite, which is the main component in enamel. Because of higher absorption in water contents of enamel, Er:YAG laser causes more micro-explosions that result in an undesirable ablation [17]. Hence, Er,Cr:YSGG laser is considered to be more effective in caries prevention with lower ablation efficacy and deeper penetration [18].

In the light of the above mentioned information, the aim of the present in-vitro study was to assess the remineralizing effect of Er,Cr:YSGG laser application combined with CPP-ACPF after erosive

demineralization by Coca-Cola in primary teeth.

Materials and Methods

Hundred sound primary teeth (exfoliated/extracted for orthodontic reasons) were collected from hospitals/private practices. Initially, teeth were cleaned with wet gauze to remove blood/debris. Disinfection was carried out through immersion in 5% sodium hypochlorite for 1 hour [19]. Following the guidelines for infection control, teeth being disinfected were kept in a container with a secure lid to prevent leaking and labelled with the biohazard symbol (Figure 1) [20].

Figure 1: Disinfection of extracted teeth

Afterwards, teeth were rinsed in distilled water and air dried for 3 seconds [21]. Then, conventional visual inspection was performed under illumination and magnification lens with a blunt probe. Teeth with cracks/restoration/hypoplasia/hypomineralization were discarded [22]. Next, extracted teeth were stored collectively in a well-sealed container, frozen at -20°C until their use (1 month). A wet cotton roll was placed in each container, without contacting the teeth (Figure 2) [21]. For proper handling, extracted teeth should be maintained in a hydrated surrounding [20]. Before starting the experiment, Teeth were defrosted to room temperature over 24 hours [21].

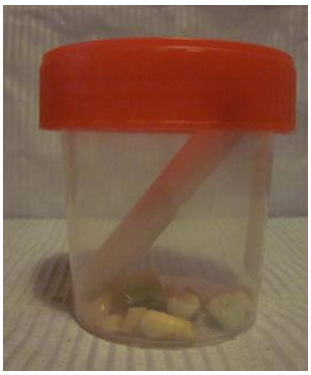


Figure 2: Storage of extracted teeth

DIAGNOdent readings

After defrosting, each tooth was air dried for 3 seconds [21]. Following the manufacturer's instructions, Probe type B (for smooth surfaces) of DIAGNOdent (KaVo, Biberach, Germany) was calibrated against its ceramic reference (Figure 3).

Figure 3: Calibration of the probe

Then, the probe was placed in light contact perpendicular to the tooth surface. The probe tip slowly scanned all four surfaces (buccal, mesial, lingual and distal) with a uniform motion for 5 sec on each surface, to ensure covering the entire area. The tip was tilted along its vertical axis to collect fluorescence from all directions to record the highest value [9]. The mean of the highest reading for each surface was recorded and used as a reference baseline for each tooth. The device was recalibrated after every 10th reading. A single, trained examiner (DE) was responsible for recording the DD values. Only teeth with readings ≤ 5 were considered intact and were included in this study (Figure 4) [13]. Hence, a total final sample size of 50 was established. Afterwards, teeth were placed in artificial saliva at an incubator at 37°C. Measurements were repeated at the end of the experiment.

Figure 4: Baseline reading

Sample Grouping

Teeth were randomly divided into 5 groups (n = 10) using a permuted block randomisation technique and were placed in coded containers. A double-blind investigation was fulfilled since coding was strictly maintained during DD measurements and statistical evaluation. Groups were assigned to 5 different treatment modalities as follows: Group I: Artificial saliva, (Saliva natural, Medac, UK) Group II: CPP-ACPF (MI Paste Plus, GC Corp, USA), Group III: Er,Cr:YSGG (Waterlase iPlus, USA), Group IV: CPP-

ACPF + Er,Cr:YSGG, and Group V: Er,Cr:YSGG + CPP-ACPF.

Erosive cycles

After grouping, teeth were immersed in 20 ml of refrigerated freshly opened Coca-Cola drink for 10 min. Then, specimens were rinsed thoroughly by distilled water, air dried with an air syringe for 3 seconds and replaced in artificial saliva at room temperature. This erosive challenge was performed 5 times/day with 3 hours interval (9 am, 12 pm, 3 pm, 6 pm and 9 pm). Those timing imitated 3 meals and in-between snacks and were repeated for 5 consecutive days. Moreover, the enamel of primary teeth becomes more susceptible to erosion at a high frequency of acid exposure (4 times/day) [2]. Coca-Cola and artificial saliva were refreshed for each cycle [19].

MI-Paste Plus application

CPP-ACPF paste was applied to teeth in groups II, IV and V. As recommended by the manufacturer, a sufficient amount of paste was smeared evenly on all tooth surfaces using a brush and left undisturbed for 5 minutes (Figure 5). Afterwards, teeth were rinsed in distilled water and replaced in artificial saliva [9]. As recommended, the paste was applied twice per day; before the first erosive challenge and after the last one.

Figure 5: MI-Paste Plus application

Er,Cr:YSGG laser irradiation

Teeth in groups III, IV and V were irradiated with Er,Cr:YSGG under the supervision of the faculty staff at Misr International University (MIU). MGG6 Sapphire tip (diameter of 600 μm-9 mm long) was placed in the hand piece according to the manufacturer instructions. Laser tip was inspected for scratches/contamination under magnification (Viqsy x30, Japan). Wavelength-specific protective eyewear was worn by the operator (OD 5+).

The device was adjusted at the following parameters: the power of 0.75 W, the energy density of 8.5 J/cm^2, energy per pulse of 12.5 mJ, the pulse width of 140 μs in (H mode), and frequency of 20 Hz without air/water cooling [23]. Teeth were removed from artificial saliva, air dried for 3 sec. And immediately radiated to avoid drying and resultant corruption of results [21]. Laser tip was kept perpendicular to the tooth surface and at a standardised distance of 1 mm. This was achieved by fixing an endodontic file with a rubber stop positioned at 1 mm to ensure a constant spot size (Figure 6).

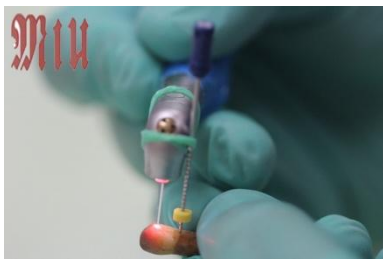


Figure 6: Tip was kept 1mm away

The laser was applied twice a day, before the first erosive challenge and after the last one. Each tooth surface was radiated horizontally and vertically. The handpiece was moved slowly for 10 sec in each direction to promote homogenous radiation covering the whole surface. Regular inspection to laser tip was performed every 5 teeth. The scratched tip was replaced while the contaminated tip was cleaned by cotton moistened with alcohol (Figure 7) [24].

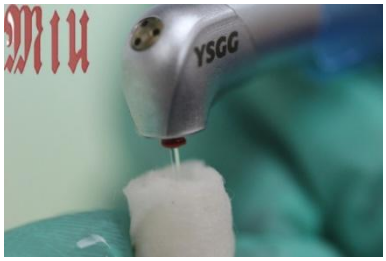


Figure 7: Cleaning of the contaminated tip

Statistical methodology

All data collected were tabulated and statistically compared using the SPSS software (ver. 21). Data were described using minimum-maximum, mean, standard deviation, median and inter-quartile range. Percentage change (%) was calculated as: [Measurement (after) – Measurement (before)] x 100/Measurement (before). Kolmogorov-Smirnov test (KS) of normality revealed a significance in the distribution of the variables, so the non-parametric statistics was adopted. Accordingly, box and whiskers plot was performed, and Wilcoxon Signed Ranks test was executed to compare DD readings at baseline and after the different treatment regimens. Kruskal-Wallis test was carried out to check for possible overall statistical differences between readings of the 5 subgroups. Then, Post-hoc pair-wise comparisons were carried out using Dunn-Sidak test for multiple comparisons. An alpha level was set to 5% with a significance level of 95%, and a beta error was accepted up to 20% with a power of study of 80%.

Results

The minimum-maximum DD readings, mean ± standard deviation, median and interquartile range at baseline and after treatment, as well as, the percentage change is represented in Table 1. Percentage change was calculated to evaluate the change in DD readings over time for better comparison.

Table 1: DIAGNOdent readings at baseline, after treatment and percentage change

	Groups					Test of significance *p* (value)
	Artificial Saliva (n = 10)	CPP-ACPF (n = 10)	Er,Cr: YSGG (n = 10)	CPP-ACPF + Er,Cr:YSGG (n = 10)	Er,Cr:YSGG+ CPP-ACPF (n = 10)	
Baseline						$X^2_{(df=4)(KW)}$= 0.2
Min-Max	2-5	1-5	3-5	1-5	1-5	*P* = 1.00
Mean ± SD	3.8 ± 1.0	3.6 ± 1.6	3.9 ± 0.9	3.6 ± 1.2	3.7 ± 1.3	
Median	4	4	4	4	4	
IQR	3-5	3-5	3-5	3-4	3-5	
KS	D = 0.18, *p* = 0.20	D = 0.21, *p* = 0.20	D = 0.25, *p* = 0.08	D=023,*p*=013	D=020,*p*=020	
After treatment						$X^2_{(df=4)(KW)}$= 40.9
Min-Max	13-23	9-15	8-14	5-8	3-9	*P* = 0.00*
Mean ± SD	18.0 ± 3.6	12.6 ± 2.0	11.4 ± 1.8	6.6 ± 1.2	6.4 ± 1.8	
Median	17.5^{abc}	13.0^{abc}	11.5^{abcde}	6.5^{cde}	6.5^{cde}	
IQR	15-21	11-14	10-13	6-8	5-8	
KS	D = 0.20, *p* = 0.20	D=028,*p*=002*	D = 0.13, *p* = 0.20	D=020,*p*=020	D=013,*p*=020	
Percentage change						$X^2_{(df=4)(KW)}$= 30.0
Min-Max	225-667	120-1200	100-300	50-400	50-200	*P* = 0.00*
Mean ± SD	409.2 ± 176.9	376.0 ± 351.1	202.2 ± 64.8	110.3 ± 107.5	83.3 ± 43.2	
Median	330.0^{abc}	225.0^{abc}	212.5^{abcde}	70.8^{cde}	70.8^{cde}	
IQR	250-600	160-367	160-250	60-100	60-80	
KS	D = 0.25, *p* = 0.07	D = 0.31, *p* = 0.00*	D = 0.14, *p* = 0.20	D=034,*p*=000*	D=033,*p*=000*	
Test of significance *p* (value)	$Z_{(WSR)}$= 2.8 *P* = 0.005*	$Z_{(WSR)}$= 2.8 *P* = 0.005*	$Z_{(WSR)}$= 2.8 *P* = 0.005*	$Z_{(WSR)}$= 2.8 *P* = 0.004*	$Z_{(WSR)}$= 2.9 *P* = 0.004*	#$Z_{(WSR)}$= 6.2 *P* = 0.00*

KS: Kolmogorov-Smirnov test; WSR: Wilcoxon Signed Ranks test; KW: Kruskal-Wallis test; *: Statistically significant (p < 0.05); #: Paired sample test for all samples (n = 50); Different superscript letters indicate significant pair-wise comparison.

KS test of normality showed significance in DD readings after treatment with CPP-ACPF (*p* = 0.02) and in values of percentage change after treatment with CPP-ACPF, applied alone and in combination with Er,Cr:YSGG (*p* = 0.00). This indicates that DD readings are not normally distributed and there is distortion in mean values. Therefore, it was recommended to use the median as a representative value for the study sample, as well as non-parametric tests for comparisons. Accordingly, box and whiskers graph was plotted to describe data distribution (Figure 8).

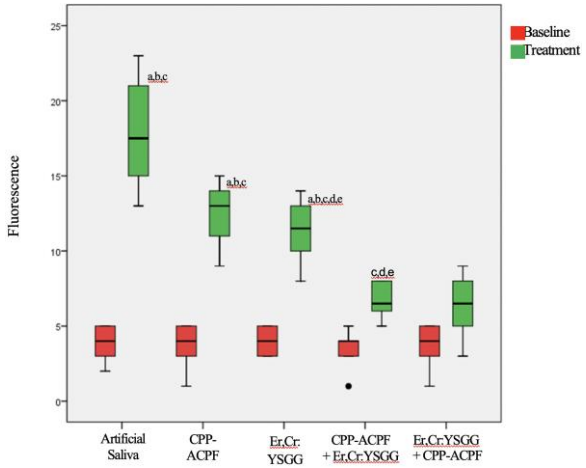
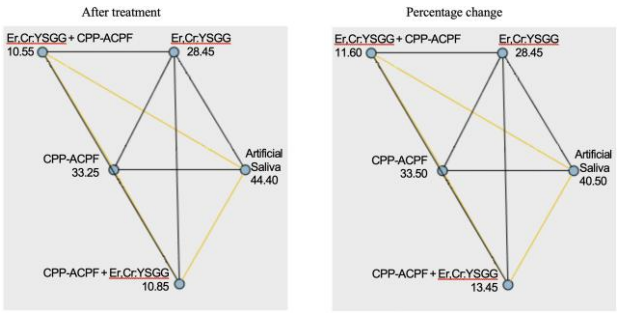


Figure 8: Box and whisker plot; (Different superscript letters indicate significant pair-wise comparison)

At baseline, there was no significant difference in DD readings between all test groups (*p* = 1.00), as sample size compromised only of teeth with readings ≤ 5. There was a significant difference in DD readings between baseline and after erosive-treatment cycles in each group (*p* = 0.004 and *p* = 0.005) and collectively (*p* = 0.00).

There was an increase in DD readings after erosive-treatment cycles in all test groups. The highest reading was in samples immersed in artificial saliva (Median = 17.5), and the lowest was in those subjected to combined CPP-ACPF and Er,Cr:YSGG laser application, regardless the sequence used (Median = 6.5). Similar to the percentage change values, the highest median was for samples immersed in artificial saliva (330%), while the lowest median was for samples with combined CPP-ACPF and Er,Cr:YSGG laser treatment, regardless the sequence used (70.8%).

Pair wise comparison between groups



Each node shows the sample average rank

Figure 9: Multiple comparisons of Dunn orders

There was a significant difference in DD readings, as well as, percentage change between groups after erosive-treatment cycles (*p* = 0.00). Pair-wise comparisons revealed that there was no significant difference between samples immersed in artificial saliva and after CPP-ACPF or Er,Cr:YSGG application.

Table 2: After treatment

Sample 1 — Sample 2	Test Statistics	Std. Error	Std. Test Statistics	Sig.	Adj. Sig.
Er,Cr:YSGG + CPP-ACPF — CPP-ACPF + Er,Cr:YSGG	0.300	6.497	0.046	0.963	1.000
Er,Cr:YSGG + CPP-ACPF — Er,Cr:YSGG	17.900	6.497	2.755	0.006	0.059
Er,Cr:YSGG + CPP-ACPF — CPP-ACPF	22.700	6.497	3.494	0.000	*0.005*
Er,Cr:YSGG + CPP-ACPF — Artificial Saliva	33.850	6.497	5.210	0.000	*0.000*
CPP-ACPF + Er,Cr:YSGG — Er,Cr:YSGG	17.600	6.497	2.709	0.007	0.067
CPP-ACPF + Er,Cr:YSGG — CPP-ACPF	22.400	6.497	3.448	0.001	*0.006*
CPP-ACPF + Er,Cr:YSGG — Artificial Saliva	33.550	6.497	5.164	0.000	*0.000*
Er,Cr:YSGG — CPP-ACPF	4.800	6.497	0.739	0.460	1.000
Er,Cr:YSGG — Artificial Saliva	15.950	6.497	2.455	0.014	0.141
CPP-ACPF — Artificial Saliva	11.150	6.497	1.716	0.086	0.861

Similarly, there was no significant difference between samples treated by Er,Cr:YSGG used alone or combined with CPP-ACPF. However, there was a significant difference between samples immersed in artificial saliva or treated with CPP-ACPF application

and those subjected to combined treatment CPP-ACPF along with Er,Cr:YSGG (Figure 9).

Table 3: Percentage change

Sample 1 — Sample 2	Test Statistics	Std. Error	Std. Test Statistics	Sig.	Adj. Sig.
Er,Cr:YSGG + CPP-ACPF — CPP-ACPF + Er,Cr:YSGG	1.850	6.512	0.284	0.776	1.000
Er,Cr:YSGG + CPP-ACPF — Er,Cr:YSGG	16.850	6.512	2.587	0.010	0.097
Er,Cr:YSGG + CPP-ACPF — CPP-ACPF	21.900	6.512	3.363	0.001	*0.008*
Er,Cr:YSGG + CPP-ACPF — Artificial Saliva	28.900	6.512	4.438	0.000	*0.000*
CPP-ACPF + Er,Cr:YSGG — Er,Cr:YSGG	15.000	6.512	2.303	0.021	0.213
CPP-ACPF + Er,Cr:YSGG — CPP-ACPF	20.050	6.512	3.079	0.002	*0.021*
CPP-ACPF + Er,Cr:YSGG — Artificial Saliva	27.050	6.512	4.154	0.000	*0.000*
Er,Cr:YSGG — CPP-ACPF	5.050	6.512	0.775	0.438	1.000
Er,Cr:YSGG — Artificial Saliva	12.050	6.512	1.850	0.064	0.643
CPP-ACPF — Artificial Saliva	7.000	6.512	1.075	0.282	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same; Asymptomatic significance (2-sided tests) are displayed. The significance level is 0.05.

Discussion

Both caries and erosion arise due to acidic attack on enamel — caries results from bacterial acids with a predilection for sites of plaque accumulation and fissures. In contrast, erosive lesions are caused by the acid of non-bacterial origin repeatedly 'washing' the enamel surface; at sites free of plaque [1].

A literature review by Taji and Seow added the incisal surface of maxillary teeth as a common site of erosion [1]. Similar to previous studies, the incisal/occlusal surface was not included in any intervention/measurement to avoid the confusion between attrition and erosion especially during the mixed dentition stage. The strong correlation between the prevalence of erosion and the frequent consumption of soft drinks at bedtime can be justified by the low salivary flow and the lack of remineralising factors such as brushing with fluoride toothpaste [5], [6].

Many studies revealed that DD has good reproducibility with a high degree of sensitivity and specificity in smooth surface lesions in primary teeth [13], [14]. Virajsilp et al., compared DD with bitewing in proximal caries diagnosis for primary teeth using the histological examination as a gold standard. The study revealed a stronger correlation between histological findings and DD reading (r = 0.85) than that with bitewing (r = 0.79). The sensitivity and specificity for DD in initial lesions were 0.75 and 0.89 respectively, compared to 0.69 and 0.55 for bitewing evaluation. Moreover, both inter- and intraexaminer reliabilities using DD were very high (0.97 and 0.99 respectively), indicating high reproducibility [25].

On the contrary, Mepparambath et al. compared the accuracy of DD to bitewing radiography in detecting proximal caries in primary teeth in-vivo. It was concluded that DD is not good at detecting initial enamel lesions in the proximal surface of primary teeth with low sensitivity (0.39). This controversy can be explained by the higher cut off limits for DD followed by Mepparambath et al. besides, DD was not compared to histological gold standard [12].

In the present study, primary teeth from children aged 6-13 years were collected from the public as well as private centres to include different genetic and socioeconomic variations. Unlike previous researches, primary teeth used in this study were not sectioned [19], [26]. Similar to Subramaniam and Pandey, primary teeth were used as a whole to avoid exerting mechanical stress on the tooth which may affect DD readings and to allow scanning of all axial surfaces for more accurate measurements [22].

Collected teeth were cleaned with wet gauze only, and rotating brush polishing was avoided in order not to interfere with DD readings [27]. Storing teeth frozen at -20°C was favoured over other commonly used mediums (formalin-thymol, chlorhexidine-sodium hypochlorite-salivary substitute-saline), as it does not significantly change the fluorescence readings [28].

A review by Western and Dicksit concluded that autoclaving, and formalin could be efficiently used for sterilisation of extracted teeth. Their use was discouraged, as autoclaving disrupts organic matrix of the tooth and formalin possess a major health hazard as a potential carcinogen [29]. The authors of the current study aimed for intermediate disinfection time, as performed by a previously [19]. This predilection was to avoid any possible change in LF readings, as well as any likely alteration in mechanical properties [28]. Besides, the authors view complete sterilisation to be only critical in the case of microbial studies, and that handling disinfected teeth with proper personal protective attire fulfils adequate infection control measures.

Thymol was also used to disinfect primary teeth in previous researches studying laser assisted remineralisation [22]. However, the authors of this study preferred the use of sodium hypochlorite due to its superior efficacy [29]. Teeth were air dried for only 3 sec before DD measurements as prolonged drying increases the fluorescence readings [21]. In this present study, the authors preferred to use a refrigerated beverage to mimic a real-life situation. The current study used artificial saliva at 37°C as a storage medium to simulate the oral environment.

For caries preventive treatment, laser irradiation is not intended to ablate the surface but only change the morphological/chemical composition of enamel instead. The authors of the current study deliberately avoided using air/water cooling with the subablative laser parameters by previous studies [23], [24]. This is explained in reviews by Ana et al., and Ramalho et al., through the strong absorption of Erbium lasers in water. Laser energy is rapidly and efficiently converted into thermal energy in water particles-either contained in enamel or existing freely

at the surface – resulting in abrupt vaporisation of water droplets and the removal of hard tissue. This thermal ablation is recognised to be hydrokinetic explosive mechanism meaning that it is water-mediated rather than by direct vaporisation of hydroxyl apatite crystals itself [16], [17].

However, other studies implemented the air/water cooling parameters to avoid the excessive increase of temperature beyond the ablation threshold or causing pulpal damage [22], [26]. Previous studies measured the temperature after Er,Cr:YSGG application under the same parameters without air/water mist at the enamel surface (212 ± 23°C) and in the pulp (2.1°C) [30], [31]. While other studies reported the ablation temperature for enamel is at 600 ± 50°C and that a temperature rises above 5.5°C in the pulp is a potential for loss of vitality [18], [32]. Therefore, the parameters used in the present study permit safe temperature rise for surface changes without causing pulpal trauma.

Studies investigating the simultaneous application of fluoride and laser to enhance acid resistance on primary teeth are scarce. Subramaniam and Pandey studied the cumulative effect of Er,Cr:YSGG laser (4W) and CPP-ACP, while Molaasadollah et al., (0.5W) observed its conjunction with APF [22], [26]. In the present study, the DD readings at baseline are by Kamath et al., [11].

The current study concluded that combining Er,Cr:YSGG irradiation with CPP-ACPF application significantly increased enamel remineralisation of primary teeth - regardless the sequence of application used.- when compared to either CPP-ACPF or artificial saliva used alone. It is difficult to compare the results of the present study because none of the previously mentioned investigations used DD as a method of evaluation. However, the results of this present study are in agreement with previous studies. It was concluded that combining Er,Cr:YSGG and APF-regardless the sequence of application used – significantly reduced the amount calcium ion lost significantly increased calcium fluoride retained after demineralisation, when compared to APF used alone. These researches used untreated enamel surface as the control group, unlike the current study, in which artificial saliva was adopted to simulate oral condition [33], [34].

Comparing with previous studies evaluating Er,Cr:YSGG assisted fluoride treatment on acid resistance in primary teeth, the findings of this study is in accordance to Subramaniam and Pandey-despite the different laser parameters used-who concluded that repeated Er,Cr:YSGG irradiation followed by CPP-ACP application significantly increased surface hardness of primary enamel more than CPP-ACP applied alone [22]. However, the findings of this study oppose to Molaasadollah et al., in which irradiation by Er,Cr:YSGG laser followed by the APF application was comparable to applying APF alone in reducing the area of WSL in primary teeth measured under a stereomicroscope. This disagreement can be attributed to the different laser parameters/material used, a single application of surface treatment, a different method of assessment [26].

The results of this study showed that the application of CPP-ACPF were comparable to immersion in artificial saliva, this is in accordance to previous studies [35], [37]. Gade justified this finding to be due to the short duration of the investigation (7 days), the absence of biofilm that enhances the adherence of CPP-ACPF and the lack of frequent demineralization that activates the CPP-ACPF [35]. However, other studies found no statistical difference under pH cycling for 30 days and after incubation with Streptococcus mutans for biofilm formation [36], [37].

The authors of the current study further suggest that the insignificant difference between artificial saliva and CPP-ACPF may attributed to the presence of xylitol in the composition of Saliva natura. This explanation is supported by Chunmuang et al., who explained that xylitol is able to form complexes with calcium ions, which results in its strong remineralizing potential [38]. The authors of this study preferred using the commercially available salivary substitute (Saliva natura) over a laboratory prepared artificial saliva due to the lack of calcium and phosphate in its composition when compared to previous studies [9], [11], [36]. The authors of the present study suggest that the insignificant difference between the Er,Cr:YSGG and all the other groups can be overcomed by increasing the sample size.

CO_2 laser has higher absorption coefficient by hydroxyapatite crystals than Er,Cr:YSGG [16]. Anaraki et al., and Kaur et al. compared acid resistance of both lasers. CO_2 laser had significantly higher surface hardness than Er,Cr:YSGG and showed significantly the least calcium ions released after demineralization. However, SEM analysis of enamel surface radiated with CO_2 laser appeared rough and cracked, which might act as retentive areas for plaque. In contrast to Er,Cr:YSGG group which revealed a smooth and glossy surface devoid of any cracks, making enamel impervious to acidic dissolution [39], [40].

More in-vivo/in-vitro studies with larger sample size need to be carried out to specify Er,Cr:YSGG parameters giving the best results under various methods of assessment and to identify the sequence/method of the combined fluoride application i.e. laser should be applied before, after or through fluoride treatment. Long term studies are needed to assess the efficiency of single verses multiple application of surface treatment. Further investigations are essential to compare the effectiveness of Er,Cr:YSGG and CO_2 laser to preventing demineralisation.

Based on the findings of this study and within its limitations, the current study concluded the following:

1) Combining Er,Cr:YSGG laser and CPP-ACPF paste significantly increased enamel remineralisation

2) Er,Cr:YSGG laser irradiation either before or after CPP-ACPF application did not affect remineralisation.

3) Saliva naturally and CPP-ACPF application had a comparable effect on remineralisation.

4) The effect of Er,Cr:YSGG laser application on remineralisation was similar to all other treatment options.

References

1. Taji S, Seow WK. A literature review of dental erosion in children. Aust Dent J. 2010; c55(4):358-67.

2. Lussi A. Dental erosion: from diagnosis to therapy. Karger, 2006. https://doi.org/10.1159/isbn.978-3-318-01331-3 PMCid:PMC2063555

3. Kazoullis S, Seow WK, Holcombe T, Newman B, Ford D. Common dental conditions associated with dental erosion in school children in Australia. Pediatr Dent. 2007; 29(1):33-9. PMid:18041510

4. Moimaz SA, Araújo PC, Chiba FY, Garbín CA, Saliba NA. Prevalence of deciduous tooth erosion in childhood. Int J Dent Hyg. 2013; 11(3):226-30. https://doi.org/10.1111/idh.12020 PMid:23506560

5. Al-Majed I, Maguire A, Murray JJ. Risk factors for dental erosion in 5-6 year old and 12-14 year old boys in Saudi Arabia. Community Dent Oral Epidemiol. 2002; 30(1):38-46. https://doi.org/10.1034/j.1600-0528.2002.300106.x PMid:11918574

6. Habib M, Hottel TL, Hong L. Prevalence and risk factors of dental erosion in American children. J Clin Pediatr Dent. 2013; 38(2):143-8. https://doi.org/10.17796/jcpd.38.2.4300111x4321l313 PMid:24683778

7. Huang LL, Leishman S, Newman B, Seow WK. Association of erosion with timing of detection and selected risk factors in primary dentition: a longitudinal study. Int J Paediatr Dent. 2015; 25(3):165-73. https://doi.org/10.1111/ipd.12109 PMid:24766533

8. Li H, Zou Y, Ding G. Dietary factors associated with dental erosion: a meta-analysis. PLoS One. 2012; 7(8):e42626. https://doi.org/10.1371/journal.pone.0042626 PMid:22952601 PMCid:PMC3432030

9. Jayarajan J, Janardhanam P, Jayakumar P, Deepika. Efficacy of CPP-ACP and CPP-ACPF on enamel remineralization - An in vitro study using scanning electron microscope and DIAGNOdent®. Indian J Dent Res. 2011; 22(1):77-82. https://doi.org/10.4103/0970-9290.80001 PMid:21525682

10. Louwerse C, Kjaeldgaard M, Huysmans MC. The reproducibility of ultrasonic enamel thickness measurements: an in vitro study. J Dent. 2004; 32(1):83-9. https://doi.org/10.1016/j.jdent.2003.08.007 PMid:14659722

11. Kamath P, Nayak R, Kamath SU, Pai D. A comparative evaluation of the remineralization potential of three commercially available remineralizing agents on white spot lesions in primary teeth: An in vitro study. J Indian Soc Pedod Prev Dent. 2017; 35(3):229-37. https://doi.org/10.4103/JISPPD.JISPPD_242_16 PMid:28762349

12. Mepparambath R, S Bhat S, K Hegde S, Anjana G, Sunil M, Mathew S. Comparison of proximal caries detection in primary teeth between laser fluorescence and bitewing radiography: An in vivo study. Int J Clin Pediatr Dent. 2014;7(3):163-7. https://doi.org/10.5005/jp-journals-10005-1257 PMid:25709294 PMCid:PMC4335105

13. Mendes FM, Nicolau J. Utilization of laser fluorescence to monitor caries lesions development in primary teeth. J Dent Child (Chic). 2004; 71(2):139-42.

14. Mendes FM, Siqueira WL, Mazzitelli JF, Pinheiro SL, Bengtson AL. Performance of DIAGNOdent for detection and quantification of smooth-surface caries in primary teeth. J Dent. 2005; 33(1):79-84. https://doi.org/10.1016/j.jdent.2004.10.010 PMid:15652172

15. Rallan M, Chaudhary S, Goswami M, Sinha A, Arora R, Kishor A. Effect of various remineralising agents on human eroded enamel of primary teeth. Eur Arch Paediatr Dent. 2013; 14(5):313-8. https://doi.org/10.1007/s40368-013-0085-9 PMid:24068490

16. Ana PA, Bachmann L, Zezell DM. Lasers effects on enamel for caries prevention. Laser Phys. 2006; 16(5):865-75. https://doi.org/10.1134/S1054660X06050197

17. Ramalho KM, Hsu CY, De Freitas PM, Aranha AC, Esteves-Oliveira M, Rocha RG, de Paula Eduardo C. Erbium lasers for the prevention of enamel and dentin demineralization: A literature review. Photomed Laser Surg. 2015; 33(6):301-19. https://doi.org/10.1089/pho.2014.3874 PMid:26067939

18. Perhavec T, Diaci J. Comparison of heat deposition of Er:YAG and Er,Cr:YSGG lasers in hard dental tissues. J Laser Health Acad. 2009; 2:1-6.

19. Agrawal N, Shashikiran ND, Singla S, Ravi KS, Kulkarni VK. Atomic force microscopic comparison of remineralization with casein-phosphopeptide amorphous calcium phosphate paste, acidulated phosphate fluoride gel and iron supplement in primary and permanent teeth: An in-vitro study. Contemp Clin Dent. 2014; 5(1):75-80. https://doi.org/10.4103/0976-237X.128672 PMid:24808700 PMCid:PMC4012123

20. Kohn WG, Harte JA, Malvitz DM, Collins AS, Cleveland JL, Eklund KJ. Guidelines for infection control in dental health care settings-2003. J Am Dent Assoc. 2004; 135(1):33-47. https://doi.org/10.14219/jada.archive.2004.0019 PMid:14959873

21. De Benedetto MS, Morais CC, Novaes TF, De Almeida Rodrigues J, Braga MM, Mendes FM. Comparing the reliability of a new fluorescence camera with conventional laser fluorescence devices in detecting caries lesions in occlusal and smooth surfaces of primary teeth. Lasers Med Sci. 2011; 26(2):157-62. https://doi.org/10.1007/s10103-010-0757-1 PMid:20157753

22. Subramaniam P, Pandey A. Effect of erbium, chromium: yttrium, scandium, gallium, garnet laser and casein phosphopeptide-amorphous calcium phosphate on surface micro-hardness of primary tooth enamel. Eur J Dent. 2014; 8(3):402-6. https://doi.org/10.4103/1305-7456.137656 PMid:25202223 PMCid:PMC4144141

23. Ana PA, Tabchoury CP, Cury JA, Zezell DM. Effect of Er,Cr:YSGG laser and professional fluoride application on enamel demineralization and on fluoride retention. Caries Res. 2012; 46(5):441-51. https://doi.org/10.1159/000333603 PMid:22739669

24. De Freitas PM, Rapozo-Hilo M, Eduardo Cde P, Featherstone JD. In vitro evaluation of erbium, chromium:yttrium-scandium-gallium-garnet laser-treated enamel demineralization. Lasers Med Sci. 2010; 25(2):165-70. https://doi.org/10.1007/s10103-008-0597-4 PMid:18787759

25. Virajsilp V, Thearmontree A, Aryatawong S, Paiboonwarachat D. Comparison of proximal caries detection in primary teeth between laser fluorescence and bitewing radiography. Pediatr Dent. 2005; 27(6):493-9. PMid:16532891

26. Molaasadollah F, Asnaashari M, Mashhadi Abbas F, Jafary M. In vitro comparison of fluoride gel alone and in combination with Er,Cr:YSGG laser on reducing white spot lesions in primary teeth. J Lasers Med Sci. 2017; 8(4):160-5. https://doi.org/10.15171/jlms.2017.29 PMid:29071020

PMCid:PMC5642162

27. Lussi A, Reich E. The influence of toothpastes and prophylaxis pastes on fluorescence measurements for caries detection in vitro. Eur J Oral Sci. 2005; 113(2):141-4. https://doi.org/10.1111/j.1600-0722.2004.00195.x PMid:15819820

28. Kaul R, Kaul V, Farooq R, Wazir ND, Khateeb SU, Malik AH, Masoodi AA. Cut off values of laser fluorescence for different storage methods at different time intervals in comparison to frozen condition: A 1 year in vitro study. J Conserv Dent. 2014; 17(2):124-8. https://doi.org/10.4103/0972-0707.128043 PMid:24778506 PMCid:PMC4001266

29. Western JS, Dicksit DD. A systematic review of randomized controlled trials on sterilization methods of extracted teeth. J Conserv Dent. 2016; 19(4):343-6. https://doi.org/10.4103/0972-0707.186457 PMid:27563183 PMCid:PMC4979281

30. Ana PA, Blay A, Miyakawa W, Zezell DM. Thermal analysis of teeth irradiated with Er,Cr:YSGG at low fluences. Laser Phys Lett. 2007; 4(11):827-34. https://doi.org/10.1002/lapl.200710060

31. Ana PA, Velloso WF Jr, Zezell DM. Three-dimensional finite element thermal analysis of dental tissues irradiated with Er,Cr:YSGG laser. Rev Sci Instrum. 2008; 79(9):093910. https://doi.org/10.1063/1.2953526 PMid:19044431

32. Zach L, Cohen G. Pulp response to externally applied heat. 1965; 19:515-30.

33. Moslemi M, Fekrazad R, Tadayon N, Ghorbani M, Torabzadeh H, Shadkar MM. Effects of Er,Cr:YSGG laser irradiation and fluoride treatment on acid resistance of the enamel. Pediatr Dent. 2009; 31(5):409-13. PMid:19947136

34. Zezell DM, Ana PA, Benetti C, Goulart VP, Bachmann L, Tabchoury CPM, Cury JA. Compositional and crystallographic changes on enamel when irradiated by Nd:YAG or Er,Cr:YSGG lasers and its resistance to demineralization when associated with fluoride. In: Rechmann P, Fried D, editors. Lasers in Dentistry. 16th ed. Proc SPIE, 2010:1-12. https://doi.org/10.1117/12.842967

35. Gade V. Comparative evaluation of remineralization efficacy of GC tooth mousse plus and enafix on artificially demineralized enamel surface: An in vitro study. Indian J Oral Health Res. 2016; 2(2):67-71. https://doi.org/10.4103/2393-8692.196097

36. Oliveira GM, Ritter AV, Heymann HO, Swift E Jr, Donovan T, Brock G, Wright T. Remineralization effect of CPP-ACP and fluoride for white spot lesions in vitro. J Dent. 2014; 42(12):1592-602. https://doi.org/10.1016/j.jdent.2014.09.004 PMid:25260438 PMCid:PMC5551488

37. Oliveira PR, Coutinho TC, Portela MB, Paula VC, Tostes MA. Influence of biofilm formation on the mechanical properties of enamel after treatment with CPP-ACP crème. Braz Oral Res. 2017; 31:e84. https://doi.org/10.1590/1807-3107bor-2017.vol31.0084 PMid:29185603

38.Chunmuang S, Jitpukdeebodintra S, Chuenarrom C, Benjakul P. Effect of xylitol and fluoride on enamel erosion in vitro. J Oral Sci. 2007; 49(4):293-7. https://doi.org/10.2334/josnusd.49.293 PMid:18195513

39. Anaraki SN, Serajzadeh M, Fekrazad R. Effects of laser-assisted fluoride therapy with a CO2 laser and Er,Cr:YSGG laser on enamel demineralization. 2012; 34(4):92-6.

40. Kaur T, Tripathi T, Rai P, Kanase A. SEM evaluation of enamel surface changes and enamel microhardness around orthodontic brackets after application of CO2 laser, Er,Cr:YSGG laser and fluoride varnish: An in-vivo study. J Clin Diagn Res. 2017; 11(9):59-63. https://doi.org/10.7860/JCDR/2017/30292.10603

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Apr 15; 7(7):1193-1197.
https://doi.org/10.3889/oamjms.2019.287
eISSN: 1857-9655
Dental Science

Comparative Study Clarifying the Usage of PEEK as Suitable Material to Be Used as Partial Denture Attachment and Framework

Sherif Aly Sadek[1,2*]

[1]Department of Prosthodontics, Faculty of Oral and Dental Medicine, Cairo University, Cairo, Egypt; [2]Department of Prosthodontics, Alfarabi Private College for Dentistry and Nursing, Jeddah, Kingdom of Saudi Arabia

Citation: Sadek SA. Comparative Study Clarifying the Usage of PEEK as Suitable Material to Be Used as Partial Denture Attachment and Framework. Open Access Maced J Med Sci. 2019 Apr 15; 7(7):1193-1197. https://doi.org/10.3889/oamjms.2019.287

Keywords: Removable Partial Denture (RPD); PEEK Material; Attachment system; Strain gauges

***Correspondence:** Sherif Aly Sadek. Department of Prosthodontics, Faculty of Oral and Dental Medicine, Cairo University, Cairo, Egypt; Department of Prosthodontics, Alfarabi Private College for Dentistry and Nursing, Jeddah, Kingdom of Saudi Arabia. E-mail: sherifalysadek@gmail.com

Received: 08-Mar-2019; **Revised:** 07-Apr-2019; **Accepted:** 09-Apr-2019; **Online first:** 13-Apr-2019

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Utilization of attachments in a removable partial denture is highly essential not only as a line of treatment but also as it has a remarkable impact on the denture's durability during the function. The attachment should act as a stress breaking system preserving the abutment teeth.

AIM: This consideration aimed to verify the convention of polyether ether ketone (PEEK) material as an attachment.

MATERIAL AND METHODS: Four groups of different materials for both attachments and partial denture framework were fabricated and tested using strain gauges to compare between them according to the strains originated around both the abutment teeth and edentulous area.

RESULTS: PEEK material is one of the esthetic materials used for fabrication of the framework of the RPD. On using it as a precision attachement is shows favorable stress distribution decreasing the strains around the abutment teeth and the alveolar ridge especially distal to the abutment teeth that was significantly reduced in comparison with the other treatment options.

CONCLUSION: Utilization of PEEK material as both an attachment and framework decline the strains performed around the abutment teeth and over the edentulous ridge.

Introduction

One of the challenging cases in Oral prosthodontics is treating a distal extension case removable partial denture (Kennedy's Class I and Class II). The best treatment plan for restoring such case is implant supported prosthesis, although it is not feasible in some cases either due to insufficient bone or economic reasons [1]. Hence, a castable removable partial denture is mostly preferred.

One of the affordable non-invasive solutions for rehabilitation of Kennedy's Class I and Class II cases is the removable partial denture which requires careful attention and meticulous treatment planning for restoring both esthetics and functional patient's desires [2], [3], [4]. Moreover, the simplicity of the framework design is extensively essential together with logical principles to diminish the associated complains that most commonly take place with removable partial denture rehabilitation [5], [6].

Attachments solved the esthetic problems associated with employing clasps especially in the anterior region [7]. A semi-precision attachment utilised with cast metal framework removable partial denture can enhance tremendous roles as both retention and esthetics that increase patient's self-confidence [5], [6], [8].

Since enhancing the clinical success with semi-precision attachment, it is recommended to involve at least two abutment teeth in distal extension cases aiming to eliminate the hazards impacts of excessive abutment loading by utilising an adjustable retentive plastic insert [9], [10], [11].

Rehabilitating Kennedy's Class II cases with a unilateral partial denture rises the patient's comfort and diminishing the adaptation period as the major connector's absence has major merit compared to the conventional removable partial denture [12].

The newly introduced PEEK [Polyether ether ketone] material in the dental market can be a nonmetallic alternative material for removable partial denture framework construction [13], [14]. Despite that limited contemplates employed PEEK as a denture base material, but some proved that such matter is of superior mechanical criteria including superior flexure behaviour with highest ability to spring back to its original shape after the load is released. These studies revealed the applicability of PEEK material in dentistry allowing offering the patient a metal-free restoration with superior mechanical properties [15], [16], [17], [18].

In-vitro studies provide the merit of repeating the considerations under the same circumstances together with the similar supporting teeth and mucosa which is not available in the in-vivo ones [19]. Thus, comparative would be more accurate and practical if they were laboratory performed.

One of the devices used to calibre the in-vitro contemplates is the Strain gauge which measures the objects' strain. This gauge comprises a flexible insulating backing that supports a metallic foil pattern which is attached with suitable adhesive. When applying a load to the object, the object is deformed which makes the foil to be deformed causing its electrical resistance to change [20], [21].

Hence, this in-vitro contemplate aimed is to evaluate the strain induced by different prosthetic options utilising PEEK material in lower Kennedy's Class II case.

Material and Methods

A mandibular model fabricated for educational purposes and constructed from acrylic resin* (Nissin dental products Inc. Kyoto Japan) was utilised as a master model replicating the anatomical features of both the teeth and soft tissues. Molars on one side of the model were removed, and their sockets were sealed with base plate wax* (Cavex set up regular, Modeling Wax, Cavex Holland.) to represent Kennedy class II partially edentulous cases.

This master model was duplicated to epoxy resin one by flashing technique using epoxy resin* (Kemapoxy 150 JM, CBM International). Then, four different treatment prosthetic options were fabricated forming four different groups.

Group 1: On the edentulous side, the first and second premolars were reduced and prepared for full coverage metal crowns with lingual ledge attached to its cast OT CAP unilateral attachment*(OT Unilateral, Rhein 83srl, Bologna, Italy). Then unilateral metal cast partial denture* (Cobalt-Chromium metal framework, vita, Switzerland) was constructed as for Kennedy class II cases according to the manufacturer's instructions.

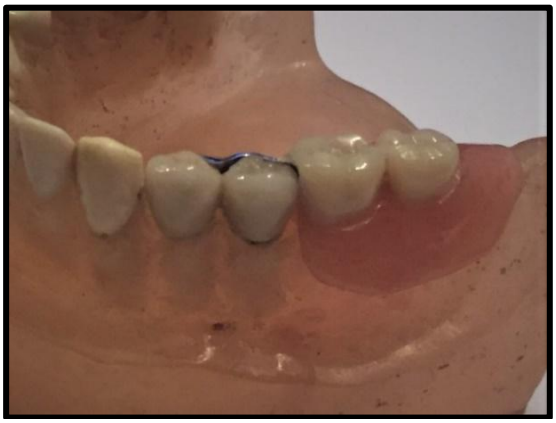


Figure 1: Full coverage metal crowns with lingual ledge attached to it cast OT CAP unilateral attachment, and unilateral metal cast partial denture

Group 2: On the reduced premolars, a full coverage metal crowns with lingual ledge attached to it OT CAP unilateral attachment were cast. Then thermoplastic PEEK* (BioHpp PEEK, Bredent, Germany) material used for fabrication of the unilateral partial denture. For the esthetic purpose the Visio.lign* (Visio. align, Bredent, Germany.) was used for the buccal flange of the partial denture used for Kennedy class II cases by the lost wax technique according to the manufacturer's guidelines.

Group 3: On the reduced premolars, full coverage PEEK crowns with lingual ledge attached to it OT CAP unilateral attachment were constructed. Then thermoplastic PEEK material unilateral partial denture with Visio.lign for buccal flange was constructed as for Kennedy class II cases.

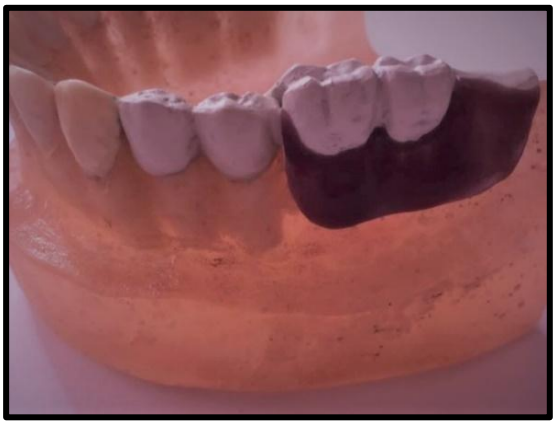


Figure 2: full coverage PEEK crowns with lingual ledge attached to it cast OT CAP unilateral attachment and Then thermoplastic PEEK material unilateral partial denture with Visio.lign for buccal flange

Group 4: On the reduced premolars, full coverage PEEK (polyether ether ketone) crowns with lingual ledge attached to it OT CAP unilateral attachment were constructed. Then unilateral metal cast partial denture was constructed as for Kennedy class II cases.

A pencil was utilised to delineate the crest of the residual ridge on the cast. Micro OT CAP parallometer mandrel* (Parallometer key for O T cap.) was employed to take the attachment from the kit and insert it into the mandrel tip carefully without squeezing the attachment between the mandrel head. Then, the mandrel was connected to parallometer to position the attachment little to the lingual aspect of the ridge to improve esthetics in final restoration and to beast functionality.

The ledge was prepared on the lingual surface of the premolars wax pattern to receive a lingual bracing arm. The attachment was positioned carefully and fixed with wax or adhesive component. The positional ring was applied over the attachment. Then, the crowns-attachment assembly was checked on the model, and the completed wax pattern was spread, invested and cast into either metal or PEEK crowns.

UNI Box was fit exactly on the attachment and flushed smoothly with abutment wax coping. Then a layer of wax was adapted on the residual cast ridge before applying the saddle and then joining the castable connector to UNI Box by resin to reinforce the structure. The completed wax pattern was spread, invested and cast into either metal or PEEK partial framework.

Electrical strain gauges using a fully digitalised universal testing machine (LLOYD) were then utilised to record the microstrains generated upon using the four different designs investigated in this consideration. Five strain gauges were employed to calibre the microstrains produced at the buccal, lingual, mesial and distal surfaces of the splinted abutments with each design next to reducing the acrylic resin surrounding the first and second premolars on the edentulous side and one at the mid of the edentulous area beneath the partial framework.

A spring type loading device capable of applying a wide range (Ascending load is applied at the acrylic bite block from 0 to 200 N with the loading tip of the device on the loading point of the acrylic block) of static loads was used to load the prosthesis. Then, all the collected data were tabulated collected and statistically analysed.

Statistical analysis

Data were presented as mean, standard deviation (SD) and Standard Error (SE). Data explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests. The strain showed a parametric distribution, so One Way ANOVA used to compare between tested groups followed by a post hoc test for pairwise comparison.

The significance level was set at $P \leq 0.05$.

Statistical analysis was performed with IBM® SPSS® (SPSS Inc., IBM Corporation, NY, USA) Statistics Version 24 for Windows.

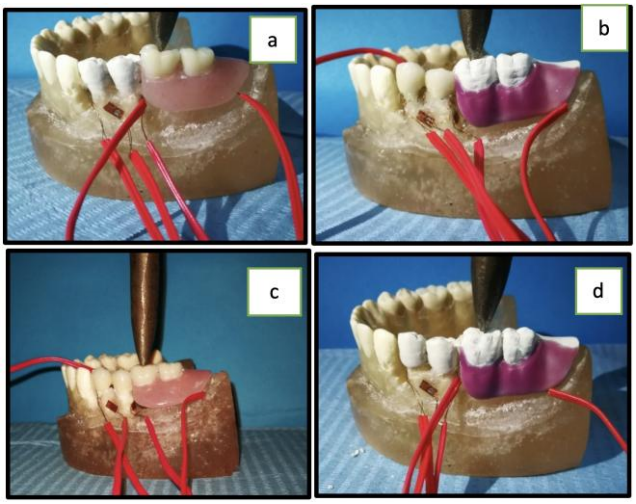


Figure 3: a- Group 4, b- Group 2, c- Group1, d- Group 3

Results

Mean, and standard deviation (SD) for the difference in Strain for different tested groups were presented in Table 1.

Table 1: Mean and standard deviation (SD) for Strain for different tested groups

		Material								p-value
		M-M		M-P		P-P		P-M		
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Strain	Mesial	182.50^{b}	6.45	100.00^{a}	4.08	23.75^{d}	4.79	25.00^{c}	4.08	≤0.001*
	Buccal	305.00^{a}	4.08	352.50^{b}	6.45	102.50^{c}	6.45	162.50^{b}	6.45	≤0.001*
	Distal	230.00^{a}	4.08	1013.75^{d}	11.09	407.50^{c}	6.45	417.50^{b}	6.45	≤0.001*
	Lingual	138.75^{a}	4.79	120.00^{a}	4.08	75.00^{b}	4.08	115.00^{a}	4.08	≤0.001*
	Ridge	632.50^{a}	6.45	227.50^{b}	6.45	462.50^{c}	6.45	568.75^{b}	8.54	≤0.001*
	Total	297.75	180.86	362.75	346.51	214.25	187.65	257.75	208.25	0.140 NS

** = Significant, NS = Non-significant.*

Correlation between Distal and Ridge Strain is shown in Table 2.

Table 2: Pearson correlation coefficient for different tested areas

		Mesial	Buccal	Distal	Lingual
Ridge	Pearson Correlation	0.168	-0.369	-0.963**	0.205
	Sig. (2-tailed)	0.533 NS	0.159 NS	≤0.001*	0.447 NS
	N	16	16	16	16

** = Significant, NS = Non-significant.*

Discussion

The unilateral designed partial denture has a significantly greater movement than a bilateral designed partial denture. So, a meticulous design should be achieved by employing a stress equalising design to decline the stress on the abutments [22].

From previous in-vitro investigations [12], [22] they recommend involving at least two vital and periodontally healthy abutments upon using extra-coronal attachment system. As the extra-coronal attachment introduce more stress at terminal abutment tooth when compared with clasp retained the denture.

The extra-coronal precision attachment is indicated for long span distal extension bases as it provides a removable prosthesis with improved esthetics and improved comfort with less post-operative adjustments [23].

RHEIN 83 OT CAP is an extra-coronal attachment used at the distal end of the splinted abutments and cast with the crowns. The male component design is a sphere with a flat head, and female component is retentive nylon caps which are colour-coded according to different retentive properties [24].

In one of the researches that compare different designs of unilateral partial dentures with extra-coronal attachments and different lengths of distal extension bases, it was concluded that the amount of stresses affects the abutments in the short saddle design are far lower than the long saddle one. So, it is recommended to use extra-coronal attachments in short Kennedy Class II cases unilaterally designed restoring two missing teeth [25].

To achieve the gingival colour of the PEEK framework of the partial denture, Visio-like can be used, and other materials as acid etching composite material can be applied [26]. The PEEK framework should not be cast to full contour to counteracts the esthetic point of view of PEEK framework from low translucency and greyish pigmentation.

Four different treatment options were designed in this in-vitro study to compare the stress distribution around the splinted abutment teeth and over the edentulous ridge. By using the strain gauge evaluation method, the PEEK-PEEK treatment option expresses the most favourable stress distribution in all aspects especially around the abutment teeth and this outcome coincides with the researches about the favourable of the PEEK material [13], [15], [17], [18], [27], [28] both biologically and mechanically especially for its flexure behaviour which is the main cause of its favourable distribution of stresses.

From the outcomes of this in-vitro study, it can be accomplished that PEEK material proved to be one of the non-metallic materials of choice due to its superior properties regarding both biologically and mechanically for restoring short unilateral partial free end edentulous cases with extra-coronal precision attachment.

References

1. Carr AB, Brown DT. McCracken's Removable Partial Prosthodontics-E-Book. Elsevier Health Sciences, 2010:12.

2. Gunne HJ. The effect of removable partial dentures on masticatory function and dietary intake. Acta Odontol Stand. 1985; 43:269-278. https://doi.org/10.3109/00016358509046507

3. D. J. Witter et al. The effect of removable partial dentures on the oral function in shortened dental arches. J Oral Rehabil. 1989; 16:27-33. https://doi.org/10.1111/j.1365-2842.1989.tb01314.x PMid:2746404

4. Partielle adjoint. Rev Mens Suisse. Restoration of the partially edentulous mouth-a comparison of overdentures, removable partial dentures, fixed partial dentures and implant treatment. Odontostomatol. 1995; 105:507-511.

5. Zajc D, Wichmann M, Reich S, Eitner S. A prefabricated precision attachment: 3 years of experience with the Swiss Mini-SG system. A prospective clinical study. Int J Prosthodont. 2007; 20:432-434. PMid:17695879

6. Johannes Schmitt, Manfred Wichmann, PhD, Stephan Eitner, PhD, Jorg Hamel. Five-year clinical follow-up of prefabricated precision attachments: A comparison of uni and bilateral removable dental prostheses Quintessence Int. 2011; 42:413-418. PMid:21519561

7. Jr Shillingburg HT, S Hobo, LD Whitsett, R Jacobi, Se Brackett. Fundamentals of fixed prosthodontics. Chicago: Quintessence. 1997; 531-2.

8. Den Haan R, et al. (Semi-)precision attachments for cast metal frame removable partial dentures. Nederland's tijdschrift voor tandheelkunde. 2011; 118(2):93-100. https://doi.org/10.5177/ntvt.2011.02.10290

9. Altay OT, Tsolka P, Preiskel HW. Abutment teeth with extracoronal attachments: The effects of splinting on tooth movement. Int J Prosthdont. 1990; 3:441-448. PMid:2088381

10. Yeung AL, Lo EC, Chow TW, Clark RK. Oral health status of patients 5-6 years after placement of cobalt-chromium removable partial dentures. J Oral Rehabil. 2000; 27:183-189. https://doi.org/10.1046/j.1365-2842.2000.00512.x PMid:10784329

11. El-Charkawi H.G., Zekry K.A. and El-Wakad M.T. Stress analysis of two osseointegrated implants supporting distal extension prosthesis. AL-Azhar Dent J. 1996; 7:347-362.

12. Patrnogić V, Todorović A, Sćepanović M, Radović K, Vesnić J, Grbović A Free-end saddle length influence on stress level in unilateral complex partial denture abutment teeth and retention elements. Vojnosanit Pregl. 2013; 70(11):1015-22. https://doi.org/10.2298/VSP110603028P

13. Stawarczyk B, Beuer F, Wimmer T, Jahn D, Sener B, Roos M, Schmidlin PR. Polyetheretherketone-a suitable material for fixed dental prostheses? J Biomed Mater Res B Appl Biomater. 2013; 101:1209-16. https://doi.org/10.1002/jbm.b.32932 PMid:23564476

14. Costa-Palau et al. Use of Polyetheretherketone in the fabrication of a maxillary obturator prosthesis: A clinical report. J Prosthet Dent. 2014; 112(3):680-2. https://doi.org/10.1016/j.prosdent.2013.10.026 PMid:24630397

15. Muhsin SA, Wood DJ, Hatton PV, Johnson A, Sereno N. The Effect of Processing Conditions on the Flexural Strength of Polyetheretherketone (PEEK) Used as Innovative Denture Base

Material. The 2nd International PEEK Meeting, USA-Washington DC, 23-24th April, 2015.

16. Godara A, Raabe D, Green S. The influence of sterilisation processes on the micromechanical properties of carbon fiber-reinforced PEEK composite for bone implant applications. Acta Biomater. 2007; 3(2):209-20. https://doi.org/10.1016/j.actbio.2006.11.005 PMid:17236831

17. Sagomonyants KB, et al. The in vitro response of human osteoblasts to Polyetheretherketone (PEEK) substrates compared to commercially pure titanium Biomaterials. 2008; 29:1563-72. https://doi.org/10.1016/j.biomaterials.2007.12.001 PMid:18199478

18. Schwitalla AD, et al. Flexural behavior of PEEK materials for dental application. Dent Mater. 2015; 31(11):1377-84. https://doi.org/10.1016/j.dental.2015.08.151 PMid:26361808

19. Asundi, A. & Kishen, A. A strain gauge and photoelastic analysis of in vivo strain and in vitro stress distribution in human dental supporting structures. Archives of oral biology. 2000; 45(7):543-50. https://doi.org/10.1016/S0003-9969(00)00031-5

20. Dahab IA, El-Gendy AA, Eltorky IR. In vitro stress analysis study of different prosthetic options using single posterior implant for management of mandibular unilateral distal extension saddle. Tanta Dental Journal. 2015; 12(1):7-15. https://doi.org/10.1016/j.tdj.2014.07.004

21. Karl M, Dickinson A, Holst S, Holst A. Biomechanical methods applied in dentistry: a comparative overview of Photo elastic examinations, strain gauge measurements, finite element analysis and three-dimensional deformation analysis. Eur J Prosthodont Restor Dent. 2009; 17(2):50-7. PMid:19645304

22. Schmitt J, Wichmann M, Eitner S, Hamel J, Holst S. Five-year clinical follow-up of prefabricated precision attachments: A comparison of uni-and bilateral removable dental prostheses. Quintessence International. 2011; 42(5):413-18. PMid:21519561

23. Feinberg E. Diagnosing and prescribing therapeutic attachment-retained partial dentures. The New York state dental journal. 1982; 48(1):27-29. PMid:7031533

24. Gupta N, Bhasin A, Gupta P, Malhotra P. Combined Prosthesis with Extracoronal Castable Precision Attachments. Case reports in dentistry. 2013;2013.

25. Patrnogi V, et al. Free-end saddle length influence on stress level in unilateral complex partial denture abutment teeth and retention elements Vojnosanit Pregl. 2013; 70(11):1015-22. https://doi.org/10.2298/VSP110603028P

26. Stawarczyk B, Beuer F, Wimmer T, Jahn D, Sener B, Roos M, Schmidlin PR. Polyetheretherketone-A suitable material for fixed dental prostheses? J Biomed Mater Res Part B. 2013:101B:1209-16. https://doi.org/10.1002/jbm.b.32932 PMid:23564476

27. Tekin S, Cangül S, Adıgüzel Ö, Değer Y. Areas for use of PEEK material in dentistry. International Dental Research. 2018; 8(2):84-92. https://doi.org/10.5577/intdentres.2018.vol8.no2.6

28. Skirbutis G, Dzingutė A, Masiliūnaitė V, Šulcaitė G, Žilinskas J. A review of PEEK polymer's properties and its use in prosthodontics. Stomatologija. 2017; 19(1):19-23. PMid:29243680

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Apr 15; 7(7):1198-1202.
https://doi.org/10.3889/oamjms.2019.246
eISSN: 1857-9655
Public Health

Association between Malondialdehyde and Glutathione (L-gamma-Glutamyl-Cysteinyl-Glycine/GSH) Levels on Workers Exposed to Benzene in Indonesia

Abdul Rohim Tualeka[1*], Tri Martiana[1], Ahsan Ahsan[2], Syamsiar S. Russeng[3], Wulan Meidikayanti[4]

[1]Department of Occupational Health and Safety, Faculty of Public Health, Airlangga University, Surabaya, East Java, Indonesia; [2]Faculty of Nursing, University of Brawijaya, Malang, Indonesia; [3]Department of Occupational Health and Safety, Faculty of Public Health, Hasanuddin University, Makassar, Indonesia; [4]Faculty of Public Health, Airlangga University, Surabaya, East Java, Indonesia

Citation: Tualeka AR, Martiana T, Ahsan A, Russeng SS, Meidikayanti W. Association between Malondialdehyde and Glutathione (L-gamma-Glutamyl-Cysteinyl-Glycine/GSH) Levels on Workers Exposed to Benzene in Indonesia. Open Access Maced J Med Sci. 2019 Apr 15; 7(7):1198-1202. https://doi.org/10.3889/oamjms.2019.246

Keywords: Benzene; MDA; Glutation; Inhalation; Shoe Worker

***Correspondence:** Abdul Rohim Tualeka. Department of Occupational Health and Safety, Faculty of Public Health, Airlangga University, Surabaya, East Java, Indonesia. E-mail: abdul-r-t@fkm.unair.ac.id

Received: 16-Jan-2019; **Revised:** 25-Mar-2019; **Accepted:** 26-Mar-2019; **Online first:** 12-Apr-2019

Funding: This article was supported by Activity Budget Plans 2017, Faculty of Public Health, Airlangga University

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

Abstract

BACKGROUND: Chemicals that enter the body, especially benzene, will undergo a detoxification process. Unfortunately, at the detoxification process, sometimes benzene can produce free radicals. Free radical oxidation of lipids produces MDA compounds (malondialdehyde). To overcome these free radicals, the body will adapt to produce Glutathione (GSH) enzymes.

AIM: The purpose of this study was to analyse the relationship between benzene concentration, MDA levels and glutathione enzymes in Shoe-Maker Home Industry workers exposed to benzene for more than 10 years.

METHODS: Measurement of benzene concentration using a gas chromatography-flame ionisation detector (GC-FID). MDA levels used a modified spectrophotometric and GSH method of thiobarbituric acid (TBA) test.

RESULT: The results showed that the majority of respondents had benzene concentrations still below the TLV value, mean of MDA levels were 6.94 mg/ml, while GSH was 4.54 mg/ml. Benzene concentration did not have a significant correlation with MDA and glutathione levels, whereas MDA levels had a strong correlation with glutathione levels ($p = 0.000$; $r = -0.947$).

CONCLUSION: Workers should always use PPE and always eat foods that contain lots of glutathione enzymes such as spinach or broccoli to reduce the impact of free radicals from benzene inhalation.

Introduction

Benzene is a liquid that is colourless and has a sweet smell, evaporates very rapidly in the air, and is difficult to dissolve in water [1], [2]. Benzene is also a raw material for making plastics, resins, synthetic fibres, dyes, detergents, medicines, pesticides, and components of crude oil, gasoline, and cigarette smoke [3], [4]. Pathways to benzene exposure can be through the skin, respiratory tract, mouth and then to the digestive tract [5].

A person who is exposed to high levels of benzene can experience several signs and symptoms, including drowsiness, dizziness, rapid or irregular heartbeat, headaches, tremors, confusion, unconsciousness, until death [6]. Excessive benzene in the body can become free radicals that can reduce blood cell production [7].

Free radicals are compounds or atoms that have one or more unpaired electrons [8]. Free radicals oxidise some of the body macromolecules such as proteins, nucleic acids and lipids [9]. Free radical oxidation of proteins, nucleic acids, and lipids each produces carbonyl compounds, MDA (malondialdehyde) and deoxyguanosine P [10].

To overcome free radicals, the body needs

antioxidants. Antioxidants are obtained from outside the body (food) or produced by the body itself (endogenous) [11]. Examples of endogenous antioxidants are superoxide dismutase, glutathione (GSH), catalase and glutathione peroxidase. One antioxidant that is often measured to see the impact of an increase in free radicals in the body is GSH [12], [13]. The Glutathione molecular formula is $C_{10}H_{17}N_3O_6S$, with a molecular weight of 307.3235 g/mol. As an antioxidant for the body, glutathione is a tripeptide consisting of amino acids; glutamate, cysteine, and glycine [14]. The content of glutathione is found in most of the body's cells, but the most are in the liver. The thiol (SH) group of cysteine functions as a proton donor and is responsible for the biological activity of glutathione. Suggested food sources because they contain glutathione as antioxidants namely asparagus, spinach, broccoli, garlic, kale, onions, watercress, cabbage, Brussels sprouts, some herbs like turmeric, cinnamon, watermelon, avocado, grapes, peaches, oranges, walnuts, granola, turkey and chicken meat, cottage cheese and yoghurt [13].

Several studies have shown that benzene exposure can increase free radicals and reduce the body's antioxidant status, especially GSH enzymes. Research in Jakarta and Iraq shows that gas station workers exposed to benzene are more susceptible to DNA damage due to free radicals. Research on the relationship of benzene, MDA and glutathione concentrations in shoe Home Industry workers still does not exist in Indonesia.

Therefore, this study aims to analyse the relationship of benzene concentration, MDA levels and glutathione enzymes in Home Industry workers exposed to benzene.

Material and Methods

The type of research used is cross-sectional. Subjects are workers in the Tambak Osowilangun shoe industry in Surabaya. The inclusion criteria in this study were male and female workers who had worked in the shoe industry in Tambak Osowilangun for > 10 years and were willing to be used as research respondents. The study sample was 25 people.

The variables calculated were benzene levels, MDA levels, GSH levels, and measurements of benzene concentration at five points in the industry. The research subject was chosen after the person was willing to participate in the study by first describing the benefits and inconvenience of participating in the study. Willingness to participate in the study was made in writing through informed consent, and this study had received prior ethical approval by the Public Health Faculty Ethics Committee, Airlangga University with ethics number 516 KEP-K.

Measurement of length of work, average work every day, and work time in a week are obtained through in-depth interviews with respondents. Then, the measurement of benzene concentration in the work environment using a measurement method of NIOSH 1501 with an activated carbon (charcoal) pipe which uses a gas chromatography-flame ionisation detector (GC-FID) technique using NIOSH 1501 standard.

MDA measurements were carried out using a modified spectrophotometry method of thiobarbituric acid (TBA) test. A total of 400 µl of the sample was reacted with 200 µl of trichloroacetic acid (TCA) 20% for deproteination. Then the cortex and centrifuge at a speed of 5000 rpm for 10 minutes. The supernatant formed was taken and 400 µl TBA 0.67% was added. Then the sample was vortexed and incubated in a water heater at 96°C, 10 minutes then lift and cool at room temperature. Then read the absorption at a wavelength of 530 nm. The sample is taken immediately after the employee's work shift is finished [14]. The normal average level for MDA is 2.61 µmol/L [15].

GSH measurement with a sample of blood taken from the left arm cubital vein as much as 2 mL of research subjects. The blood is then centrifuged at 2000 rpm for 3 minutes to get a plasma. Then the plasma is stored in a refrigerator -20°C, before GSH examination. GSH levels were measured by mixing 50 plasma with 1.78 mL phosphate buffer 0.1 M pH 8- and 0.2-mL TCA 5%. The mixture was then centrifuged at 1500 g for 5 minutes, a temperature of 40°C. The supernatant was then added with 0.01 mL DNTB and left for 1 hour. The mixture is then examined using spectrophotometry at a wavelength of 412 nm to determine plasma GSH levels [16]. GSH normal level is 3.8-5.5 µmol/L [17].

Statistical analysis using Pearson and Spearman's Rank correlation test with an alpha of 0.05. The closer to 1, the stronger the correlation between variables and vice versa.

Results

Characteristics of Shoe Maker Worker

The majority of respondents have a high school senior education background (45.80%) and male sex (56%) and do not have smoking habits (60%). The majority of respondents, as many as 56% still have benzene concentrations still under TLV standard (0.01 mg/ml). In Figure 1, shows that the average MDA level in the respondent's body is 6.94 mg/ml.

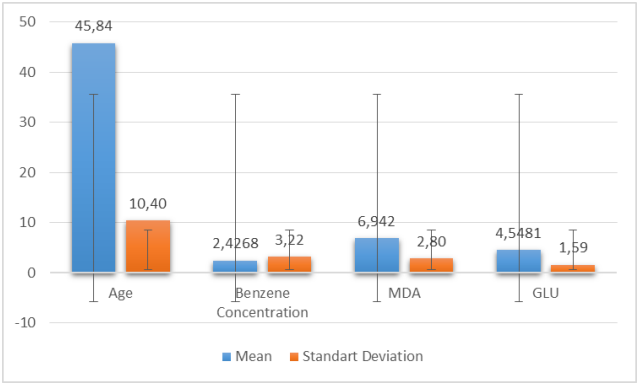


Figure 1: Average distribution and standard deviation of variables of age, benzene concentration, MDA and glutathione levels

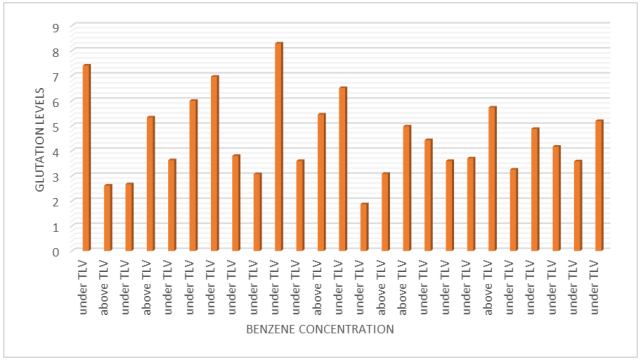


Figure 3: Graph of the Relationship between Benzene Concentration and Glutathione Level

The highest MDA level in the respondent's body was 12.73 mg/ml, while the low level was 2.93 mg/ml. Figure 1 also shows that the average level of glutathione in the body of the respondent was 4.54 mg/ml. The highest level of glutathione in the body of the respondent was 8.28 mg/ml, while the lowest level was 1.86 mg/ml.

Correlation between Benzene, MDA and Glutathione Concentration in Shoe Home Industry Worker

Correlation between Benzene concentration and MDA

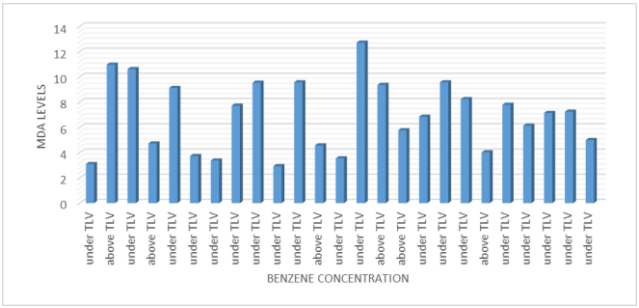


Figure 2: Graph of Relationship between Benzene Concentration and MDA Level

Figure 2 shows that there is no trend curve between the relationship between benzene concentration and MDA level. This is by the statistical test which states that there is no correlation between benzene concentration and MDA level (p = 1,000; r = 0.000). Correlation between Benzene Concentration with Glutation Level

Figure 3 shows that there is no tendency curve between the relationship of benzene concentration and glutathione level. This is by the statistical test which states that there is no relationship between benzene concentration and glutathione level (p = 1,000; r = 0.000).

Relationship of MDA Levels with Glutathione

Figure 4 shows that there is an inverse linear relationship between levels of MDA and Glutathione which means that the greater the MDA level, the lower the level of glutathione in the body. This is consistent with the statistical test which states that there is a strong relationship between MDA levels and glutathione levels (p = 0.000; r = -0.947).

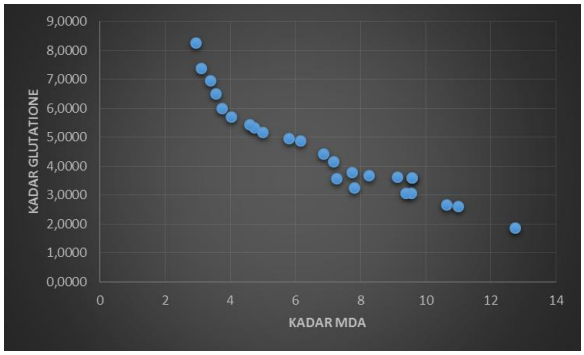


Figure 4: Graph of Relationship between MDA Levels and Glutathione Levels

Discussion

The majority of respondents have male gender and have a high school education background (high school). Benzene concentration in the majority of respondents has a value under the Threshold Limit Value (TLV). The Threshold value for the concentration of benzene according to the Regulation of the Minister of Manpower and Transmigration of the Republic of Indonesia Number 13 of 2011 concerning the Physics and Chemical Factor Threshold Value at the Workplace is 0.5 ppm (1.6 mg/m^3) [18].

Malondialdehyde (MDA) is a compound that can describe the activity of free radicals in cells so that it is used as one of the indications of oxidative

stress caused by free radicals [19]. Another study reinforces this statement by stating that the mediator Malondialdehyde (MDA) is a final product of fat peroxidation which is used as a biological biomarker of fat peroxidation and can describe the degree of oxidative stress [20]. The average MDA level in the respondent's body was 6.94 mg/ml, and this value showed a higher value than previous studies in Indonesia (0.731 nmol/mL). Statistical tests also showed no significant relationship between benzene and MDA levels in shoe workers. This contrasts with similar research [10]. But the thing that distinguishes this research from the previous one is the location/place of research. The previous research location was located at a gas station which was known to have higher levels of benzene than the shoe industry factory which was only exposed to benzene in the glueing process. Other factors that influence can be too small a large sample and less exposure to benzene.

Glutathione (lgamma-glutamyl-cysteinyl-glycine) is a tripeptide consisting of glutamic acid, cysteine, and glycine. The compound has a sulfhydryl/thiol group (-SH) found in the amino acid cysteine. The sulfhydryl group causes GSH to act as a powerful electron donor (nucleophile) in counteracting free radicals. GSH can decompose H_2O_2 to H_2O with the help of glutathione peroxidase enzymes [12], [21]. These compounds are metabolised by the liver to produce free radicals such as superoxide anions (O_2-), hydroxyl radicals (OH-) and semicunionan radicals. GSH works to counteract these free radicals to prevent or reduce cell damage [22]. The longer you work at the benzene exposure site, the more exposure to benzene, toluene and xylene compounds will accumulate and are more likely to reduce the body's antioxidants [11].

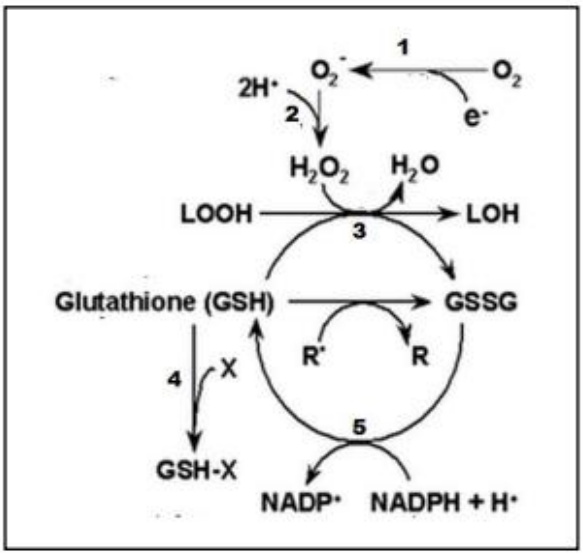


Figure 5: Role of GSH to Prevent Free Radicals 16 GSH helps the enzymes glutathione peroxidase and superoxide dismutase to break free radicals (LOOH, O_2 -, R ', X); 1. NADPH Oxidase; 2. Superoxide Dismutase; 3. Glutathione Peroxidase; 4. Glutathione S-Transferase; 5. Glutathione Reductase

GSH has a role as an antioxidant by reducing free radicals directly or as a cofactor of antioxidant enzymes such as glutathione peroxidase (Figure 1) and glutathione transhydrogenase [23]. The main function of GSH is to detoxify drugs, xenobiotics or pesticides catalysed by GSH-S-transferase enzymes. GSH also plays a role in maintaining thiol groups (-SH) in essential proteins, by reducing disulfide bonds in proteins, which are catalysed by the enzyme thioltransferase [12], [23]. However, the results of the statistical test analysis stated that there was no significant relationship between benzene concentration and glutathione. This has similarities with previous studies [16]. The thing that can be a significant factor of this correlation is the possibility that there are many other antioxidants (glutathione peroxidase, SOD and catalase) which play a role in reducing free radicals [8], [12].

MDA and glutathione levels have a strong significant correlation and have a reciprocal relationship which means that the higher the MDA level, the lower the level of glutathione in the worker's body. This has similarities with another study which states that there is an increase in MDA levels and a decrease in Glutathione levels in cement workers [24]. Even studies in Jordan and Poland in automobile workers showed an increase in MDA levels by 48% and a decrease in GSH levels by 16-25% [25], [26]. High MDA levels indicate higher free radicals as well. This benzene radical can suppress detoxification enzymes, one of which is GSH. The most dangerous forms of free radicals benzene are superoxide (O_2) anion, hydroxyl radical (OH) and hypochlorite acid (HOCl) and hydrogen peroxide (H_2O_2). Also, benzene free radicals can damage blood cells, can cause lipid peroxidation which can cause liver fibrosis [27]. What can be done to reduce benzene exposure in workers is to use PPE regularly such as masks, especially during the glueing process. Another approach is taken by consuming foods rich in detoxification enzymes, especially GSH which is usually found in the majority of vegetables such as asparagus, spinach, broccoli, garlic, kale, and onions [13].

In conclusion, the majority of respondents have male gender, high school education background, benzene concentration is still below the TLV value, the average MDA level is 6.94 mg/ml, and the average glutathione level is 4.54 mg/ml. Benzene concentration did not have a significant correlation with MDA and glutathione levels; on the contrary, MDA levels had a strong correlation with glutathione levels. The influencing factor was the benzene threshold which was still too small, so it still did not show high levels of GSH and MDA. Conversely, MDA and GSH have a strong correlation because the more benzene that becomes free radicals (MDA), the free radicals will directly interfere with the work of biotransformation enzymes (detoxification), one of which is GSH. Workers should always use PPE and always eat foods that contain lots of glutathione

enzymes such as vegetables (spinach or broccoli) to reduce the impact of free radicals from benzene inhalation.

Acknowledgements

The authors would like thank the rector of Airlangga University. The authors would like to acknowledge workers at Shoe Industry Romokalisari Surabaya, East Java Indonesia and thanks to Wulan Meidikayanti and Fathimatul Tualeka for editing this article.

References

1. ATSDR. Toxicological Profile for Benzene. Atlanta, Georgia: ATSDR, 2007.

2. Tualeka AR, Jalaludin J, Salesman F, Wahyu A, Tukiran T, Setiawan S, Wibrata DA, Hasyim HN. Risk Analysis Characterization of Benzene and Demographic Factors toward Immunoglobulin A. Open Access Maced J Med Sci. 2018; 6(12):2381. https://doi.org/10.3889/oamjms.2018.488

3. Nirmawati S, Tualeka AR, Adi AC. Effect of Food Containing High Fe (Iron) Intake to Urinary Trans, Trans-muconic Acid (Tt-ma) Levels on Workers Exposed to Benzene. Indian Journal of Public Health Research & Development. 2018; 9(1):53-57. https://doi.org/10.5958/0976-5506.2018.00010.4

4. Smith, MT. Advances in Understanding Benzene Health Effects and Susceptibility. California: Division of Environmental Health Sciences, School of Public Health, University of California, Berkeley, 2010.

5. Putri, YRP. Benzene in Urban Mara. Thesis, Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Indonesia, Depok, 2011. [In Indonesian]

6. Central Disease Center (CDC). Facts about Benzene. 2013. Retrieved October 11, 2018, from https://emergency.cdc.gov/agent/benzene/basics/facts.asp.

7. Kamal A, Malik RN. Hematological evidence of occupational exposure to chemicals and other factors among auto-repair workers in Rawalpindi, Pakistan. Osong Public Health Res Perspect. 2012; 3(4):229-238. https://doi.org/10.1016/j.phrp.2012.10.003 PMid:24159519 PMCid:PMC3747659

8. Birben E, Sahiner UM, Erzurum S, Sackesen C, Kalayci O. Oxidative stress and antioxidant defense. WAO J. 2012; 5:9-19. https://doi.org/10.1097/WOX.0b013e3182439613

9. Kunwar A, Priyadarsini KI. Free radicals, oxidative stress, and importance of antioxidants in human health. J Med Allied Sci. 2011; 1(2):53-60.

10. Ariyani R. Study of detection DNA-adduct 8- hidroksi-2'-deoksiguanosin as a biomarker of cancer risk on worker at some Gas Station Jakarta. Essay. Jakarta: Indonesia University, 2009.

11. Odewabi AO, Ogundahunsi OA, Oyalowo M. Effect of exposure to petroleum fumes on plasma antioxidant defense system in petrol attendants. J Pharmacol Toxicol. 2014; 5(2):83-88. https://doi.org/10.19026/bjpt.5.5461

12. Lushchak VI. Glutathione homeostasis and functions: potential targets for medical interventions. J Amino Acids. 2012; 2012:1-26. https://doi.org/10.1155/2012/736837 PMid:22500213 PMCid:PMC3303626

13. Cadenas E, Jones PD. Bioavailability of Glutathione. In: Cadenas, E, Packer L, editors. Handbook of Antioxidants. 2nd. ed. New York: Marcel Dekker, Inc., 2002:549-64.

14. Zainuri M, Wanandi SI. Specific Activity of Manganese Superoxide Dismutase (MnSOD) and Catalase in Rat Liver Induced Systemic Hypoxia: Its Relationship with Oxidative Damage. J Health Res Dev Media. 2012; 22(2):87-2.

15. Singh Z, Karthigesu IP, Singh P, & Rupinder KAUR. Use of malondialdehyde as a biomarker for assessing oxidative stress in different disease pathologies: a review. Iranian J Pub Health. 2015; 43(3):7-16.

16. Safyudin S & Subandrate S. Glutathione level (GSH) of the blood of SPBU employees in Palembang City. J Med Health: Scientific Publication, Faculty of Medicine, Sriwijaya University. 2015; 2(3):277-81.

17. Tim Guilford. Holistic Primary Care. What Every Doctor Should Know About Glutathione. Accessed on https://www.holisticprimarycare.net/topics/topics-o-z/vitamins-a-supplements/1421-what-every-doctor-should-know-about-glutathione.html [October 24, 2018]

18. The Republic of Indonesia. Regulation of the Minister of Manpower and Transmigration No. 13 of 2011 concerning the Threshold Value of Physical and Chemical Factors in the Workplace. Jakarta: State Secretariat, 2011. [In Indonesian]

19. Asni E, Harahap IP, Prijanti AR, Wanandi SI, Jusman SWA, Sadikin M. Effect of hypoxia on malondialdehyde levels, reduced glutathione and catalase activity of rat kidneys. Indonesian Medicine Magazine. 2009; 59(12):595-600.

20. Rahardjani KB. Relationship between Malondialdehyde (MDA) and Outcome of Neonatorum Sepsis. Sari Pediatri. 2016; 12(2):82-7. https://doi.org/10.14238/sp12.2.2010.82-7

21. Al-Fartosy AJM, Awad NA, Shanan SK. Biochemical correlation between some heavy metals, malondialdehyde and total antioxidant capacity in blood of gasoline station workers. Int Res J Environment Sci. 2014; 3(9):56-60.

22. Li C, Zhou HM. The role of manganese superoxide dismutase in inflammation defense. Enzyme Res. 2011; 2011:1-6. https://doi.org/10.4061/2011/308730 PMid:22145076 PMCid:PMC3226318

23. Wu G, Fang YZ, Yang Z, Lupton JR, Turner ND. Glutathione metabolism and its implications for health. J Nutr. 2004; 134:489-2. https://doi.org/10.1093/jn/134.3.489 PMid:14988435

24. Orman A, Kahraman A, Çakar H, Ellidokuz H, Serteser M. Plasma malondialdehyde and erythrocyte glutathione levels in workers with cement dust-exposure silicosis. Toxicology. 2005; 207(1):15-20. https://doi.org/10.1016/j.tox.2004.07.021 PMid:15590118

25. Kasperczyk A, Słowińska-Łożyńska L, Dobrakowski M, Zalejska-Fiolka J, Kasperczyk S. The effect of lead-induced oxidative stress on blood viscosity and rheological properties of erythrocytes in lead-exposed humans. Clin Hemorheol Microcirc. 2014; 56(3):187-5. PMid:23370159

26. Shraideh Z, Badran D, Hunaiti A, & Battah A. Association between occupational lead exposure and plasma levels of selected oxidative stress-related parameters in Jordanian automobile workers. Inter J Occup Med Environ Health 2018, Jul 4;31(4):517-525. https://doi.org/10.13075/ijomeh.1896.01243

27. Ellah AB, Rushdi M, Okada K, Yasuda J. Oxidative stress and bovine liver diseases: Role of glutathione. Japanese Journal of Veterinary Research. 2007; 54(4):163-73.

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Apr 15; 7(7):1203-1208.
https://doi.org/10.3889/oamjms.2019.249
eISSN: 1857-9655
Public Health

The Assessment of Drug Utilization Study of Anticancer Drugs Using WHO Prescribing Indicators in a Government Tertiary Care Hospital of the Hyderabad - Karnataka Region of India

Asmatanzeem Bepari[1*], Nayana Sakre[2], Ishrat Rahman[3], Shaik Kalimulla Niazi[4], Asmabi M. Dervesh[5]

[1]Department of Basic Health Sciences, College of Medicine, Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia; [2]Department of Pharmacology, Mandya Institute of Medical Sciences, Mandya, Karnataka, India; [3]Department of Basic Health Sciences, College of Dentistry, Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia; [4]Department of Preparatory Health Sciences, Riyadh Elm University, Riyadh, Saudi Arabia; [5]Department of Biochemistry, Prakash Institute of Medical Sciences and Research, Urun-Islampur, Sangli, Maharashtra, India

Citation: Bepari A, Sakre N, Rahman I, Niazi SK, Dervesh AM. The Assessment of Drug Utilization Study of Anticancer Drugs Using WHO Prescribing Indicators in a Government Tertiary Care Hospital of the Hyderabad - Karnataka Region of India. Open Access Maced J Med Sci. 2019 Apr 15; 7(7):1203-1208. https://doi.org/10.3889/oamjms.2019.249

Keywords: WHO prescribing indicators; Anticancer drugs; Drug utilisation review; Pre-chemotherapy drugs

***Correspondence:** Asmatanzeem Bepari. Department of Basic Health Sciences, College of Medicine, Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia. E-mail: dr.asmatanzeem@gmail.com

Received: 10-Feb-2019; **Revised:** 26-Mar-2019; **Accepted:** 28-Mar-2019; **Online first:** 13-Apr-2019

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Cancer is a major burden and threat to global society. A wide range of chemotherapeutic agents is extensively used to treat cancer at different stages. Inappropriate drug use may also lead to the raised cost of medical care, adverse drug effects, and patient mortality. Hence, in recent years, drug utilisation studies have become a potential tool to be used in the evaluation of different health care systems including cancer.

AIMS: The objectives of the study were to identify the various types of cancer, the commonly prescribed drugs, rational use of anticancer drugs, and analyse the prescribing indicators in a tertiary care government hospital of India.

MATERIAL AND METHODS: Newly diagnosed cancer and/or known case of carcinoma of either sex which required treatment/on treatment with chemotherapy aged > 18 yrs admitted in Radiotherapy Department from April 2016 to September 2016 were included in the study and analysed for prescribing indicators.

RESULTS: The head & neck cancers were the prevalent cancers observed with more preponderance among males. Most of the patients were prescribed with a single anticancer drug. Cisplatin was the most commonly used cytotoxic drug followed by carboplatin, and antimetabolites. The most commonly used adjuvant drugs in our study were anti-emetics and anti-peptic ulcer drugs. Over 82% of anticancer agents were taken from the essential drug list and were prescribed in generic names, indicating rational use.

CONCLUSION: Over 82% of anticancer agents were taken from the essential drug list and were prescribed in generic names, indicating rational use.

Introduction

Cancer is a major burden and threat to global society. It is one of the leading causes of death in both developed and developing countries [1]. A survey conducted by the World Health Organization (WHO) indicated that 8.2 million people succumbed from cancer in 2012, and it may rise to 19 million by 2025 [2]. In India, the estimated number of people living with this disease is around 2.25 million, and the estimated numbers of new cancers are about 1.1 million per year [3]. More than 0.6 million die because of cancer each year [3], and approximately 42% of cancers are tobacco related [4]. The main modalities used for its treatment include surgery, radiation, chemotherapy, immunotherapy, and hormones. The decision of therapy depends upon patient factors, tumour factors, and treatment factors [5]. A wide range of chemotherapeutic agents is extensively used to treat cancer at different stages. Chemotherapy refers to the usage of antineoplastic drugs to treat cancer as a standardised treatment regimen [6]. It is the only therapy which acts systematically to reduce

the disease from the entire body [5]. These drugs usually act on rapidly dividing cells and are either cell cycle specific or non-specific [7].

The drug use for the treatment of diseases is a complicated process since optimal benefits of drug therapeutics in patient care may not be obtained because of under-use, overuse or misuse of the drugs. Inappropriate drug use may also lead to the raised cost of medical care, adverse drug effects, and patient mortality. Hence, in recent years, DUS has become a potential tool to be used in the evaluation of different health care systems including cancer [8]. The evaluation of drug utilisation of anticancer drugs is necessary as their irrational use has generated a significant health problem in the current medical practice. Drug utilisation has been defined by the WHO as the marketing, distribution, prescription, and use of drugs in a society with specific emphasis on the resulting medical and social consequences [9]. Drug utilisation research promotes the rational use of drugs in the population [9]. Monitoring of drug utilisation patterns helps to improve the therapeutic efficacy, provides feedback to the prescriber to assure rational use of medicines and decrease the adverse drug reactions. The ultimate goal of drug utilisation research is to assess whether drug therapy is rational or not. Therefore, the present study aimed to analyse and evaluate the trends and patterns of prescribing anticancer drugs.

The objectives of the study were to identify the various types of cancer, the commonly prescribed drugs, rational use of anticancer drugs, and analyse the prescribing indicators in a tertiary care government hospital of India.

Material and Methods

Study design

The study was an observational, prospective, non-interventional study

Study site

The study was conducted in the cancer radiotherapy department of Vijayanagara Institute of Medical Sciences, Ballari, one of the major government tertiary care hospitals for cancer treatment near the Hyderabad- Karnataka region. The study was performed after receiving the necessary ethical clearance from the Institutional Ethics Committee.

Study Duration

It was carried out for six months, period from April 2016 to September 2016

Inclusion criteria

Newly diagnosed cancer &/or known case of carcinoma of either sex which required treatment/on treatment with chemotherapy aged > 18yrs admitted in the Radiotherapy Department from April 2016 to September 2016 was included in the study. The written informed consent was taken before the start of the study. All the patients were observed for the complete duration of the study.

Exclusion criteria

Patients who were pregnant or having insufficient records and data were excluded from the study.

Sample size calculation

The sample size was designed based on the average number of inpatients admitted and registered in the earlier six months of the study period. A total number of 144 patients were recruited in the study from April 2016 to September 2016.

Data collection

Data were collected and entered in specially designed patient data entry forms. The prescription parameters needed for the study were recorded.

Data analysis

The data were entered in Microsoft Excel (Windows 7; Version 2007). The collected data of demographic and clinical variables were analysed using descriptive statistics such as frequencies and percentage, were represented in tables and figures. WHO core prescribing indicators was compiled at the end of the study to know the number of prescriptions with polypharmacy, per cent of prescriptions with injectables, per cent of drugs prescribed from Essential Drugs list.

Results

Age

The majority of patients (36.8%) were in the age group of 56-65 years followed by 18.05% in 46-55 years (26 patients), 17.36% > 65 years (25 patients),

14.58% in 36-45 years (21 patients), 9.02% in 26-35 years (13 patients) and 4.16% in 18-25 years (6 patients). The mean age of the participants was found to be 53.86 years.

Sex

Males constituted 54.86% (79 patients), and females constituted 45.13% (65 patients) of the total study population.

Distribution of cancer

Table 1, shows the system-wide distribution of cancer among study participants. The head and neck cancers were more predominant (46.7%), followed by gastrointestinal cancer (24.1%), reproductive system (14.51%), breast (11.29%) and respiratory cancers (3.22%).

Table 1: System-wise distribution of cancers

Organ System name	% pts
Gastrointestinal	24.1
Head and neck cancers	46.52
Breast cancers	11.29
Reproductive system	14.51
Respiratory	3.22

The organ-wise distribution is shown in Figure 1.

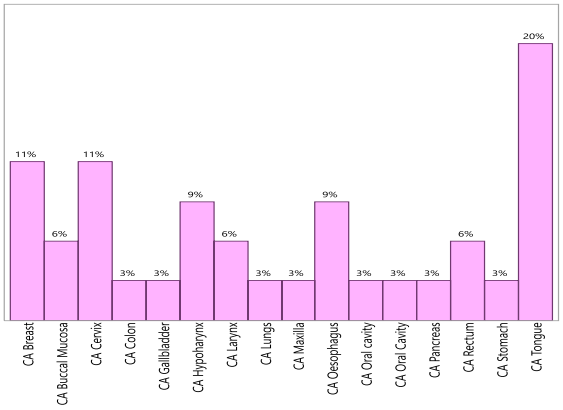


Figure 1: Organ-wise distribution of the cancers

Drug utilisation pattern: Out of 144 cancer patients analysed, 65.27% patients (94) was prescribed with single anticancer drug therapy while the remaining patients were given multiple anticancer drugs therapy.

The treatment regimens used in different organ system cancer is shown in Table 2.

Table 2: The different treatment regimens used in cancer patients

Organ System name	% pts	Treatment regimens used
Gastrointestinal	24.1	1. Cisplatin/Carboplatin + Radiation therapy 2.Oxaliplatin+Capacetabine 3. 5-FU(5-Fluorouracil) + Radiation therapy 4.5FU + Oxaliplatin(FOLFOX) 5. DCF-docetaxel, cisplatin + 5-fluorouracil 6. GEMOX- Gemcitabine + oxaliplatin
Head and neck cancers	46.52	1.Cisplatin/Carboplatin (+ o r-) Radiation therapy 2.Cisplatin+ Paclitaxel (+or-) Radiation Therapy
Breast cancers	11.29	1.Doxorubicin+Cyclophosphamide+Radiation Therapy 2. Paclitaxel
Reproductive system	14.51	1. Cisplatin+ Radiation therapy 2. Carboplatin+ Paclitaxel
Respiratory	3.22	Carboplatin+ Paclitaxel

The anticancer drugs, the adjuvant drugs and other supportive drugs pattern is depicted in Figures 2, 3 and Tables 3, 4 and 5.

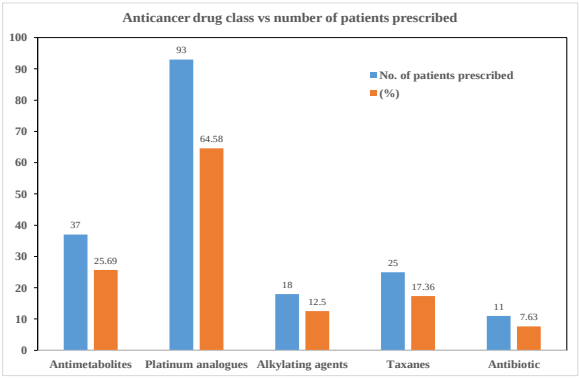


Figure 2: Anticancer class- wise usage of the drugs

Discussion

Anticancer drugs utilisation

Totally 184 anticancer drugs were given to 144 study participants. The average number of these drugs in our study was 1.27 per patient. The present research showed that the most common class of cytotoxic agents prescribed was platinum compounds (64.58%).

Table 3: Prescription pattern of each anticancer drug

Name of Anticancer drug	Number of pts prescribed(n)	(%)
5-Fluorouracil	16	8.7
Capecitabine	13	7.07
Carboplatin	38	20.65
Cisplatin	45	24.46
Cyclophosphamide	18	9.78
Docetaxel	8	4.35
Doxorubicin	11	5.98
Gemcitabine	8	4.35
Oxaliplatin	10	5.43
Paclitaxel	17	9.24
	184	100

Amongst the platinum analogues, the most commonly used drug was cisplatin that comprised 24.46%, followed by carboplatin and oxaliplatin which formed 20.65% and 5.43% respectively of all anticancer drugs. Cisplatin was used with radiotherapy in 33.3% of patients for the management of carcinoma of tongue, cervix, oesophagus and hypopharynx which were the most common types of cancers in the study population.

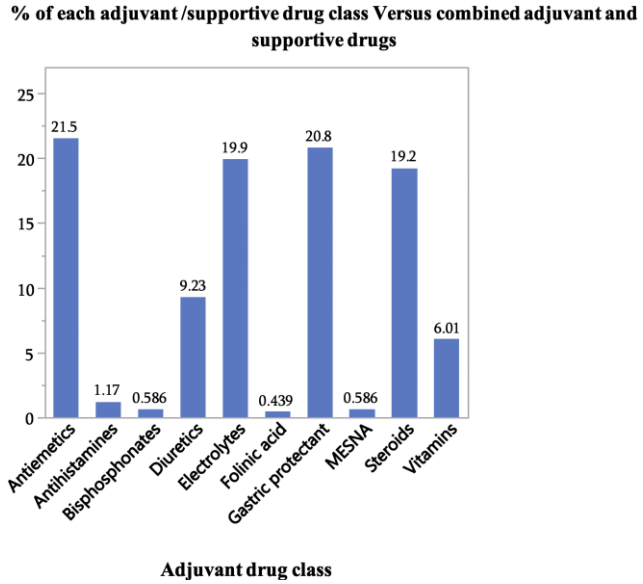


Figure 3: Drug class-wise usage of adjuvant & supportive drugs

Carboplatin with radiotherapy was the second common standard regimen in cancer of the tongue and oral cavity in the present study. The antimetabolites were the next commonly prescribed anticancer drug class accounting for 25.69% of all anticancer drugs. 5-Fluorouracil was most common antimetabolite given (43.24%).

Table 4: Prescription pattern of each adjuvant drug

Drug name	Number of pts given	(%)
Aprepitant	4	0.586
Dexamethasone	131	19.2
Furosemide	63	9.23
Potassium Chloride	67	9.82
Leucovorin	3	0.439
MESNA	4	0.586
Magnesium sulphate	69	10.11
Ondansetron	143	20.96
Pantaprozole	60	8.79
Rantac	82	12.01
Chlorpheniramine	8	1.17

The other antimetabolites used were capecitabine (35.13%) and gemcitabine (21.6%). They were used in gastrointestinal cancers like carcinoma of rectum, colon and stomach. The use of cisplatin (18.5 %) and 5-FU (13 %) were also higher in a comparable study conducted by Dave et al., at PDU Govt. Medical College and Hospital, Gujarat [10]. Similar findings were seen in other related studies of Mary Rohini et al., 2015, Goyal et al., 2014 and Darshan et al., 2014 [11], [12], [13].

Table 5: Prescription pattern of other supportive drugs

Drug name	Number of pts given	(%)
Multivitamin injection	41	6.01
Sucracure	3	0.439
Zoledronic acid	4	0.586
Total- Adjuvant & other supportive drugs	682	100

After antimetabolites, taxanes and alkylating agents were the 3rd and 4th commonest class of agents used. Amongst the taxanes which accounted for 17.36% anticancer drug use, the commonest taxane used was paclitaxel (68%), followed by docetaxel (32%). They were prescribed in carcinoma of the breast with secondaries, cervix, lung and stomach.

Adjuvant and supportive drugs utilisation pattern

Cancer chemotherapy includes anticancer medicines accompanied by adjuvant and supplementing therapeutic measures. These additional medications other than the anti-neoplastic drugs are used for reducing the adverse effects seen with the cancer chemotherapy. In total, 682 adjuvant drugs were given to 144 study participants. The average number of these drugs in our study were 4.73 per patient. The prescribing pattern of the different adjuvant and supportive drugs used is shown in Table 4 and 5. The antiemetics were the most commonly prescribed pre-chemotherapy drug accounted for 21.54% of total adjuvant drugs given, followed by the drugs used to reduce gastric acidity which constituted 20.8%. Inj Ondansetron was the most common used antiemetic dug which was administered intravenously in almost all patients, and Aprepitant was used in only in 4 patients of the study. On an average 90.9% of the patients were prescribed dexamethasone along with the chemotherapy. This adjuvant steroid, when used with chemotherapy, minimises the adverse effects like nausea and vomiting caused by chemotherapy medications. Also, it has found to improve appetite, decrease inflammation at the cancer site, and also decrease the elevated blood calcium levels (which is connected with some bone cancer cases) [14].

Cisplatin/carboplatin-based chemotherapy regimen causes hypomagnesemia and hypokalemia due to renal magnesium (Mg) and potassium (K) losses. Therefore potassium chloride and magnesium sulphate were given to almost all the patients receiving a cisplatin/carboplatin-based chemotherapy regimen. These patients also have a high risk of renal tubular dysfunction and a cumulative impairment in renal function, manifested by a decline in the glomerular filtration rate [15]. To prevent the development of nephrotoxicity, forced hydration in the form of saline infusion before, on the day of chemotherapy, and following cisplatin [16] and diuresis, by furosemide was given to the patients in our study. Although some researchers have already reported the effect of furosemide on reducing the

renal toxicity, its effect on the prevention of nephrotoxicity is still controversial [17]. It has been reported that furosemide protects renal function, while it worsens renal histopathology [18]. However, it is discovered that prophylactic magnesium supplementation, in addition to curbing adverse effects that occur directly from magnesium deficiency, can minimise the severity of cisplatin-induced renal damage without interfering with the anticancer effect of the drug [19]. Magnesium performs an essential role in the preservation of intracellular K^+ loss too. Therefore, in the present study, furosemide was always co-prescribed with MgSO4 to reduce the nephrotoxicity of cisplatin.

CPM was used as an antiallergic among eight patients. Chlorpheniramine maleate has found to be effective, patient convenient and very useful in preventing allergic reaction due to paclitaxel [20]. Mesna was used along with cyclophosphamide to prevent hemorrhagic cystitis. The other drugs prescribed included zoledronic acid in breast cancer with secondaries in bones. Administration of anticancer drugs is associated in some patients with severe acid reflux diseases which require the prescription of proton pump inhibitors, H2 antagonist and antacid prophylactically as well as therapeutically to the patients [21]. In our study, as gastric protectants, Ranitidine or Pantaprozole were given intravenously. Inj Ranitidine formed 12.01%, followed by Inj Pantaprozole which was 8.79% of the total prechemotherapy drugs in all the study groups. Inj Ranitidine was given in 82 patients and Inj Pantaprozole in 60 patients.

Table 6: WHO prescribing indicators

Prescribing Indicators	In patients
Number of drugs prescribed per patient	6.01
Percentage of drugs prescribed by Generic name	76.7%
Number of anti-cancer drugs prescribed per patient	1.27
Number of adjuvant/supportive drugs prescribed per patient	4.73
Percentage of drugs prescribed from national essential drug list	82.2%

The prescribing indicator shows that the average number of cytotoxic drugs per prescription was 1.277, which was near to study conducted by B. Sajeev Kumar et al., (1.73) [22] and relatively lower than that of comparable studies conducted in Brazil (2.4), Jordan (2.3) and in other places in India (2.7) [23]. The average number of drugs per prescript was 6.01. The number of adjuvant/supportive drugs prescribed per patient was 4.73. The percentage of drugs prescribed by the Essential Drug List was 82%. The drugs were prescribed based on the hospital formulary and supplied on a nonprofit basis by the government. The percentage of drugs ordered by the generic name was 77%. Prescribing medicines by generic name must be strengthened since generic medicines are as efficient as brand ones, and they cost less which lowers the medical expenditure.

In conclusion, the head & neck cancers were the prevalent cancers observed with more preponderance among males. Most of the patients were prescribed with a single anticancer drug. Cisplatin was the most commonly used cytotoxic drug followed by carboplatin, and antimetabolites. The most commonly used adjuvant drugs in our study were anti-emetics and anti-peptic ulcer drugs. Over 82% of anticancer agents were taken from the essential drug list and were prescribed in generic names, indicating rational use. WHO promotes that more such drug utilisation studies are needed in every health care setting to assess and assure the rational drug use.

Acknowledgement

We thank Dr K. Laxminarayana N., Professor and H.O.D, Department of Pharmacology, Vijayanagara Institute of Medical Sciences, Ballari and Dr Meher Kumar, Head of Department, Department of Radiotherapy, Vijayanagara Institute of Medical Sciences, Ballari for their encouragement and the support for our study.

References

1. WHO. The global burden of disease: 2004 update. Geneva: World Health Orga¬nization, [updated 2008

2. Cancer. World Health Organization: WHO. Available from: http://who.int/cancer/en.

3. American Cancer Society. Chemotherapy Principles 2013. Available from: http://www.cancer.org/treatment/treatmentsandsideeffects/treatmenttypes/chemotherapy/chemotherapyprinciplesanindepthdiscussionofthetechniquesanditsroleintreatment/ chemotherapy-principles-what-is-chemo

4. Times of India. 48% cancers due to tobacco chewing 2011. Available from: https://timesofindia. indiatimes.com/city/chandigarh/48-cancers-due-to-tobacco-chewing/articleshow/10649252. cms?

5. Haskell CM. Introduction. Cancer treatment. 4th edn. WB Saunders, Philadelphia, USA 1995; pp: 3-9.

6. Malhotra V, Perry MC. Classical chemotherapy: mechanisms, toxicities and the therapeutic window. Cancer Biol Ther. 2003; 2:S2-4. https://doi.org/10.4161/cbt.199 PMid:14508075

7. Rang HP, Dale MM, Ritter JM, Flower RJ. Rang and Dale's Pharmacology. Philadelphia, Elsevier Publisher, 2007:718-38. https://doi.org/10.1016/B978-0-443-06911-6.50056-6

8. Sachdeva PD, Patel BG. Drug utilization studies- Scope and future perspectives. IJPBR. 2010; 1:11-7.

9. World Health Organization. WHO: Introduction to drug utilization research/WHO International Working Group for Drug Statistics Methodology, WHO Collaborating Centre for Drug Statistics Methodology, WHO Collaborating Centre for Drug Utilization Research and Clinical Pharmacological Services. Geneva, WHO,

2003. Available from: http://www.whocc.no/filearchive/publications/drug_utilization_research.pdf

10. Dave DJ, Pillai A, Shah DV, Agarwal S, Goel A. An analysis of utilization pattern of anticancer drugs in diagnosed cases of carcinoma in a tertiary care teaching hospital. Int J Basic Appl Med Sci. 2014; 4:251-59.

11. Pentareddy MR, Suresh AV, Shailendra D, Subbaratnam Y, Prasuna G, Naresh DT, Rajsekhar K. Prescription pattern of anticancer drugs in a tertiary care hospital. Journal of Evidence based Medicine and Healthcare. 2015; 2(20):3001-9. https://doi.org/10.18410/jebmh/2015/435

12. Yash N Goyal, Krunal C Solanki, Rusva A Mistry, Niasrg D Joshi, Anil P Singh, Maganlal V Gajera. Pattern of adverse drug reactions due to cancer chemotherapy in tertiary care teaching hospital in Gujarat. International Journal of Scientific Research. 2014; 3(1):333-335. https://doi.org/10.15373/22778179/JAN2014/112

13. Darshan J Dave, Ajita Pillai, Dimple V Shah, Sneha Agrawal and Anilkumar Goel. An analysis of Utilization Pattern of Anticancer drugs in diagnosed cases of Carcinoma in a tertiary care teaching hospital.International Journal of Basic and Applied Medical Sciences. 2014; 4(1):251-259.

14. Hesketh PJ. Defining the emetogenicity of cancer chemotherapy regimens: relevance to clinical practice. Oncologist. 1999; 4:191-6. PMid:10394587

15. Wolters Kluwer Health-Up-to-date. Cisplatin nephrotoxicity, 2013. Available from: http:// www.uptodate.com/contents/cisplatin-nephrotoxicity

16. Losonczy G, Mathe C, Muller V, Szondy K, Moldvay J. Incidence, risk factors and prevention of cisplatin induced nephrotoxicity in patients with lung cancer. Magy Onkol. 2010; 54:289-96. https://doi.org/10.1556/MOnkol.54.2010.4.3 PMid:21163759

17. Cornelison TL, Reed E. Nephrotoxicity and hydration management for cisplatin, carboplatin, and ormaplatin. Gynecologic oncology. 1993; 50(2):147-58. https://doi.org/10.1006/gyno.1993.1184 PMid:8375728

18. Lehane D, Winston A, Gray R, Daskal Y. The effect of diuretic pre-treatment on clinical, morphological and ultrastructural cis-platinum induced nephrotoxicity. International Journal of Radiation Oncology* Biology* Physics. 1979; 5(8):1393-9. https://doi.org/10.1016/0360-3016(79)90677-1

19. Lajer H, Daugaard G. Cisplatin and hypomagnesemia. Cancer Treat Rev. 1999; 25:47-58. https://doi.org/10.1053/ctrv.1999.0097 PMid:10212589

20. Harada T, Doi M, Yamada Y, Akase T. Evaluation of short-time premedication with d-chlorpheniramine maleate injection for paclitaxel-induced hypersensitivity reaction. Gan to kagaku ryoho. Cancer & chemotherapy. 2008; 35(8):1347-51. PMid:18701846

21. The Scott Hamilton CARES initiative. Heartburn and chemotherapy. Available from: http:// chemocare.com/chemotherapy/side-effects/heartburn.aspx#.U0sDNPmSwd4

22. Kumar BS, Maria S, Shejila CH, Udaykumar P. Drug Utilization Review and Cost Analysis of Anticancer Drugs Used in a Tertiary Care Teaching Hospital. Indian Journal of Pharmaceutical Sciences. 2018; 80(4):686-93. https://doi.org/10.4172/pharmaceutical-sciences.1000408

23. Khan MK, Thapa RK, Adhikari DS, Rajbhandari M, Dwa P, Shrestha S, et al. Evaluation of cancer prevalence and cytotoxic medication prescribing in central region of Nepal. Kathmandu Univ J Sci Eng Technol. 2013; 9:189-99.

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Apr 15; 7(7):1209-1213.
https://doi.org/10.3889/oamjms.2019.255
eISSN: 1857-9655
Public Health

Factors Affecting Uncontrolled Blood Pressure among Elderly Hypertensive Patients in Pekanbaru City, Indonesia

Mitra Mitra*, Wulandari Wulandari

Department of Public Health, Hang Tuah Institute of Health Science, Pekanbaru, Indonesia

Citation: Mitra M, Wulandari W. Factors Affecting Uncontrolled Blood Pressure among Elderly Hypertensive Patients in Pekanbaru City, Indonesia. Open Access Maced J Med Sci. 2019 Apr 15; 7(7):1209-1213. https://doi.org/10.3889/oamjms.2019.255

Keywords: Elderly; Hypertension; Uncontrolled blood pressure

***Correspondence:** Mitra Mitra. Department of Public Health, Hang Tuah Institute of Health Science, Pekanbaru, Indonesia. E-mail: mitra@htp.ac.id

Received: 11-Feb-2019; **Revised:** 29-Mar-2019; **Accepted:** 31-Mar-2019; **Online first:** 13-Apr-2019

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: The highest prevalence of hypertension is among older adults. Although older adults with hypertension have already controlled their blood pressure by taking antihypertensive drugs, hypertension will still occur when not balanced with a healthy lifestyle. Data from Pekanbaru Municipality Health Department in 2016 showed that hypertension was the most common disease in Pekanbaru City.

AIM: The purpose of this study was to determine the factors that influence uncontrolled blood pressure among elderly patients with hypertension at Harapan Raya Pekanbaru Health Center in 2017.

MATERIAL AND METHODS: The study employed a cross-sectional design. The sample size comprised 116 elderly patients with hypertension who visited Harapan Raya Community Health Center. The sample criteria were patients aged ≥ 60 years and taking hypertension medication, and subjects were selected using consecutive sampling. The variables collected were demographic characteristics of elderly patients (age, education, sex, and occupation), physical activity, smoking habit, coffee and tea consumption and sodium intake. Statistical analysis involved the chi-square test and multiple logistic regressions.

RESULTS: The proportion of elderly hypertensive patients with uncontrolled blood pressure was 52.6%. The most influential variables concerning uncontrolled blood pressure were smoking habit (P-value: 0.004, OR: 5.2 95% CI: 1.71-15.8), no routine for taking antihypertensive drugs (P-value = 0.029 OR = 2.96 95% CI: 1.11-7.86) and sodium intake (P-value: 0.044 OR: 0.264 95% CI: 0.072-0.967).

CONCLUSION: The dominant factor influencing uncontrolled blood pressure among the elderly was smoking. Health workers at the Community Health Center are expected to provide education and counselling for elderly patients with hypertension to control their blood pressure by taking antihypertensive drugs regularly and quitting smoking.

Introduction

Hypertension is a medical condition characterised by increased constriction of arterial blood vessels resulting in blood flow resistance that increases blood pressure in blood vessels [1]. The classification of hypertension according to Joint National Committee VII is systolic and diastolic blood pressure over 140/90 mmHg [2]. Elderly hypertensive patients are naturally susceptible to stroke and other cardiovascular complications [3]. Hypertension is a chronic and asymptomatic disease, requiring optimal control and medication adherence especially among the elderly to reduce the risk of cardiovascular, cerebrovascular and renal diseases [4].

Blood pressure increases with age and is a common condition among the elderly. The Framingham Heart Study reported that the prevalence of hypertension increased from 27.3% among patients under 60 years to 74.0% among those over 80 (5). A Global Brief on Hypertension Report, WHO (2013) showed that nearly one billion people worldwide suffer from high blood pressure (hypertension), and two-thirds of them occur in developing countries [6]. Indonesia is one of the developing countries with a prevalence of hypertension of 25.8%, while in Riau it totals 20.9% [7]. Based on the 2016 Pekanbaru City

Health Office report from 20 health centres in Pekanbaru City, the highest number of cases of hypertension in Harapan Raya Health Center was 5570 [8]. Visits of elderly patients with hypertensive to Harapan Raya Health Center in 2016 amounted to 35.2% of all elderly visits [9].

The purpose of the study was to determine the factors that influence uncontrolled blood pressure among elderly patients with hypertension at Harapan Raya Health Center in 2017.

Material and Methods

Study Design and Sampling Procedure

The study employed a cross-sectional design. The study was conducted at Harapan Raya Public Health Center, Pekanbaru City from May to July 2017. The population in this study was determined based on the average number of monthly elderly visits. The elderly visits at Harapan Raya Public Health Center in 2016 totalled 4,213, of which 1,482 were elderly patients with hypertensive. The subjects in this study comprised elderly patients with hypertension living in the work area of Harapan Raya Health Center Pekanbaru City, aged 60 years and over and taking antihypertensive drugs. The determination of sample size was based on a hypotheses test for population proportion (one side test) from Lameshow (1997) [10] with respect to $\alpha = 5\%$, $\beta = 20\%$ and related research conducted by Wahyuningsih and Astuti, resulting in a minimum sample of 116 elderly subjects [11].

Instrument Development and Data Collection Procedures

The sampling technique used in this study comprised consecutive sampling, conducted by selecting elderly subjects by inclusion criteria and domiciles in the work area of Harapan Raya Public Health Center. Inclusion criteria comprised elderly subjects aged 60 years and over and willing to be a respondent by signing a consent form. They should have a history of hypertension and be taking antihypertensive drugs. The history of hypertension and antihypertensive drug data were obtained from medical records of Harapan Raya Community Health Center. The dependent variable was uncontrolled blood pressure among elderly patients with hypertension. The independent variables collected were socio-demographic characteristics, taking antihypertensive drugs, smoking habit, coffee and tea consumption, physical activity, and sodium intake. Data were collected by interview questionnaires and checking blood pressure using a stethoscope. Uncontrolled blood pressure was defined when elderly systolic blood pressure is > 140 mmHg, and diastolic is > 90 mmHg [2].

Smoking habits were obtained from interviews about the behaviour of elderly smokers in the past year. Physical activity was determined using a daily elderly activity measured using the calculation of PAL (Physical Activity Level) and Physical Activity Ratio (PAR). Food and Agriculture Organization (FAO)/World Health Organization (WHO)/United Nation University (UNU) [12] stated that the magnitude of physical activity performed by a person within 24 hours is stated in PAL. PAR was defined as the amount of energy spent over a certain time conducting an activity. The classification level of physical activity was defined as lighter when the PAL was < 1.7, moderate when the value of PAL was 1.7 to 1.99 and heavy when the value of PAL was > 1.99 [12]. Sodium intake was obtained from food recall in 2 x 24 hours by calculating how much sodium in milligrams was consumed daily by recording an over the standard when sodium intake was ≥ 2400 mg/day and sufficient when it was < 2400 mg/day [13]. Coffee and tea consumption was obtained from interviews using questionnaires about coffee and tea consumption habits, using sufficient and over the standard categories. Over the standard category was determined when the elderly subjects confirmed a habit of drinking three glasses or more of tea or coffee daily. Otherwise, it was categorised as sufficient [14].

Ethics Considerations

Data were collected after obtaining permission from the study program and the Riau Province Unity Board. A research permit was also obtained from the Pekanbaru Municipality Health Department to collect data at Harapan Raya Community Health Center. Before collecting the data, the researcher explained to respondents about the steps of implementing the data collection. When the respondents agreed, then they would sign a consent form.

Data Processing and Analysis

The data were collected and checked by the researcher to assess the completeness and clarity of the respondents' answers. The next step was to encode each variable. Data were processed using a computer program including data on sodium intake. The data were analysed using univariate analysis to determine the distribution of the characteristic frequency of each variable and bivariate analysis using the chi-square test. Simple logistic regression was employed to screen for multivariate modelling ($p < 0.25$). Multivariate analysis used multiple logistic regressions to identify dominant factors that influenced uncontrolled blood pressure among the elderly. The significance level used was $p < 0.05$.

Results

Sociodemographic Characteristics

Table 1 shows that respondents in this study totalled 116 elderly subjects. The proportion of elderly patient with hypertension with uncontrolled blood pressure was 52.6%. The average age of the elderly was 64.05 years ranging from 60 to 77 years. The majority obtained low education levels, elementary and junior high school, 72.4%, and most were women (57.8%). The elderly generally did not work (72.4%). Most elderly did not take antihypertensive drugs routinely (70.7%). The majority reported low physical activity levels (90.5%). Most elderly were nonsmokers in the past one year (70.7%). The elderly had a habit of consuming coffee or tea at the sufficient category (74.1%) and consuming sodium at the sufficient category (65.5%). Bivariate analysis with chi-square test showed that factors significantly correlated with uncontrolled blood pressure among elderly patients with hypertension were age, the routine of taking antihypertensive drugs, smoking status and sodium intake ($p < 0.05$).

Table 1: Sample characteristics and factors associated with uncontrolled blood pressure among elderly patients with hypertension (n = 116)

Characteristics	Blood Pressure				Total		P value	OR $_{crude}$ (95% CI)
	Controlled		Uncontrolled					
	n	%	n	%	n	%		
Elderly	55	47.4	61	52.6	116	100.0		
Age (years)*								
≤ 62	36	61.0	23	39.0	59	50.9	0.005	3.130
> 62	19	33.3	38	66.7	57	49.1		(1.465-6.691)
Education level								
Low	36	42.9	48	57.1	84	72.4	0.166	0.513
High	19	59.4	13	40.6	32	27.6		(0.224-1.174)
Sex								
Male	19	38.8	30	61.2	49	42.2	0.160	0.545
Female	36	53.7	31	46.3	67	57.8		(0,258-1.153)
Occupation status								
Unemployed	42	50.0	42	50.0	84	72.4	0.487	1.462
Working	13	40.6	19	59.4	32	27.6		(0.641-3.335)
Routinely antihypertensive drugs								
Yes	21	61.8	13	38.2	34	29.3	0.046	2.281
No	34	41.5	48	58.5	82	70.7		(1.005-5.175)
Physical Activity								
Moderate	8	72.7	3	27.3	11	9.5	0.147	3.291
Low	47	44.8	58	55.2	105	90.5		(0.827-13.101
Smoking status								
Nonsmoker	45	54.9	37	45.1	82	70.7	0.022	2.919
Smoker	10	29.4	24	70.6	34	29.3		(1.240-6.873)
Coffee and tea consumption								
Sufficient	44	51.2	42	48.8	86	74.1	0.247	1.810
Overstandard	11	36.7	19	63.3	30	25.9		(0.770-4.253)
Sodium intake								
Sufficient	27	35.5	49	64.5	76	65.5	0.001	0.236
Overstandard	28	70.0	12	30.0	40	34.5		(0.104-0.538)

* mean ± sd: 64.05 ± 4.6 median: 62 min-max: 60-77 95% CI: 63.2-64.9.

Multivariate analysis with multiple logistic regressions was used to determine the dominant factors that influenced controlled blood pressure among elderly patients with hypertension. Multivariate modelling with 6 models found that dominant variables influencing uncontrolled blood pressure among elderly with hypertension were not taking antihypertensive drugs routinely and smoking and sodium intake. Smoking was a dominant variable for uncontrolled blood pressure affecting the elderly. Elderly smokers had 5 times the risk of uncontrolled blood pressure than nonsmokers (OR: 5.203). The elderly who did not take antihypertensive drugs routinely were 3 times more at risk of uncontrolled blood pressure than the elderly who regularly took medicine (OR: 2.963). Sodium intake had an inverse association relationship. Education and age of the elderly were confounding variables. The Omnibus test value obtained was $p < 0.001$ meaning that the result of multivariate modelling was relevant. These five independent variables (age, education level, taking antihypertensive drugs routinely and sodium intake) could explain uncontrolled blood pressure among elderly patients with hypertension by 31.6%.

Table 2: Multivariate analysis with binary logistic regression of factors affecting uncontrolled blood pressure among elderly patients with hypertension

Variable	Category	B	p-value	Adj OR	95% CI
Age	≤ 62 y	1		Ref	
	> 62 y	1.250	0.066	3.490	0.921-13.224
Education	Low	1		Ref	
	High	0.453	0.438	1.573	0.501-4.945
Routinely	Yes	1		Ref	
antihypertensive drugs	No	1.086	0.029	2.963	1.117-7.860
Smoker	Not a smoker	1		Ref	
	Smoker	1.649	0.004	5.203	1.714-15.798
Sodium intake	sufficient	1		Ref	
	Overstandard	-1.333	0.044	0.264	0.072-0.967

Omnibus Test (Chi Square) $p < 0.001$; Cox & Snel R Square: 0.237; Nagelkerke R Square: 0.316; Hosmes-Lemeshow (GoF): 2.620 (7) $p > 0.05$.

Discussion

The results showed that the proportion of uncontrolled blood pressure among elderly patients with hypertension was 52.6%. Based on multivariate analysis with multiple logistic regressions, the dominant factor affecting uncontrolled blood pressure was smoking. Other significantly related variables included taking antihypertensive drugs irregularly. Sodium intake in this study demonstrated an inverse association. The level of elderly education was a confounding variable of the smoking habit.

Hypertension will become a serious health problem if uncontrolled. Controlling hypertension among the elderly as self-care for hypertensive patients includes consuming recommended antihypertensive drugs, monitoring blood pressure regularly and modifying lifestyle to maintain health such as performing physical activities, stopping smoking, reducing salt consumption and increasing intake of vegetables and fruit [15]. However, implementing self-care by hypertensive patients had not been maximised. The study of Oliveria suggested that although knowledge of hypertension is quite good, hypertensive patients do not have a thorough understanding of their blood pressure conditions, such as a lack of awareness in blood pressure control [16]. At least 50% of patients receiving prescribed antihypertensive drugs do not take them as recommended [17]. Irregularity in taking antihypertensive drugs will have an impact on

elevating blood pressure [17].

The prevalence of hypertension increases with increasing age. One-half of people aged between 60 and 69 years have hypertension, and this increases to 60 to 70% among people over age 70 [18]. Research by Hazarika et al., and Alam et al., showed the trend of increasing prevalence of hypertension in the age group over 60 years [3], [19]. Hypertension is highly prevalent among the elderly. Several epidemiological surveys conducted in the USA and Europe concluded that hypertension prevalence among the elderly ranged between 53% and 72% [20]. Arterial stress (decrease in arterial wall adherence) is considered an inevitable consequence of ageing which causes elevated blood pressure levels [21]. Among older people with hypertension, endothelial cell damage increases with systolic blood pressure and fibrinogen levels indicate prothrombotic status. The condition shows that uncontrolled hypertension among the elderly causes organ damage [22].

Smoking increases blood pressure [23]. Several studies have shown a significant association of smoking with the incidence of hypertension [23], [24]. Toxic chemicals such as nicotine and carbon monoxide exploited through cigarettes entering the bloodstream can damage the endothelial lining of the arteries, resulting in the process of atherosclerosis and high blood pressure [20]. Research by Ragueneau showed smoking caused a significant increase in systolic blood pressure and diastolic by + 7% and 10%, respectively [26]. Blood pressure and heart rate increase during smoking. This effect is particularly associated with nicotine.

The increase in blood pressure is caused by increased cardiac output and total peripheral vascular resistance. A rise in blood pressure immediately occurs before any increase in circulating catecholamine. Among hypertensive patients, blood pressure decreases the effect of partial beta-blockers that can be eliminated by tobacco smoking, whereas alpha-receptor inhibitors seem to retain antihypertensive properties among smokers. This creates a paradox that smoking increases blood pressure [26].

Elderly hypertensive sufferers are required to take antihypertensive drugs to control their blood pressure. Controlling blood pressure by taking antihypertensive drugs is an attempt to prevent the occurrence of hypertension among the elderly. Antihypertensive medication use and blood pressure control among US adults with hypertension significantly has increased over the past 10 years [27]. In this study, the elderly who did not regularly take antihypertensive drugs totalled 70.1%. Reducing systolic and diastolic blood pressure can be achieved by non-pharmacological (lifestyle measures) as well as pharmacological means [27]. The commitment of the elderly in taking medicine according to a doctor's recommendation is needed to control blood pressure and prevent complications of hypertension.

The success of therapy is not only determined by the correct diagnosis and drug selection, but also by adherence to the therapy. To improve health and prevent the occurrence of diseases caused by hypertension, the elderly need to perform self-care by taking medicationsby the prescription, monitoring blood pressure and reducing sodium intake [28]. Various efforts are required to improve patient adherence to the consumption of antihypertensive drugs to control their blood pressure and prevent complications of hypertension. Research by Yang showed that 44.6% of elderly hypertensive patients could control their hypertension. Many hypertensive patients do not know their condition, and the level of control and treatment remains relatively low, most likely due to improper use of antihypertensive drugs and unhealthy lifestyle choices [29].

In this study, an inverse association was found between sodium intake and controlled blood pressure. The possibility of bias in recalling information of sodium consumption might be due to the subjects' age and educational background, making it difficult to gather information related to what was actually consumed by them daily. In addition, it would be likely caused by the elderly routine in taking antihypertensive medication although the intake of sodium was high.

The proportion of elderly with uncontrolled blood pressure at Harapan Raya Community Health Center, Pekanbaru City was 52.6%. Factors associated with uncontrolled blood pressure among elderly hypertensive patients included smoking habit, irregularity in taking antihypertensive drugs and sodium intake. In this study, sodium intake exhibited an inverse association relationship. Education and age of the elderly were confounding variables. Awareness of elderly patients with hypertension to consume antihypertensive drugs remains low. More effort is needed by health workers at the Public Health Center is to provide more intensive education and counseling on the importance of controlling blood pressure, regularly taking antihypertensive drugs and quitting smoking.

Acknowledgements

The authors would like to thank the Head of Pekanbaru Municipality Health Department and Head of Harapan Raya Public Health Center who has given permission to collect data, and to the respondents involved in this research.

References

1. Ladecola C, Davisson RL. Hypertension and Cerevascular Dysfunction. Cell Metab NIH Public Acces. 2008; 7(6):476-84. https://doi.org/10.1016/j.cmet.2008.03.010 PMid:18522829 PMCid:PMC2475602

2. JNC-VII. The Seventh Report of The Joint National Comitee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. 2004.

3. Hazarika NC, Biswas D, Mahanta J. Hypertension in the Elderly Population of Assam. Journal of the Association of Physicians of India. 2003; 51:567-73.

4. Lionakis N, Mendrinos D, Sanidas E, Favatas G, Georgopoulou M, Lionakis N, et al. Hypertension in the elderly. World Journal of Cardiology. 2012; 4(5):135-47. https://doi.org/10.4330/wjc.v4.i5.135 PMid:22655162 PMCid:PMC3364500

5. Alhawassi TM, Krass I, Pont LG. Hypertension in Older Persons : A Systematic Review of National and International Treatment Guidelines. The Journal of Clinical Hypertension. 2015; 17(6):486-92. https://doi.org/10.1111/jch.12536 PMid:25827023

6. WHO. A global brief on Hypertension. Geneva, 2013.

7. Ministry of Health-RI. Hypertension. Jakarta: Pusdatin, 2015.

8. Pekanbaru Municipality Health. Pekanbaru Municipality Health Departement Profil 2016. Pekanbaru, 2015.

9. Community Health Centrer Harapan Raya. Community Health Center Harapan Raya Profil. Pekanbaru, 2016.

10. Lwanga S, Lemeshow S. Sample size determination in health studies. Geneva: WHO, 1997.

11. Wahyuningsih, Astuti E. Factors Affecting Hypertension in the Elderly. Journal Ners and Midwifery Indonesia. 2013; 1(3):71-5.

12. FAO/WHO/UNU. Human energy requirements. Rome, 2001.

13. Almatsier S. Basic Principles of Nutrition Science. Jakarta: Gramedia Pustaka Utama, 2009.

14. Uiterwaal CSPM, Verschuren WMM, Bueno-de-mesquita HB, Ocké M, Geleijnse JM. Coffee Intake and Incidence of hypertension. The American Journal of Clinical Nutrition. 2007; 85(2):718-23.

15. Peltzer K, Phaswana-mafuya N. Hypertension and associated factors in older adults in South Africa. Cardiovascular Journal of Africa. 2013; 24(3):66-71. https://doi.org/10.5830/CVJA-2013-002 PMid:23736129 PMCid:PMC3721893

16. Oliveria SA, Chen RS, Mccarthy BD, Davis CC, Hill MN. Hypertension Knowledge, Awareness, and Attitudes in a Hypertensive Population. J Gen Intern Med. 2005; 20:219-25. https://doi.org/10.1111/j.1525-1497.2005.30353.x PMid:15836524 PMCid:PMC1490067

17. Ministry of Health-RI. Pharmaceutical care for hypertension. Directorate of Community Pharmacy and Clinic, 2006.

18. Acelajado MC. Optimal management of hypertension in elderly patients. Integrated Blood Pressure Control. 2010; 3:145-53. https://doi.org/10.2147/IBPC.S6778 PMid:21949630 PMCid:PMC3172073

19. Alam N, Soni GP, Jain KK, Verma S, Panda PS. Prevalence and determinants of hypertension in elderly population of Raipur city, Chhattisgarh. International Journal of Research in Medical Sciences. 2015; 3(3):568-73. https://doi.org/10.5455/2320-6012.ijrms20150307

20. Hafiz M, Weta W, Ratnawati NLKA. Factors associated with the incidence of hypertension in the elderly group in the working area of UPT Puskesmas Petang I Badung District in 2016. E Jurnal Medika. 2016; 5(7):1-23.

21. Gardin JM, Arnold AM, Bild DE, Smith V, Lima JAC, Klopfenstein HS, et al. Left Ventricular Diastolic Filling in the Elderly : The Cardiovascular Health Study. The American Journal of Cardiology. 1998; 82(1):345-51. https://doi.org/10.1016/S0002-9149(98)00339-7

22. Schmieder RE. End Organ Damage In Hypertension Early detection. Deutsches Ärzteblatt International. 2010; 107(49):866-74.

23. Primatesta P, Falaschetti E, Gupta S, Marmot MG, Poulter NR. Evidence From the Health Survey for England. Hypertension. 2001; 37(1):187-93. https://doi.org/10.1161/01.HYP.37.2.187 PMid:11230269

24. Quasem I, Shetye MS, Alex SC, Kumar A, Sarma P., Thankappan K. Prevalence, awareness, treatment and control of hypertension among the elderly in Bangladesh and India : a multicentre study. Bulletin of the World Health Organization. 2001; 79(6):490-500.

25. Dewhurst MJ, Dewhurst F, Gray WK, Chaote P, Orega GP, Walker RW. The high prevalence of hypertension in rural-dwelling Tanzanian older adults and the disparity between detection, treatment and control : a rule of sixths ? Journal of Human Hypertension. 2012; 27(6):374-80. https://doi.org/10.1038/jhh.2012.59 PMid:23235367

26. Ragueneau I, Michaud P, Dcmolis J, Moryusef A, Jaillon P, Funck-brentano C. Effects of cigarette smoking on short-term variability of blood pressure in smoking and non smoking healthy volunteers. Fundam Clin Pharmacol. 1999; 13(1):501-7. https://doi.org/10.1111/j.1472-8206.1999.tb00010.x PMid:10456293

27. Gu Q, Burt VL, Dillon CF, Yoon S. Trends in Antihypertensive Medication Use and Blood The National Health and Nutrition Examination Survey, 2001 to 2010. Circulation. 2012; 126:2105-14. https://doi.org/10.1161/CIRCULATIONAHA.112.096156 PMid:23091084

28. Viera AJ, Jamieson B. How effective are hypertension self-care interventions ? The Journal of Family Practice. 2007; 56(3):229-31.

29. Yang L, Xu X, Yan J, Yu W, Tang X, Wu H, et al. Analysis on associated factors of uncontrolled hypertension among elderly hypertensive patients in Southern China : a community-based , cross-sectional survey. BMC Public Health. 2014; 14(903):1-8. https://doi.org/10.1186/1471-2458-14-903

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Apr 15; 7(7):1214-1219.
https://doi.org/10.3889/oamjms.2019.275
eISSN: 1857-9655
Public Health

Effects of Face-To-Face and Online Training on Self-Care of Middle-Aged and Elderly People with Type 2 Diabetes: A Comparative Study

Es-hagh Ildarabadi[1], Mohammad Ghasem Tabei[2], Ameneh Mosaferi Khosh[3*]

[1]Department of Nursing, Esfarayen Faculty of Medical Sciences, Esfarayen, Iran; [2]Department of Internal Medicine, Esfarayen Faculty of Medical Sciences, Esfarayen, Iran; [3]Department of Postgraduate, School of Nursing and Midwifery, North Khorasan University of Medical Sciences, Bojnurd, Iran

Citation: Ildarabadi E, Tabei MG, Mosaferi Khosh A. Effects of Face-To-Face and Online Training on Self-Care of Middle-Aged and Elderly People with Type 2 Diabetes: A Comparative Study. Open Access Maced J Med Sci. 2019 Apr 15; 7(7):1214-1219. https://doi.org/10.3889/oamjms.2019.275

Keywords: Diabetes Mellitus; Type 2; Aged; Elderly; Self Care; Education; Distance

***Correspondence:** Ameneh Mosaferi Khosh. Department of Postgraduate, School of Nursing and Midwifery, North Khorasan University of Medical Sciences, Bojnurd, Iran. E-mail: hmosaferi56@gmail.com

Received: 24-Feb-2019; **Revised:** 03-Apr-2019; **Accepted:** 04-Apr-2019; **Online first:** 13-Apr-2019

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Self-care training is one of the strategies used to control diabetes. There is some ambiguity about the appropriate method for educating middle-aged and older adults about self-care.

AIM: This study aimed to compare the effects of face-to-face and online training on self-care levels in middle-aged and older adults with type 2 diabetes.

MATERIAL AND METHODS: In a randomised clinical trial, 84 middle-aged and older adults with type 2 diabetes who had been referred to the Diabetes Clinic of Esfarayen in Iran, were evaluated. Patients who meet the inclusion criteria were randomly assigned into two groups. Diabetes self-care education (DSCE) was provided using a face-to-face training method in one group and using online training method in the other group. The summary of diabetes self-care activities (SDSCA) questionnaire was completed at baseline and 1 month after training.

RESULTS: The mean and standard deviation of self-care scores before and 1 month after training were 43.16 ± 14.94 and 65.76 ± 10.65 in the face-to-face training group, and 37 ± 10.75 and 56.82 ± 12.06 in the online training group, respectively. The differences in the self-care scores were significant both before and after the intervention in the two groups ($p < 0.05$). Although the difference was greater in the face-to-face training group than in the online training group, it was not statistically significant ($P > 0.05$).

CONCLUSION: Both face-to-face and online training had a similar effect on the self-care levels in middle-aged and older adults with type 2 diabetes. Therefore, both training methods could be used as effective techniques to meet the needs and educational requirements of middle-aged and older adults with type 2 diabetes.

Introduction

Elderly is emerging as an important and global phenomenon today due to population growth. World Health Organization (WHO) announced that older adults accounted for about 7% of the total population in Southwest Asia (including Iran) in 2000, and this figure will reach 15% by 2030 [1]. The United Nations has stated that the total population of older adults in the world was approximately 737 million in 2009. The number is expected to reach 2 billion in 2050, with two-thirds of the population living in developing countries [2]. Statistical surveys in Iran also indicate the rapid growth of the elderly population [3]. According to the 2011 census in Iran, the population over the age of 60 years was 6,159,676 and the population aged over 65 years was 4,296,769 [4]. On the other hand, aging significantly increases the risk of elderly people to chronic illness. Recent studies indicate that 80% of elderly people suffer from at least one chronic illness that puts them at greater risk of disability and death compared to others [3], [4], [5]. Nearly 40% of the elderly people in the community experience some of the constraints of chronic illness [5]. One of the most common and chronic illnesses among elderly people is diabetes. The disease has been exhibiting an alarming outbreak over the past

decade and is rapidly rising, up to 50% [6]. The WHO predicted that around 300 million people worldwide would develop diabetes until 2025 [7]. It is one of the most important causes of mortality and disability in older adults [8]. Middle age refers to the age of between 45-60 years [9]. The mortality rate for middle-aged people with diabetes is twice more than that of non-diabetic (healthy) people at the same age [10].

The International Diabetes Federation (IDF) in 2015 estimated that 1 out of 11 adults were diagnosed with diabetes (415 million) and one out of two adults (46%) was not diagnosed with diabetes. Twelve per cent of global health cost is spent on diabetes ($ 673 billion). Three-quarters (75%) of people with diabetes live in low-income and middle-income countries. Also, it has been revealed that 1.5 million people died in 2012 due to diabetes. The population of adults over the age of 65 years with type 2 diabetes in the United States is projected to reach 70 million in 2030 from 35 million in 2000 [11]. In terms of the importance of the disease, diabetes mellitus is the most important cause of blindness among people aged 25-74 years and is the leading cause of amputation in the United States. Also, 35% of people with chronic renal failure and dialysis have diabetes. Moreover, the patients with diabetes are twice more likely to experience a stroke than healthy people, so that diabetes and its related complications are the third leading cause of death in the United States [12].

One of the ways to control diabetes and reduce its complications is self-care training. The self-care promotes the quality of life and helps reduce costs. The American Diabetes Association states that people with diabetes should be trained to treat themselves to delay the onset of diabetes-related complications [7]. One of the methods for patients' education is e-learning, which is the use of computers and the internet. To enter the site for access to educational content, there are courses and communication tools that in turn can be considered convergence of education and the internet [7], [8]. In the current society, the internet has increasingly been considered as a highly practical tool, and there is an increase in the number of people who receive their health information through the internet. This suggests that the internet may be of help in changing the behaviour of the community to prevent and control type 2 diabetes. Internet-based education is well known for the prevention and treatment of chronic diseases [13]. Another way of teaching is face-to-face training, which is one of the most common methods of education in the healthcare system. This method allows for two-person discussions and a change in behaviour. However, this method of training needs to spend more time, and it is impossible in crowded centres [14]. Unlike face-to-face interactions, web-based interventions can target a wider audience without adding more per-user fees and is available to users all day long [13].

Many elderly patients with diabetes experience complications of this disease. Considering the importance of diabetes and prevention of complications and effective control of blood sugar and the need for self-care education as well as ambiguity about the appropriate method for educating middle age and elderly people, this study was conducted to compare the effects of face-to-face and online training on self-care levels in elderly and middle-aged people with type 2 diabetes.

Material and Methods

The present randomised clinical trial was conducted on middle-aged and older adults with type 2 diabetes who had been referred to the Diabetes Clinic of Esfarayen, Iran, in 2017. Due to the lack of a similar study, 15 people from each of the online training group and the face-to-face training group were selected for a pilot study to estimate the sample size. Using the results of the pilot study, the sample size was calculated to be 38 for each group. The study was conducted during the second half of 2017. To conduct this study, the researchers were referred to the Diabetes Clinic of Esfarayen with the approval of the ethics committee of North Khorasan University of Medical Sciences. The medical records of the relevant patients were delivered to the researchers in coordination with the clinic. Patients were selected using a purposive and convenience sampling method. The inclusion criteria were as follows: age ≥ 45 years; a history of at least 6 months of diabetes; minimal reading and writing skills and skills in using internet; no history of other chronic diseases including hypertension and cardiovascular disease; fluent in the Persian language; resided in the city of Esfarayen and able to communicate (i.e., there were no communication barriers, such as deafness or blindness). The exclusion criteria were death or hospitalisation during the study period; absent for more than two face-to-face training sessions; missed more than two online training sessions; and an unwillingness to voluntarily participate in the study. A brief explanation of the research objectives and methodology was given to the middle-aged and older adults before the intervention and written informed consent was obtained from all patients.

A total of 84 eligible patients were contacted using the phone number noted in their medical records. The necessary arrangements were made to provide the intervention, set the hours of the class, and obtain written consent from the participants. It is worth noting that no course had been previously held in the diabetes clinic. The research units were encoded from 1 to 82; patients with the odd codes were assigned randomly to the face-to-face training group (n = 42), and the even codes were assigned to the online training group (n = 42). The participants

completed a demographic profile and answered the summary of diabetes self-care activities (SDSCA) questionnaire before the intervention. The SDSCA self-report questionnaire contained 15 items about the self-care criteria of the patients over the past 7 days. It covered various aspects of the treatment regimen of people with diabetes: general and diabetic diet (five questions), exercise (two questions), blood sugar testing (two questions), use of insulin injections or anti-diabetic medications (one question), foot care (four questions), and smoking (one question) [15]. The SDSCA score for each behaviour, except smoking, ranged from 0 – 7; the score for smoking behaviour ranged from 0 to 1. A total adherence score, which ranged from 0 – 99, was obtained by adding each score [15]. In a study by Hamadzadeh et al., eight faculty members confirmed the content validity index (CVI) of the SDSCA questionnaire. The reliability of the SDSCA was evaluated using Cronbach's alpha, which was 0.88 [15].

The educational content of the training sessions was related to diabetes self-care education (DSCE) and included information on nutrition, exercise, foot care, adherence to the drug regimen, and self-monitoring of blood glucose. The materials were extracted from national medical and nursing books, including the risk assessment for heart attack, stroke and cancer, family care guides, which were reviewed and published by the Ministry of Health and Medical Education in 2017, and Nurse and Diabetes (National Disease Prevention and Control Program), Ministry of Health and Medical Education, Deputy of Health, Center for Disease Control, and the Department of Endocrinology and Metabolism [16].

The educational topics covered during the sessions were as follows. Session 1 discussed diabetes and its types, diagnosis, and symptoms. Session 2 discussed appropriate diet for diabetic patients (a diet with low fat and high fibre contents and a limited amount of carbohydrates), while session 3 provided information about the possible treatments for diabetes and methods of self-monitoring of blood glucose (twice-daily, pre-lunch/pre-dinner). Session 4 discussed the importance of exercise in patients with diabetes, while in session 5, foot care and the complications of smoking in patients with diabetes has been discussed. Session 6 provided information on how to inject and maintain insulin levels, and the necessary tests that are involved. The educational content was the same for both groups. The training for the online training group lasted 3 weeks. A training file containing videos and text was sent every 3 days through a social network. People were requested to send a message to the researchers after receiving and studying the file. To prevent the contamination of information, individuals were asked not to distribute the class content to other groups. Before starting the online training, the researchers made sure that the participants had a mobile phone and the skills to use it. The participants were then taught how to receive and study the files. One month after completing the training, a post-test questionnaire was given to both groups. The collected data were categorised and coded in tables and analysed with SPSS version 24 software. Statistical tests, such as chi-squared test, independent t-test, and paired t-test, Fisher's exact test, Mann–Whitney, Wilcoxon test, and McNemar's test were used for data analysis.

Results

A total of 84 middle-aged and older adults were enrolled in the study (34 women and 8 men were in the face-to-face training group, and 31 women and 11 men were in the online training group). The mean age of the participants was 55.35 years; most participants were women (77.4%). Also, 61.9% had a primary level of education, and 96.4% were married. Other demographic and clinical information of the participants in the groups is presented in Table 1.

Table 1: Demographic and clinical profile of middle-aged and older adults with type 2 diabetes in the face-to-face and online training groups

Variables	Categories	Face-to-face training N (%)	Online training N (%)	P-value
Sex	Female	34 (81.34)	31 (74)	0.149
	Male	8 (19)	11(24)	
Duration of disease		6.96 ± 5.82	6.35 ± 4.07	0.816
Occupational status	Employee	9 (21)	21 (50)	0.01
	Housekeeper	19 (45)	14 (33)	
	Retiree	4 (10)	3 (7)	
	Free	10(24)	4(10)	
Marital status	Married	41 (98)	38 (95.0)	0.611
	The divorced and deceased spouse	1(2)	2 (5)	
Drug consumption	Oral medication	37 (88)	40 (95)	0.433
	Insulin	5(12)	2 (5)	
Familial history	Yes	22(52)	15(36)	0.272
	No	20(48)	27(64)	
Educational level	Uneducated	12 (29)	13 (31)	>0.99
	< High school	14 (33)	13 (31)	
	≥ High school	11 (26)	8 (19)	
	≥ Bachelor	5(12)	8 (19)	
Family relationship	Father	9 (41)	4 (27)	0.259
	Mother	6 (27)	8(53)	
	Sibling	6 (27)	3 (20)	
Economic level	Poor	11 (26)	10 (23)	0.99
	Average	25 (60)	24 (58)	
	Good	6 (14)	8 (19)	
Self-monitoring of blood glucose	Yes	23 (55)	20 (48)	0.511
	No	19 (45)	22 (52)	
Complications	Renal	1 (2.4)	0 (0)	0.369
	Ocular	6 (14.6)	10 (25)	
	Other	1 (2.4)	0 (0)	
	No	33 (80.5)	30 (75)	
Assistance to self-care	Spouse	13 (31)	6 (14)	0.026
	Child	8 (19)	5 (12)	
	Neighbor	2 (5)	1 (2)	
	Individual	19 (45)	30(72)	

Most patients in the face-to-face training group were housekeepers (45%), while 50% of the people in the online training group were employees; thus, the distribution of occupations was heterogeneous in the two groups. The Kruskal-Wallis test was used to evaluate the effect of occupation on the total DSCE score before training. There was no significant relationship between occupation and DSCE ($\chi^2 = 1.46$; P = 0.48).

There was a significant improvement in the self-care scores for adherence to diet for patients in both groups after training (P < 0.001). However, there was no significant difference in the scores for adherence to diet between the two groups before training (P = 0.15). The score was significantly higher after training in the face-to-face group compared with the online training group (Table 2).

Table 2: Comparison of self-care scores for adherence to diet in the face-to-face and online training groups

Diet	Before training	After training	T-value	P-value
Face-to-face training group	19.54 ± 7	25.47 ± 4.32	-6.09	< 0.001*
Online training group	17.55 ± 5.56	22.27 ± 4.78	-6.09	< 0.001*
T-value	1.42	3.18		
P-value	0.15**	0.002**		

*Paired t-test **Independent t-test.

The self-care scores for the exercise of the patients in both groups were studied before and after training (Table 3). The results showed that the scores improved significantly in both groups (P < 0.001). The score in the face-to-face training group both was significantly higher than in the online group before and after training (P = 0.003). To examine and control the effect of the exercise score before training, the difference in the exercise scores before and after training in both groups was assessed using the Mann-Whitney U test. There was no significant difference in the changes in exercise scores between the two groups after training (P-value = 0.56 and Mann-Whitney U test = 779).

Table 3: Comparison of self-care scores for exercise in the face-to-face and online training groups

Exercise	Before training	After training	Z-value	P-value
Face-to-face training group	5.61 ± 4.17	7.76 ± 3.37	-3.7	*< 0.001
Online training group	2.9 ± 2.76	5.4 ± 2.65	-4.96	*< 0.001
Mann-Whitney U test	-2.95	-3.32	67	140
P-value	0.003**	0.001**		

* Wilcoxon ** Mann–Whitney

The mean self-care scores in both groups were significantly increased after training (Table 4). There was a significant difference in the self-care scores of the groups before the training. To control and compare the scores before and after the two training methods, the difference in the scores before and after the training was calculated using the Wilcoxon test. The mean difference was 22.59 ± 11.55 in the face-to-face training group and 19.82 ± 10.88 in the online training group; there was no significant difference between the two groups (z = 694 and P-value = 0.17).

Table 4: Mean self-care scores in the face-to-face and online training groups

Mean self-care score	Before training	After training	Z-value	P-value
Pace-to-face training group	43.16 ± 14.94	65.76 ± 10.65	5.59	< 0.001*
Online training group	37 ± 10.75	56.82 ± 12.06	5.44	< 0.001*
Mann-Whitney U test	606.5	483.5		
P-value	0.03	0.001		

* Wilcoxon ** Mann–Whitney.

Discussion

The present study was conducted to compare the effects of face-to-face and online training on the self-care levels in middle-aged and older adults with type 2 diabetes. The results showed that both approaches increased the self-care scores of the participants. After the intervention, the face-to-face training group showed a statistically significant increase in the mean self-care scores. This indicated the effectiveness of face-to-face training on changing the behaviour and increasing the levels of self-care in middle-aged and older adults with type 2 diabetes. In concurrence with this study, Azizi et al., showed that self-care education increased the control of drug complications and haemoglobin A_1c levels in patients with diabetes type1, which proved the effectiveness of training sessions [17]. The mean self-care scores in middle-aged and older adults with type 2 diabetes in the online training group were also compared before and after the intervention. Although the study period was short, the results showed there was a significant increase in the scores after the intervention, which suggested that online training was effective for increasing self-care levels. Noahi et al. examined the effect of e-learning on self-care knowledge, attitude, and performance of patients with type 2 diabetes in Kerman, Iran. The results indicated there was a significant difference in the mean scores in the three areas before and after the intervention, as well as a decrease in blood sugar and glycosylated haemoglobin levels after the intervention, which highlighted the effectiveness of online training [16]. A study by Ralston et al. also agreed with the results of the current study [18]. Studies have shown that self-care training programs using new educational models can promote the quality of life of older adults with diabetes type 2. It has been shown that self-care training improved the quality of life of older adults with diabetes, which agreed with the present study [19].

One of the aspects of self-care that was assessed in this study was adherence to the diet. Patients' self-reported adherence to the recommended diet in both groups was significantly improved after training. The self-care score for adherence to diet showed no significant difference between the two groups before the training but was significantly higher in the face-to-face training group after the training was completed. This result reflected the greater effect that face-to-face training had in improving the adherence to a recommended diet of the participants. This result was consistent with the findings reported by Norris et al., [20]. The study by Oshvandi et al. also is in line with the results of this study. The researchers examined the effect of self-care education using the teach-back method on self-care behaviours in patients with type 2 diabetes. The results showed that education increased adherence to diet and other self-care behaviours [21]. In line with the present study, Zamanzadeh et al., reported that

distance learning provided by nurses for patients with diabetes increased adherence to appropriate diets [22]. Another aspect of self-care that was investigated in this study was exercise and physical activity. The self-care scores for the exercise of the patients was determined in both groups before and after training and was found to significantly increase in both groups, which indicated the effectiveness of the training methods. Similar to this study, Naghibi et al., reported that the self-care score associated with exercise increased after training in patients with type 2 diabetes [23]. It has been indicating that self-care training increased a patient's adherence to physical activity, which agreed with the results of this study [24]. This study has some limitations. The small sample size of patients in the two groups was a limitation. Therefore, further larger studies are required to confirm the results. Another limitation was that it was not possible to have a long-term follow-up with the patients. It is recommended that future studies increase the follow-up period of the patients. Other limitations included the low literacy of elderly people with diabetes and the lack of adequate control over mobile phones and the use of social networks.

Based on the results of this study, face-to-face training and online training had the same effect on the level of self-care in middle-aged and older adults with type 2 diabetes. Therefore, face-to-face training and online training can be considered to be effective resources for improving self-care and promoting the health and well-being of elderly and middle age people.

Acknowledgements

The authors express their gratitude to all the participants in the study, as well as to the head of the Diabetes Clinic of Esfarayen, the Hospital of Esfarayen, and Mrs Eydi for their valuable assistance in implementing this project. This paper was released from a Master's thesis approved in 2017 with the project code of IR.nkums.REC.1395.31, deputy of research and technology of North Khorasan University of Medical Sciences and the Esfarayen School of Medical Sciences funded the study.

References

1. Flesner MK. Care of the elderly as a global nursing issue. Nurs Adm Q. 2004; 28(1):67-72. https://doi.org/10.1097/00006216-200401000-00014 PMid:14986512

2. Beard JR, Officer A, de Carvalho IA, Sadana R, Pot AM, Michel JP, et al. The World report on ageing and health: a policy framework for healthy ageing. Lancet. 2016; 387(10033):2145-2154. https://doi.org/10.1016/S0140-6736(15)00516-4

3. Noroozian M. The elderly population in Iran: an ever-growing concern in the health system. Iran J Psychiatry Behav Sci. 2012; 6(2):1-6. PMid:24644476 PMCid:PMC3940007

4. Mousavi SM, Haghi M, Gharasi Manshadi M. Iran's Health System and Readiness to Meet the Aging Challenges. Iran J Public Health. 2015; 44(12):1716-7. PMid:26811829 PMCid:PMC4724751

5. Emami Zeydi A, Jannati Y, Darvishi Khezri H, Gholipour Baradari A, Espahbodi F, Lesani M, et al. Sleep quality and its correlation with serum C-reactive protein level in hemodialysis patients. Saudi J Kidney Dis Transpl. 2014; 25(4):750-5. https://doi.org/10.4103/1319-2442.134962 PMid:24969183

6. Corriere M, Rooparinesingh N, Kalyani RR. Epidemiology of diabetes and diabetes complications in the elderly: an emerging public health burden. Curr Diab Rep. 2013; 13(6):805-13. https://doi.org/10.1007/s11892-013-0425-5 PMid:24018732 PMCid:PMC3856245

7. Gerber BS, Brodsky IG, Lawless KA, Smolin LI, Arozullah AM, Smith EV, et al. Implementation and evaluation of a low-literacy diabetes education computer multimedia application. Diabetes Care. 2005; 28(7):1574-80. https://doi.org/10.2337/diacare.28.7.1574 PMid:15983303

8. Kharroubi AT, Darwish HM. Diabetes mellitus: The epidemic of the century. World J Diabetes. 2015; 6(6):850-67. https://doi.org/10.4239/wjd.v6.i6.850 PMid:26131326 PMCid:PMC4478580

9. Mojalli M, Karimi Moonaghi H, Khosravan S, Mohammadpure A. Dealing with coronary artery disease in early encountering: a qualitative study. Int Cardiovasc Res J. 2014; 8(4):166-70. PMid: 25614861

10. Mitnitski A, Song X, Rockwood K. Improvement and decline in health status from late middle age: modeling age-related changes in deficit accumulation. Exp Gerontol. 2007; 42(11):1109-15. https://doi.org/10.1016/j.exger.2007.08.002 PMid:17855035

11. Alberti KG, Zimmet P, Shaw J. International Diabetes Federation: a consensus on Type 2 diabetes prevention. Diabet Med. 2007; 24(5):451-63. https://doi.org/10.1111/j.1464-5491.2007.02157.x PMid:17470191

12. Harding JL, Pavkov ME, Magliano DJ, Shaw JE, Gregg EW. Global trends in diabetes complications: a review of current evidence. Diabetologia. 2019; 62(1):3-16. https://doi.org/10.1007/s00125-018-4711-2 PMid:30171279

13. McDonnell ME. Telemedicine in Complex Diabetes Management. Curr Diab Rep. 2018; 18(7):42. https://doi.org/10.1007/s11892-018-1015-3 PMid:29797292

14. Shrivastava SR, Shrivastava PS, Ramasamy J. Role of self-care in management of diabetes mellitus. J Diabetes Metab Disord. 2013; 12(1):14. https://doi.org/10.1186/2251-6581-12-14 PMid:23497559 PMCid:PMC3599009

15. Hamadzadeh S, Ezatti ZH, Abedsaeidi ZH, Nasiri N. Coping Styles and Self-Care Behaviors among Diabetic Patients. Iran Journal of Nursing. 2013; 25(80):24-33.

16. Nouhi E, Khandan M, Mirzadeh A. Effective of electronic education on knowledge attitude and self-care in patient's diabetic type 2 refer to diabetic center of Kerman University of medical science. Iranian Journal of Nursing Research. 2011; 6(22):73-80.

17. Azizi M, Arsalani N, Mohammadi Shahboulaghi F, Hosseinzadeh S, Rajab A. The effect of self-care education on the control of diabetes complications, medications and HbA1C in adolescents with type 1 diabetes. Hayat. 2017; 22(4):350-61.

18. Ralston JD, Hirsch IB, Hoath J, Mullen M, Cheadle A, Goldberg HI. Web-based collaborative care for type 2 diabetes: a pilot randomized trial. Diabetes Care. 2009; 32(2):234-9. https://doi.org/10.2337/dc08-1220 PMid:19017773 PMCid:PMC2628685

19. Kargar Jahromi M, Ramezanli S, Taheri L. Effectiveness of diabetes self-management education on quality of life in diabetic elderly females. Glob J Health Sci. 2014; 7(1):10-5.

https://doi.org/10.5539/gjhs.v7n1p10 PMid:25560339 PMCid:PMC4796384

20. Norris SL, Engelgau MM, Narayan KM. Effectiveness of self-management training in type 2 diabetes: a systematic review of randomized controlled trials. Diabetes Care. 2001; 24(3):561-87. https://doi.org/10.2337/diacare.24.3.561 PMid:11289485

21. Oshvandi K, Jokar M, Khatiban M, Keyani J, Yousefzadeh MR, Sultanian AR. The effect of self care education based on teach back method on promotion of self care behaviors in type ii diabetic patients: a clinical trial study. Iranian Journal of Diabetes and Lipid Disorders. 2014; 13(2):131-43.

22. Zamanzadeh V, Zirak M, Maslakpak MH, Parizad N. Distance education and diabetes empowerment: A single-blind randomized control trial. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2017; 11:S247-S251. https://doi.org/10.1016/j.dsx.2016.12.039 PMid:28034693

23. Naghibi SA, Asghari M, Rostami F. Investigation the Effect of Education on Self-Care Promotion in Type 2 Diabetic Patients in Noor Health Centers in 2015. Journal Of Health Research In Community. 2015; 1(2):22-8.

24. 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. PMid:28569983

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Apr 15; 7(7):1220-1225.
https://doi.org/10.3889/oamjms.2019.260
eISSN: 1857-9655
Public Health

The Effect of the Nursing Care Model Based on Culture to Improve the Care of Malnourished Madurese Children in Indonesia

Abdul Aziz Alimul Hidayat*, Musrifatul Uliyah

Department of Nursing, University of Muhammadiyah Surabaya, 60113, Surabaya, Indonesia

Citation: Hidayat AAA, Uliyah M. The Effect of the Nursing Care Model Based on Culture to Improve the Care of Malnourished Madurese Children in Indonesia. Open Access Maced J Med Sci. 2019 Apr 15; 7(7):1220-1225. https://doi.org/10.3889/oamjms.2019.260

Keywords: Health; Malnutrition; Nursing; Transcultural nursing

***Correspondence:** Abdul Aziz Alimul Hidayat. Department of Nursing, University of Muhammadiyah Surabaya, 60113, Surabaya, Indonesia. E-mail: azizhidayat@um-surabaya.ac.id

Received: 27-Mar-2019; **Revised:** 02-Apr-2019; **Accepted:** 03-Apr-2019; **Online first:** 14-Apr-2019

Funding: This research did not receive any financial support

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

Abstract

AIM: The purpose of this study is to analyse the usage of nursing care model based on culture to improve parenting practices related to malnutrition among Madurese children.

METHODS: This study uses a quasi-experimental design and a purposive sample of 102 respondents from families with toddlers who suffered from nutritional deficiency. The sample consisted of an intervention group (n = 51) and a control group (n = 51). Data were collected using a questionnaire and weight measurement.

RESULTS: A t-test found a difference between all of the variables of care, including health technology utilization, when comparing the following variables between the intervention group and the control group ($t = 14.12$, $p < 0.001$), beliefs and philosophy ($t = 10.20$, $p < 0.001$), cultural values and lifestyle ($t = 13.63$, $p < 0.001$), economic reasons ($t = 0.20$, $p = 0.837$), nursing action response based on culture ($t = 11.28$, $p < 0.001$), and care behaviors for children ($t = 16.43$, $p < 0.001$). The Wilcoxon signed-rank test found a difference between pre-intervention nursing care model based on culture and post-intervention nursing care model based on culture regarding the variable malnutrition status ($t = 16.43$, $p < 0.001$).

CONCLUSION: This study found that the application of nursing care model based on culture affects care practices. Nursing care model based on culture can be applied to families with toddlers who are malnourished because of the lack of a culture of care.

Introduction

The incidence of malnutrition and poor nutrition in Indonesia is based on research into basic health with indicators of weight for age (W/A) and stands at 18.4%. This is a high incidence compared to other Southeast Asian countries. The Madurese are members of a tribe in Indonesia spread over four districts. One is Sumenep, which has an 8.6% incidence of overall malnutrition and a 20.9% malnutrition rate among children.

The incidence is high because the Madurese are a patriarchal society in which women do not have significant positions which can be seen from the weak bargaining position of women relative to men. Consequently, women do not have access to health care even when they are pregnant or postpartum. The Madurese trust the dukun (traditional medicine) more than the health care workers. They trust the shaman and the advice of parents or elders. Moreover, most Madurese still live with their relatives (especially parents), so treatment decisions depend on parents or relatives. The Madurese culture prohibits foods derived from animal sources (eggs and fish) and vegetables (pineapple and eggplant) during pregnancy. These restrictions have a

negative impact on infant growth and the nutritional status of children [1].

Also, it is the culture of the Madurese mothers to feed bananas mixed with rice to babies at the age of one week [2]. It is assumed that if a child starves, they were not given bananas mixed with rice. To make it worse, it is the culture of the Madurese that when a child is sick, the mother takes medicine. Finally, the Madurese would feed the husband rather than the children because the husband is considered the breadwinner [3]. These problems are also prevalent in Cameroon where the incidence of infant malnutrition is also due to sociocultural factors, among others, improper feeding, lack of the mother's knowledge, lack of access and the distribution of health services [4]. These problems have also been prevalent in Iran among children aged 0-4 years who were found to have nutritional problems due to the mother's education, behaviour and parenting practices [5]. Kenya also has a similar problem with the cultural influence on feeding practices among children under the age of four [6]. Benin also has the same problem where there is a relationship between ethnicity and malnutrition among children [7].

To solve the problems above, we may turn to the application of nursing care models based on culture (transcultural nursing). This model is a form of humanistic nursing care focusing on the behaviour of individuals and groups as well as the process involved in maintaining or improving the behavior of health or alleviating pain both physically and psychoculturally according to the cultural background.

The implementation of transcultural nursing applies nursing interventions through three strategies: to retain, restructure, and negotiate. Transcultural nursing in Indonesia has not been implemented based on the local cultures; in particular, addressing the problem of malnutrition is necessary due to the culture of parenting that does not improve health. The purpose of this study is to describe the influence of nursing care model based on culture in improving malnutrition parenting practices for Madurese children.

Methods

Study design

The study used a quasi-experimental study to examine the effects of the Nursing Care Model based on Culture to Improve the Care of Malnourished Madurese Children (utilization of health care technology, beliefs and philosophies, cultural values and lifestyles, economic reasons, nursing response based on culture, child care behavior, and malnutrition status) in Indonesia

Setting and sample

This is a quasi-experimental study. The research sample consisted of 102 families of which there are 52 families in the treatment group (Group A) and 50 families in the control group (Group B). The malnourished children were located using the community health centre medical database. The children were selected based on inclusion criteria such as Madurese ethnicity, under the age of 5 and supportive family which can correspond in Bahasa Indonesia (writing and reading). The data on malnourished children were obtained from five community health centres in Sumenep district. The children were divided into control and treatment groups based on the data obtained. The sample inclusion criteria were families with children with poor nutrition.

Instruments

The transcultural nursing result was measured through a questionnaire regarding child care practices with cultural approaches consisting of the utilisation of medical technology, beliefs and philosophies of health, family and societal dependency, cultural values, caring styles, economic aspects and responses to transcultural and caring practices. The average score was measured.

The research instrument was composed of 50 questions using a Likert scale with the following responses: 1 = never, 2 = rarely, 3 = sometimes, 4 = often, and 5 = always.

Procedure and Data collection

This study used a purposive sampling technique. The data were obtained from questionnaires of child care practice with cultural approaches consisting of the utilisation of medical technology, beliefs and philosophies of health, family, societal dependency, cultural values, caring style, economic aspects and responses to transcultural and caring practices. The respondents provided informed consent before the study. This study was conducted over six months in 2016 at a community health centre in Sumenep district, East Java province, Indonesia.

The intervention group received the stages of the nursing care model based on culture (transcultural nursing) for two months. The subsequent stages were as follows: (1) the stage of nursing assessment based on culture was conducted for 60 minutes with the collection and identification of cultural issues related to health problems; (2) the preparation phase of the action plans, goals and

criteria results was carried out for 60 minutes and established the plans, objectives and outcomes of actions to be implemented; (3) reinforcing the idea that the culture does not conflict with the treatment of malnutrition; (4) cultivating cultural negotiation in the treatment of malnutrition; and (5) fostering cultural restructuring in the treatment of malnutrition if the culture of care is against the principles of health. Transcultural nursing is a care method that includes cultural approaches such as the cultural strengthening of nursing values unrelated to malnutrition, negotiation of nursing culture to overcome malnutrition and the restructuring of malnutrition handling culture that is not in line with medical service principals [8].

Data analysis

All of the data were loaded into the Statistical Package for the Social Sciences (SPSS) version 19 and analysed using statistical methods. Statistical analysis used a t-test for data with normal distribution and the Mann-Whitney test for non-normal data distribution. The questionnaire's validity and reliability were tested with Cronbach alpha (0.83).

Ethical consideration

This study was approved by the Human Research Protections Program, Institutional Review Board, M University of Surabaya, No. 004071017.

Results

Demographic characteristics

Table 1 shows the characteristics of the mothers and their malnourished children.

Table 1: Sample Characteristics

Variables	Intervention group (N = 51) (n, %)		Control group (N = 51) (n, %)		Test of group differences
Mother's age					$\chi^2 = 1.921$
< 20 years	3	(5.9)	1	(2.0)	$p = 0.860$
21-25 years	16	(31.4)	18	(35.3)	
26-30 years	8	(15.7)	9	(17.6)	
31-35 years	16	(31.4)	14	(27.5)	
36-40 years	5	(9.8)	4	(7.8)	
> 40 years	3	(5.9)	5	(9.8)	
Mother's education					$\chi^2 = 1.765$
Never attended school	1	(2.0)	3	(5.9)	$p = 0.623$
Did not complete primary school	12	(23.5)	9	(17.6)	
Primary school	25	(49.0)	28	(54.9)	
Junior high school	13	(25.5)	11	(21.6)	
Senior high school	0	(0.0)	0	(0.0)	
University	0	(0.0)	0	(0.0)	
Mother's job					$\chi^2 = 3.076$
Unemployed/homemaker	1	(2.0)	4	(7.8)	$p = 0.380$
Farmer	33	(64.7)	30	(58.8)	
Farmworker	14	(27.5)	16	(31.4)	
Government servant	3	(5.9)	1	(2.0)	
Child's gender					$\chi^2 = 0.056$
Male	11	(21.6)	12	(23.5)	$p = 0.813$
Female	40	(78.4)	39	(76.5)	
Child's age					$\chi^2 = 0.065$
0-1 months	0	(0.0)	0	(0.0)	$p = 0.799$
< 2-6 months	0	(0.0)	0	(0.0)	
< 7-12 months	9	(17.6)	10	(19.6)	
> 12 months	42	(82.4)	41	(80.4)	

The mothers were mostly between 21 and 25 years old (33.3%), while some of them were less than 20 years old (4.20%). Some of them received primary school education (45.0%), but a percentage of them had never attended school (4.20%). The mothers worked mostly as farmers (54.0%), and some of them were civil servants (4.0%). Most of the malnourished children were girls (79.2%), and they were more than one year old (83.3%). Some of them were between 7-12 months (16.7%).

Impact of the nursing care model base on cultural to budaya perawatan

Table 2 shows that after the two-month culture-based nursing intervention showed that the nursing care model based on culture had a statistically significant influence in increasing the utilisation of health care technology, compared to the control group ($t = 14.12$, $p < 0.001$). Participants felt that the belief of child care by utilising local culture would succeed after the intervention compared to the control group ($t = 10.20$, $p < 0.001$). Cultural values and manifestations improved after intervention compared to the control group ($t = 13.63$, $p < 0.001$). For economic reasons, there was no significant influence between the intervention group and the control group in improving child care ($t = 0.20$, $p = 0.837$). Participants also showed good nursing response based on culture after intervention compared to the control group ($t = 11.28$, $p < 0.001$). For child care behaviour experiencing positive behavioural enhancement, statistically, there was a significant difference between the intervention group and the control group ($t = 16.43$, $p < 0.001$).

Table 2: Comparison of Pre-Test and Post-Test Scores on the Culture-Based Health Care Variables of Children Between the Intervention and Control Groups

Culture-based health care variables	Pre-intervention Mean ± SD	t	*p*	Post-intervention Mean ± SD	t	*p*
Utilization of health care technology						
Intervention group (n = 51)	13.84 ± 6.24	1.36	0.175	29.53 ± 6.53	14.12	< 0.001
Control group (n = 51)	12.24 ± 5.62			12.49 ± 5.61		
Beliefs and philosophies						
Intervention group (n = 51)	16.27 ± 5.69	0.89	0.375	27.92 ± 5.39	10.20	< 0.001
Control group (n = 51)	15.33 ± 4.94			16.71 ± 5.69		
Cultural values and lifestyles	16.27 ± 5.69	0.43	0.668	32.27 ± 5.69	13.63	< 0.001
Intervention group (n = 51)	15.80 ± 5.35			16.80 ± 5.76		
Control group (n = 51)						
Economic reasons						
Intervention group (n = 51)	15.80 ± 5.35	0.06	0.864	31.80 ± 5.35	0.20	0.837
Control group (n = 51)	16.00 ± 6.14			16.04 ± 6.12		
Nursing response based on culture						
Intervention group (n = 51)	16.00 ± 6.14	0.43	0.109	31.69 ± 6.13	11.28	< 0.001
Control group (n = 51)	17.84 ± 5.34			18.67 ± 5.49		
Child care behavior						
Intervention group (n = 51)	17.84 ± 5.34	0.15	0.057	33.84 ± 5.34	16.42	< 0.001
Control group (n = 51)	15.80 ± 5.35			16.27 ± 5.45		

Impact of the nursing care model based on cultural to malnutrition status

Table 3 shows that the two-month culture-based nursing intervention showed statistically significant differences in malnutrition status between

the intervention group and the control group (t = 16.43, p < 0.001). Data shows that in the intervention group there is a change in malnutrition status. Before the intervention, there was no respondent with normal nutrition status. However, after the intervention there were 39 respondents (76.5%) who were malnourished became normal. Thus, the percentage of respondents with mild malnutrition decreased from (60.8%) to (11.8%) while those with moderate malnutrition decreased from (37.3%) to (9.8%). This is different in the control group where there was no significant change in the number of respondents with mild malnutrition and moderate malnutrition status.

Table 3: Comparison of Nutritional Status Before and After Intervention in the Intervention Group (n = 51) and the Control Group (n = 51)

Malnutrition status	Intervention group				Control group			
	Pre-intervention		Post-intervention		Pre-intervention		Post-intervention	
	n	%	n	%	n	%	n	%
Normal	0	0.0	39	76.5	0	0.0	2	3.9
Mild malnutrition	31	60.8	6	11.8	29	56.9	29	56.9
Moderate malnutrition	19	37.3	5	9.8	21	41.2	19	37.3
Severe malnutrition	1	2.0	1	2.0	1	2.0	1	2.0

Wilcoxon signed-rank test $p < .001$; Wilcoxon signed-rank test $p = .697$.

Discussion

All of the variables in the post-test, after application of the nursing model for three months, differed between the intervention group and the control group. Consequently, we concluded that the nursing model could improve parenting practices among Madurese mothers concerning nutrition. The results show that the transcultural nursing strategy can be used as an alternative to managing malnutrition among Madurese children. This was proven by the t-test values of all of the variables (p < 0.05). This suggests that the care culture factor is a health issue in the community that must be addressed especially among children in rural areas. Culture-based nursing care (transcultural nursing) can encourage families to change aspects of their culture that are unhealthy.

Leininger stated that transcultural nursing could be used in three ways: it can (1) maintain a positive culture, (2) conduct negotiations or accommodate the culture if the culture does not conflict with health care, and (3) restructure the culture if the culture causes health problems [9], [10]. In the context of Madurese children with malnutrition, all three approaches are applicable. We can maintain the family commitment to the child and encourage the family to continue using health care centre facilities and health posts held by clinic nurses. Cultural negotiations can be carried out by approaching the village leaders, clerics and villagers and offering health cadres in the form of meetings and health education [11], [12], [13].

Approaching clerics is a priority because the Madurese respect them very much. Cultural restructuring can be accomplished through empowerment programs for families and community by engaging in various social activities, educational opportunities and training activities for mothers who have children with malnutrition [14].

Also, family behaviours concerning health care are inherited from generation to generation. Habits that are “normal” can be changed if they are unhealthy. In this context, transcultural nursing can play a critical role in challenging pre-existing beliefs and encouraging positive parenting behaviour [15], [16], [17].

Several variables in this study changed, including the use of health care technology. Post-treatment intervention-based culture changed certain aspects of respondents’ perceptions of the use of technology to overcome current health problems, search for health assistance, the perception of sickness, treatment habits or coping health problems, among others. Trust and confidence after post-treatment intervention-based culture also changed. This is a belief system that includes human beliefs and images regarding the nature of God or that are considered God and regarding the supernatural. Religion influences the way a person strives to prevent disease and plays a strong role in rituals related to preventive health care. Religion outlines moral, social and dietary practices designed to keep adherents healthy and in a balanced state. Religion also plays an important role in the perception of disease prevention in its adherents. For example, for the majority of Muslims in the Madurese tribe, one of the alternatives to treatment is prayer [18]. Praying contains deep psychotherapeutic elements. Psychoreligious therapy is no less important than psychotherapy and psychiatry because praying contains spiritual powers that generate confidence and feelings of optimism (hope of healing). Self-confidence and optimism are essential for healing a disease or illness in addition to drugs and medical treatment

Changes in culture, values and lifestyles post-intervention are necessary because values are abstract concepts regarding ideas that are considered good or bad. Cultural values that are considered good and bad are formulated and defined by a culture’s adherents. These changes can improve human health via culture-based care. Several views related to transcultural nursing occurred in malnourished Madurese children, especially regarding cultural care preservation or maintenance. These include breastfeeding babies and the prohibition on taking infants outside for 40 days after birth. Cultural care accommodation or negotiation also includes less than 2 years of breastfeeding, and regarding cultural care

re-patterning or restructuring, feeding newborns with honey, formula and bananas and discarding the first milk [9].

Cultural care from Leininger's perspective on the health of children (aged 0-2 years) can provide an action plan for cultural care maintenance or preservation, including the habit of breastfeeding a baby and give formula milk, and culture related to the prohibition on taking infants outside for 40 days after birth. Breastfeeding for less than 2 years can be considered a cultural care accommodation or negotiation action plan. Regarding feeding newborns with honey, formula and bananas and discarding the first milk, postpartum/breastfeeding mothers are subjected to cultural care re-patterning or restructuring because these actions are considered detrimental to infant health [17], [19].

Cultural care is the cognitive ability to know the values, beliefs, and expression patterns that guide, support, or provide opportunities for other individuals or groups to maintain their health and improve their living conditions [20]. The purpose of transcultural nursing is to bridge the care system using by the general public with professional care through nursing care.

Thus, in transcultural nursing, nurses must be able to make nursing decisions and action plans by focusing on three principles, among them cultural care preservation or maintenance, namely the principle of helping, facilitating, or focusing on cultural phenomena to enable individuals to determine their desired lifestyle and level of health [20]. Cultural care accommodation or negotiation, namely the principles of negotiation, support, assistance or focus on cultural phenomena that reflect ways to adapt, allow creative professional action and decisions to assist clients from the culture designated to negotiate or consider health and lifestyle conditions, and cultural care repatterning or restructuring, which is the principle of reconstructing or changing to improve the health conditions and lifestyles of clients [20]. Cultural restructuring is carried out if the culture is detrimental to health status [21].

In conclusion, the nursing care model based on culture is a model-based approach to culture that plays an important role in overcoming child health problems in the community, especially when cultural factors are conflicting with healthy principles. This approach can be used in developing countries where there are many isolated regions with few accessible health care facilities and workers. Transcultural nursing can reduce infant morbidity due to malnutrition, especially among populations who maintain a local culture that is conflicting with healthy principles. Even a three-months program can make a significant difference.

References

1. Barroso MM, Salvador LM, Fagundes Neto U. Severe protein-calorie malnutrition in two brothers due to abuse by starvation. Rev Paul Pediatr. 2016; 34(4):522-7. PMid:27452429 PMCid:PMC5176076

2. Ahmad B, Anam N, Khalid N, Mohsen R, Zaal L, Jadidy E, et al. Perceptions of women of reproductive age about vitamin and folic acid supplements during pregnancy, Taibah University, Almadinah Almunawwarah, Kingdom of Saudi Arabia. Journal of Taibah University Medical Sciences. 2013; 8(3):199-204. https://doi.org/10.1016/j.jtumed.2013.08.002

3. Hanafi MI, Hamid Shalaby SA, Falatah N, El-Ammari H. Impact of health education on knowledge of, attitude to and practice of breastfeeding among women attending primary health care centres in Almadinah Almunawwarah, Kingdom of Saudi Arabia: Controlled pre-post study. Journal of Taibah University Medical Sciences. 2014; 9(3):187-93. https://doi.org/10.1016/j.jtumed.2013.11.011

4. Pemunta NV, Fubah MA. Socio-cultural determinants of infant malnutrition in Cameroon. J Biosoc Sci. 2015; 47(4):423-48. https://doi.org/10.1017/S0021932014000145 PMid:24717356

5. Noughani F, Bagheri M, Ramim T. Nutritional habits of mothers and children in the age group 0-4 years in Iran. Ecol Food Nutr. 2014; 53(4):410-8. https://doi.org/10.1080/03670244.2013.824434 PMid:24884555

6. Chege PM, Kimiywe JO, Ndungu ZW. Influence of culture on dietary practices of children under five years among Maasai pastoralists in Kajiado, Kenya. Int J Behav Nutr Phys Act. 2015; 12:131. https://doi.org/10.1186/s12966-015-0284-3 PMid:26450270 PMCid:PMC4597609

7. Chague F, Varloteaux M, Renaud C, Brune V, Enel C, Stoffel V. [Relations between ethnicity and child malnutrition in rural Benin]. Med Sante Trop. 2013; 23(3):337-43. PMid:24161528

8. Cukljek S, Juresa V, Babic J. The cross-cultural (transcultural) adaptation and validation of the nursing image questionnaire. Nurse Education Today. 2017; 48:67-71. https://doi.org/10.1016/j.nedt.2016.09.006 PMid:27718387

9. McFarland MR, Wehbe-Alamah HB. Leininger's culture care diversity and universality: Jones & Bartlett Publishers, 2014.

10. Giger JN. Transcultural Nursing-E-Book: Assessment and Intervention: Elsevier Health Sciences, 2016.

11. Vu-Augier de Montgremier M, Blanchet-Collet C, Guzman G, Moro MR. [Towards a transcultural approach to eating disorders]. Soins Psychiatr. 2016; 37(307):22-4. https://doi.org/10.1016/j.spsy.2016.09.006 PMid:27890271

12. Bjarnason D, Mick J, Thompson JA, Cloyd E. Perspectives on Transcultural Care. Nursing Clinics of North America. 2009; 44(4):495-503. https://doi.org/10.1016/j.cnur.2009.07.009 PMid:19850185

13. Im E-O, Lee Y. Transcultural Nursing: Current Trends in Theoretical Works. Asian Nurs Res. 2018; 12(3):157-65. https://doi.org/10.1016/j.anr.2018.08.006 PMid:30179700

14. Erika KA. The Effect of Transcultural Nursing, Child Healthcare Model and Transtheoretical Model Approaches to Knowledge and Culture of Family. Jurnal Ners. 2016; 9(2):8. https://doi.org/10.20473/jn.V9I22014.262-269

15. Law K, John W. Homelessness as culture: How transcultural nursing theory can assist caring for the homeless. Nurse Educ Pract. 2012; 12(6):371-4. https://doi.org/10.1016/j.nepr.2012.04.010 PMid:22658675

16. Pulido-Fuentes M, González LA, Martins MdFdSV, Martos JAF. Health Competence from a Transcultural Perspective. Knowing how to Approach Transcultural Care. Procedia - Social and Behavioral Sciences. 2017; 237:365-72. https://doi.org/10.1016/j.sbspro.2017.02.022

17. Lor M, Crooks N, Tluczek A. A proposed model of person-, family-, and culture-centered nursing care. Nurs Outlook. 2016;

64(4):352-66. https://doi.org/10.1016/j.outlook.2016.02.006 PMid:27061841

18. Drevdahl DJ. Impersonating culture: The effects of using simulated experiences to teach cultural competence. Journal of Professional Nursing. 2018; 34(3):195-204. https://doi.org/10.1016/j.profnurs.2017.10.006 PMid:29929800

19. Ogino M. Transcultural aspects. Journal of the Neurological Sciences. 2017; 381:18. https://doi.org/10.1016/j.jns.2017.08.084

20. Leininger M. Culture care theory: a major contribution to advance transcultural nursing knowledge and practices. J Transcult Nurs. 2002; 13(3):189-92; discussion 200-1. https://doi.org/10.1177/10459602013003005 PMid:12113148

21. Mixer SJ, Carson E, McArthur PM, Abraham C, Silva K, Davidson R, et al. Nurses in Action: A Response to Cultural Care Challenges in a Pediatric Acute Care Setting. J Pediatr Nurs. 2015; 30(6):896-907. https://doi.org/10.1016/j.pedn.2015.05.001 PMid:26072213

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Apr 15; 7(7):1226-1232.
https://doi.org/10.3889/oamjms.2019.289
eISSN: 1857-9655
Review Article

DOACs vs Vitamin K Antagonists: a Comparison of Phase III Clinical Trials and a Prescriber Support Tool

Alina-Maria Pirlog[1*], Cristian Daniel Pirlog[2], Marius Adrian Maghiar[3]

[1]University of Oradea, Faculty of Medicine and Pharmacy, Department of General Medicine, Oradea, Romania; [2] University of Oradea, Faculty of Medicine and Pharmacy, Department of Dentistry, Oradea, Romania; [3]University of Oradea, Faculty of Medicine and Pharmacy, Department of General Surgery, Oradea, Romania

Citation: Pirlog AM, Pirlog CD, Maghiar MA. DOACs Vs Vitamin K Antagonists - A Comparison of Phase III Clinical Trials and A Prescriber Support Tool. Open Access Maced J Med Sci. 2019 Apr 15; 7(7):1226-1232. https://doi.org/10.3889/oamjms.2019.289

Keywords: DOACs; Warfarin; Atrial Fibrillation; Oral anticoagulants; Stroke prevention

***Correspondence:** Alina-Maria Pirlog. University of Oradea, Faculty of Medicine and Pharmacy, Department of General Medicine, Oradea, Romania. E-mail: drpirlog.alina@yahoo.co.uk

Received: 13-Feb-2019; **Revised:** 07-Apr-2019; **Accepted:** 08-Apr-2019; **Online first:** 14-Apr-2019

Funding: This research did not receive any financial support

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

Abstract

AIM: The purpose of this article was to systematically review the literature assessing the efficacy and safety of phase III clinical trials for each direct oral anticoagulant versus vitamin K antagonists and to design a ''go-to'' table for the prescriber.

MATERIAL AND METHODS: A systematic review of specialist literature was conducted to identify RCTs which compared direct oral anticoagulants (DOACs) with standard warfarin treatment. Medline, Em-base, and the Cochrane databases were searched from January 2005- January 2019. The inclusion criteria were randomised controlled trials of oral anticoagulants in patients with non-valvular atrial fibrillation (NVAF). Four publications were phase III randomised control trials (RCTs) included in the final analysis.

RESULTS: Regarding the primary outcome in RELY the results were 1.69% per 100-year patients (p/y) for Warfarin compared to 1.11% p/y dabigatran etexilate 150mg BD (twice daily). In ROCKET AF the rates of the primary outcome were 2.2% p/y for warfarin compared to 1.7% p/y for rivaroxaban 20 mg OD (once daily). In ARISTOTLE trial the rates of the primary outcome were 1.60% p/y for warfarin compared to 1.27% p/y for apixaban 5 mg BD. In ENGAGE AF TIMI, the rates of the primary outcome were 1.50% p/y for warfarin compared to 1.18% p/y for edoxaban 60mg BD.

CONCLUSION: DOACs showed to be either noninferior or superior to warfarin with regards to the primary outcome with better safety patterns. Our ''go-to'' table provides a supportive tool for physicians in preventing medical errors when managing patients on oral anticoagulants.

Introduction

Atrial Fibrillation (AF) is one of the leading causes of cardiovascular morbidity and mortality worldwide [1]. The incidence and prevalence of AF have been increasing in recent years up to the point that one in four middle-aged adults in Europe and the US will develop this common cardiac arrhythmia [2], [3]. The above numbers reflect a growing number of patients requiring anticoagulants for stroke prevention.

The clinical management of patients with non-valvular AF (NVAF) has improved in recent years with the introduction of direct oral anticoagulant agents (DOACs) [4]. In the last decade, the four DOACs: dabigatran etexilate, rivaroxaban, apixaban and edoxaban have been used for the prevention of stroke and systemic embolism for people with NVAF with one or more risk factors: prior stroke or transient ischaemic attack; age 75 years or older; hypertension; diabetes mellitus; symptomatic heart failure [5].

Several characteristics distinguish DOACs from vitamin K antagonists (VKAs): rapid onset of action (1-3 h), do not require bridging with parenteral anticoagulants and there is no need for routine monitoring of anticoagulation. Additionally, DOACs have similar (7-15 h) half-lives and are partially eliminated by the kidney: 85% of dabigatran etexilate, 50% of edoxaban, 33% of rivaroxaban, and 27% of apixaban [5]. Patients who are taking Warfarin should be aware of the potential risks and benefits of switching to DOACs and their level of international

normalised ratio (INR) control taken into consideration when switching between anticoagulants [5], [6].

The availability of several drugs with similar efficacy and safety for stroke prevention in NVAF patients offers a selection for prescribers and users. Consequently, prescribers should have a good knowledge of these agents' characteristics and the trials in which their use was established to counsel and care for the growing number of patients on oral anticoagulants.

The decision to take lifelong drugs such as oral anticoagulants should be made in collaboration between a patient and their doctor after an informed discussion about the risks and benefits of all the different drugs [7]. Medical professionals, particularly busy general practitioners/family doctors can find difficulties in keeping up to date with all the current guidelines and the new emerging drugs used in medical practice. If prescribers are better informed, then they can proficiently counsel their patients and collaborate with them when initiating an oral anticoagulant.

The purpose of this article was to systematically review the literature assessing the efficacy and safety of phase III clinical trials for each DOAC versus VKAs used in stroke prevention in patients with NVAF. Also, it aimed to design a ''go-to'' table for the prescriber to make an informed decision when comparing the oral anticoagulant drugs.

Material and Methods

A systematic review of specialist literature was conducted to identify phase III randomised control trials (RCTs) in patients receiving DOACs compared with standard warfarin treatment. Medline, Embase, and the Cochrane databases were searched from January 2005- January 2019 with no language restrictions using medical keywords to identify RCTs including ''Dabigatran'', ''Rivaroxaban'', ''Apixaban'', ''Edoxaban'', ''Atrial Fibrillation'', ''Humans'', ''Randomized Controlled Trial''. After combining the results and removing duplicates, the titles and abstracts were screened in 50 studies (Figure 1).

The full text of eight publications was retrieved and evaluated for eligibility, and four articles were phase III RCTs included in the final analysis. Studies had to meet the following inclusion criteria: randomised controlled trials of VKAs and DOACs in patients with NVAF. The research was excluded if patients were not followed up, if these were not randomised trials, and if papers were guidelines or any expert opinions.

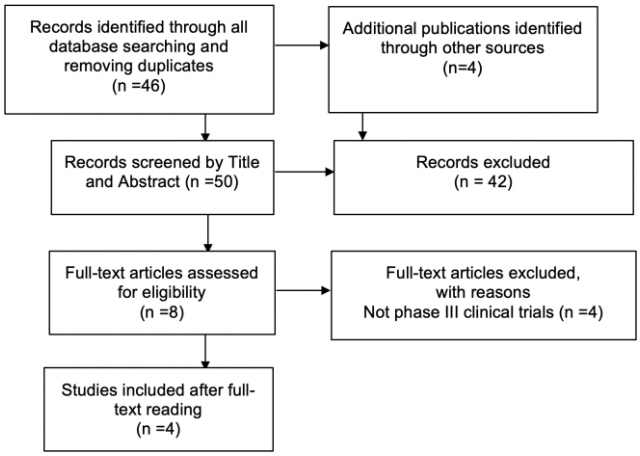


Figure 1: Prisma flow diagram illustrating the study selection process

Results

Four phases III clinical trials were evaluated, and in Table 1 and Table 2 we have compared the characteristics of phase III clinical trials for each DOAC that was on the market at the time of this study. Due to the heterogeneity of the key parameters, analysis of the statistical data was not attempted.

Table 1: Phase III clinical trials NOACs purpose and specific data characteristics

Study Name	RELY 2009	ROCKET AF 2011	ARISTOTLE 2012	ENGAGE AF TIMI 48 2013
Purpose	Dabigatran etexilate 150 mg BD or 110 mg BD to open-label dose adjusted Warfarin	Rivaroxaban 20 mg OD to dose-adjusted Warfarin	Apixaban 5 mg BD to dose-adjusted Warfarin	Edoxaban 30 mg OD and 60 mg OD to dose-adjusted Warfarin
Method-all were Prospective randomised pivotal phase III clinical trial	unblinded	double-blind, double-dummy	double-blind, double-dummy	double-blind, double-dummy
Number of patients	18113	14246	18201	21105
Follow up (years)	2	1.9	1.8	2.8
CHADS2	2.1	3.5	2.1	2.8
TTR (%)	64%	55%	62%	68%
Females (%)	37%	39%	35%	37%
Age mean (years)	71	73	70	72
Jadad score	3	5	5	5

RE-LY (Randomised Evaluation of Long-Term Anticoagulation Therapy); ROCKET AF (Rivaroxaban Once Daily Oral Direct Factor Xa Inhibition Compared with Vitamin K Antagonism for Prevention of Stroke and Embolism Trial in AF); ARISTOTLE (Apixaban for Reduction in Stroke and Other Thromboembolic Events in AF); ENGAGE AF-TIMI 48 (The Effective Anticoagulation with Factor Xa Next Generation in AF- Thrombolysis in Myocardial Infarction 48).

ROCKET-AF (rivaroxaban), ARISTOTLE (apixaban) and ENGAGE AF TIMI (edoxaban) were double-blind double-dummy trials. RELY (dabigatran etexilate) and ARISTOTLE trials had a similar number of patients of approximately 18100. The follow-up period in all trials ranged from 1.8-2.8 years. RELY and ARISTOTLE participants had an equal CHADS2

score of 2.1. ENGAGE AF TIMI and ROCKET AF participants had a CHADS2 score of 2.8 and 3.5, respectively. Time in the therapeutic range INR (TTR) varied from 64%, 55%, 62% and 68% respectively.

Table 2: Phase III clinical trials DOACs efficacy and bleeding rates

Study name and purpose	Primary Outcome	Bleeding/Mortality of 100 patients per year
RE-LY 2009	Rates of the primary outcome (stroke or systemic embolism): 1.69% p/y warfarin 1.53% p/y dabigatran etexilate 110 mg BD-noninferiority 1.11% p/y dabigatran etexilate 150 mg BD-superiority	The rate of major bleeding: 3.36% p/y warfarin 2.71% p/y dabigatran etexilate 110 mg BD 3.11% p/y dabigatran etexilate 150 mg BD The rate of hemorrhagic stroke: 0.38% p/y warfarin 0.12% p/y dabigatran etexilate 110 mg BD 0.10% p/y dabigatran etexilate 150 mg BD The mortality rate: 4.13%p/y warfarin 3.75% p/y dabigatran etexilate 110 mg BD 3.64% p/y dabigatran etexilate 150 mg BD
ROCKET AF 2011	Rates of the primary outcome (stroke or systemic embolism): 2.2 %p/y warfarin 1.7% p/y rivaroxaban 20 mg OD noninferiority	The rate of nonmajor bleeding: 14.5% p/y warfarin 14.9% p/y rivaroxaban 20 mg OD The rate of major bleeding: 3.4% p/y warfarin 3.6% p/y rivaroxaban 20 mg OD The rate of gastrointestinal bleeding: 2.2% p/y warfarin 3.2% p/y rivaroxaban 20 mg OD The rate of hemorrhagic stroke: 0.7% p/y warfarin 0.5% p/y rivaroxaban 20 mg OD The mortality rate: 2.2% p/y warfarin 1.9% p/y rivaroxaban 20 mg OD
ARISTOTLE 2012	Rates of the primary outcome (stroke or systemic embolism): 1.60% p/y Warfarin 1.27% p/y Apixaban 5 mg BD superiority	The rate of major bleeding: 3.09% p/y warfarin 2.13% p/y apixaban 5 mg BD The rate of hemorrhagic stroke: 0.47% p/y warfarin 0.24% p/y apixaban 5 mg BD The mortality rate: 3.94% p/y warfarin 3.52% p/y apixaban 5 mg BD
ENGAGE AF TIMI 48 2013	Rates of the primary outcome (stroke or systemic embolism): 1.50% p/y Warfarin 1.18% p/y Edoxaban 60 mg OD 1.61% p/y Edoxaban 30 mg OD noninferiority	The rate of major bleeding: 3.43% p/y warfarin 2.75% p/y edoxaban 60 mg OD 1.61% p/y edoxaban 30 mg OD The rate of gastrointestinal bleeding: 1.23% p/y warfarin 1.51% p/y edoxaban 60 mg OD 0.82% p/y edoxaban 30 mg OD The rate of hemorrhagic stroke: 0.47% p/y warfarin 0.26% p/y edoxaban 60 mg OD 0.16% p/y edoxaban 30 mg OD The mortality rate: 3.17% p/y warfarin 2.74% p/y edoxaban 60 mg OD 2.71% p/y edoxaban 30 mg OD

Regarding the primary outcome in RELY the results were 1.69% per 100-year patients (p/y) for warfarin compared to 1.53% p/y dabigatran etexilate 110 mg BD and 1.11% p/y dabigatran etexilate 150 mg BD. In ROCKET AF the rates of the primary outcome were 2.2% p/y for warfarin compared to 1.7% p/y for rivaroxaban 20 mg OD. In ARISTOTLE trial the rates of the primary outcome were 1.60% p/y for warfarin compared to 1.27% p/y for apixaban 5 mg BD. In ENGAGE AF TIMI the rates of the primary outcome were 1.50% p/y for warfarin compared to 1.18% p/y for edoxaban 60 mg BD and 1.61% p/y for edoxaban 30 mg BD. Taking into consideration all the important literature in oral anticoagulation for NVAF we designed a comprehensive but simple to follow ''go-to'' table (Table 3) [8], [9], [10], [11], [12], [13], [14], [15] to aid the prescribers worldwide.

Table 3: Oral Anticoagulants Specific Information A Prescriber Support Tool

	Warfarin	Dabigatran etexilate	Rivaroxaban	Apixaban	Edoxaban
Dose/Frequency	INR Dependent OD	150 mg BD	20 mg OD	5 mg BD	60 mg OD
Peak/Half-life	3-5 days Half-life 40 hours	2 days Half-life 12-14 hours	2-3 days Half-life 5-13 hours	1-2 days Half-life 8-15 hours	1-2 days Half-life 9-11 hours
Reduced dose	N/A	110 mg BD	15 mg OD	2.5 mg BD	30 mg OD
Age	N/A	Age > 80 years Also consider in > 75years	No dose adjustment	Age > 80 years	No dose adjustment
Weight	Extreme weight > 120 kg BMI > 40 kg/m2	< 50 kg	No dose adjustment	< 60 kg	< 60 kg
Renal	Not affected	CrCl < 5 0mL/min Cl CrCl < 30 mL/min 85%	CrCl < 15-49 mL/min Cl CrCl < 15 mL/min 33%	Creatinine > 133 micromoles/L or > 1.5 mg/dl Cl CrCl < 15mL/min 27%	CrCl < 15-49 mL/min Cl CrCl < 15 mL/min 50%
Interactions CAUTION IF	Abciximab Alteplase Amiodarone Amoxicillin Aprepitant Antiplatelets Azathioprine Azoles (fluco,mico, itra,vori) Barbiturates Bosentan Carbamazepine Chloramphenicol Ciprofloxacin Clarithromycin Coamoxiclav Capecitabine Danazol Disulfiram Doxycycline Erythromycin Fibrinolytics Fibrates Flucloxacillin Fluorouracil	Amiodarone Quinidine Clarithromycin Erythromycin Dose reduction: Verapamil (take at the same time)	Clarithromycin Erythromycin Fluconazole Amiodarone Quinidine	Diltiazem Naproxen	Ciclosporin Tacrolimus Ketoconazole Itraconazole Voriconazole Posaconazole Amiodarone Quinidine Dose reduction: Clarithromycin Erythromycin Dronedarone Ciclosporin Tacrolimus
Interactions AVOID IF	Fluvastatin Fluvoxamine Fenofibrate Glucagon HIV protease inhibitors Ivermectin Levofloxacin Leflunomide Lymecycline Metronidazole Mercaptopurine Mesalamine NSAIDs Ofloxacin Quinidine Quinine Paclitaxel Prostacyclin Paracetamol Phenytoin Propofol	Ketoconazole Itraconazole Voriconazole Posaconazole Dronedarone Rifampicin St John's Wort Carbamazepine Phenytoin Phenobarbital Ritonavir Anticoagulants Tacrolimus Cyclosporin	Ketoconazole Itraconazole Voriconazole Posaconazole Dronedarone Rifampicin St John's Wort Carbamazepine Phenytoin Phenobarbital Ritonavir Anticoagulants	Ketoconazole Itraconazole Voriconazole Posaconazole Dronedarone Rifampicin St John's Wort Carbamazepine Phenytoin Phenobarbital Ritonavir Anticoagulants	Ritonavir Rifampicin St John's Wort Carbamazepine Phenytoin Phenobarbital Anticoagulants
Dose reduction for DOACs if > 2 factors	Rifampicin Ribavirin SSRI SNRI Steroids St John's Wort Sulfasalazine Tamoxifen Terbinafine Vandetanib		Antiplatelets NSAIDs Systemic steroids Thrombocytopenia HASBLED > 3	History of GI bleeding Recent surgery on critical organ (brain, eye)	
Liver AVOID IF	Caution	Elevated liver enzymes > 2 upper limits of normal Hepatic impairment or liver disease expected to have any impact on survival	Hepatic disease associated with coagulopathy Cirrhotic patients with Child-Pugh B and C	Hepatic disease associated with coagulopathy Severe hepatic impairment	Hepatic disease associated with coagulopathy Elevated liver enzymes > 2 upper limits of normal total bilirubin ≥ 1.5 x upper limit of normal
Side effects	Hair loss Rare vascular calcification and skin necrosis	Oesophagitis Gastritis Duodenitis HASBLED > 3 Anaemia	Anaemia, Dizziness, Headache, Serious skin reactions (rare) Galactose intolerance	Contains lactose Anaemia	Anaemia, Dizziness, Headache High bilirubin High gamma glutamyltransferaseskin rash
Food interaction	Brocolli/Green leafs vegetables, Garlic, Ginger, Grapefruit, Cranberry, Mango, Green tea, Alcohol	X	X must be taken with food	X	X
Switching between anticoagulants	Overlap until INR > 2.0 May take 5-10 days	INR < 2.0	INR < 3.0	INR < 2.0	INR < 2.5
Compliance aid	Risk assessment	No	Yes	Yes	Yes
Swallow whole	Most brands of Warfarin tablets will disperse in water	Yes (tartaric acid)	No, can be crushed	No, can be crushed	Yes
Missed Dose	next dose as normal	up to 6h before the next dose	up to 12h before the next dose	up to 6h before the next dose	up to 12h before the next dose
Bleeding	All DOACs showed less Intracranial bleeding compared to Warfarin Antidote Vitamin K Prothrombin complex concentrate	Major bleeding D150 mg BD = W D110 mg BD < W GI bleeding D150 mg BD > W D110 mg BD = W Antidote Idarucizumab Haemodialysis	Major bleeding R = W GI bleeding R > W No antidote Prothrombin complex concentrate	Major bleeding A < W GI bleeding A = W No antidote Prothrombin complex concentrate	Major bleeding E < W GI bleeding E60mg OD > W No antidote Prothrombin complex concentrate
Specific Populations	Obese >120kg Renal/Hepatic Impairment (caution) 2nd-trimester pregnancy	110mg BD if previous GI haemorrhage High bleeding risk HASBLED>3; 150mg BD if recurrent stroke despite well managed VKA	For Asian patients use another DOAC Preference for once daily preparations	Previous GI haemorrhage High bleeding risk HASBLED>3 Elderly patients Renal impairment	Preference for once daily preparations High bleeding risk HASBLED>3 Elderly patients

Discussion

General characteristics of the four RCTs

ROCKET AF, ARISTOTLE and ENGAGE AF TIMI were double-blind, double-dummy trials, whereas RELY was an open-label trial which suggested a possible bias for this trial. The RELY trial authors state the risk of bias was reduced by the implementation of several validated procedures, including blinded evaluation of outcome events [16]. RELY and ARISTOTLE trials had a similar number of patients of more than 18100, ROCKET AF had the smallest number of participants of 14246, whereas ENGAGE AF TIMI had the largest population of 21105 which showed that all trials were large trials of high importance. The follow-up period was similar in RELY, ROCKET AF and ARISTOTLE but considerably longer in ENGAGE AF TIMI at 2.8 years. RELY and ARISTOTLE participants had an equal CHADS2 score of 2.1. However, ENGAGE AF TIMI and ROCKET AF participants had a higher CHADS2 score of 2.8 and 3.5, respectively. This was a significant finding and should be taken into account when choosing a particular DOAC for a patient.

The mean percentage of TTR was lower in ROCKET-AF (55%) compared to TTR in ARISTOTLE (62%), RE-LY (64%) and ENGAGE AF TIMI (68%). This is also an important finding and should be taken into account particularly for patients who are switching from a VKA to a DOAC. In ROCKET AF the low TTR was interpreted as poor control of the patients anticoagulant status. Age and sex of the studied population were similar in all studies ranging from 70-73 years and female percentage between 35-39%. These numbers show similarities with the general epidemiological data in AF (2). Finally, all the RCTs obtained a good Jadad score.

RE-LY

For the primary outcomes, dabigatran etexilate 150 mg twice daily was superior to warfarin, and dabigatran etexilate 110 mg twice daily was noninferior to warfarin. Major bleeding was significantly decreased with the 110 mg twice daily dose of dabigatran etexilate. However, the group on the 150 mg twice daily dose of dabigatran etexilate showed increased major bleeding events compared to warfarin. The risk of hemorrhagic stroke was also significantly lower with both the 110 mg and 150 mg doses [16]. These findings show that dabigatran etexilate was noninferior or superior (150 mg BD) when compared to warfarin, but the bleeding risk should be considered in both anticoagulants.

Interestingly, the rate of myocardial infarction was higher with both doses of dabigatran etexilate compared with warfarin but not statistically significant. A reason for this was explained by Connolly et al., (RELY) that warfarin provides better protection against coronary ischaemic events compared to dabigatran [16] (Table 2).

Dabigatran etexilate capsules contain coating with tartaric acid to enhance the gastric absorption which requires a more acidic environment. This acidity may explain the increased incidence of dyspeptic symptoms with both dabigatran etexilate doses [16]. This should be taken into consideration when prescribing dabigatran etexilate in patients with known gastro-oesophagal pathology.

ROCKET AF

For the primary outcomes, the trial demonstrated noninferiority for rivaroxaban compared with warfarin in patients with NVAF who were at moderate to high risk for stroke. Major bleeding was similarly reported for rivaroxaban and warfarin groups. However, less fatal bleeding and less intracranial haemorrhage were found in the rivaroxaban group. In contrast, gastrointestinal (GI) bleeding was more frequently reported in the rivaroxaban group [17]. Consequently, extra caution should be taken when prescribing rivaroxaban in patients with previous GI bleeding (Table 2).

At the end of the trial, patients transitioning to open-label therapy had more strokes with rivaroxaban compared with warfarin [18]. Patel et al. explained that the difficulty in transitioning from blinded trial therapy to the open-label use of a VKA could have been the cause for this [17]. Presumably many patients who had previously been assigned to the warfarin group would have already had a therapeutic INR compared to the patients in the rivaroxaban group [17]. This should be taken into account when switching between anticoagulants.

ARISTOTLE

Granger at al described it was the only study of a DOAC that showed significantly lower rates of all-cause mortality reported at 3.52% in the apixaban group compared to 3.94% in the warfarin group [19]. Apixaban has shown to be significantly more effective than warfarin, with fewer overall strokes and systemic emboli by 21%, major bleeding events by 31% and decreased mortality by 11% [19]. Consequently, further studies showed positive findings for apixaban in comparing DOACs indirectly. A meta-analysis of the above trials indicated that there were no statistically significant differences between dabigatran etexilate, rivaroxaban or apixaban in the incidence of stroke, systemic embolism and all-cause mortality [6], [18]. Additionally, apixaban was associated with a significantly lower incidence of all bleeding outcomes compared with rivaroxaban and a lower incidence with clinically relevant non-major bleeding compared to dabigatran etexilate 150 mg twice daily [18].

ENGAGE AF TIMI

This was the largest DOAC trial, and it showed that both once-daily regimens of edoxaban were noninferior when compared with warfarin regarding the primary outcome. Of note, the follow-up period in this trial was long, and the TTR was higher compared to the previous three DOAC trials. This illustrated good management of patients on oral anticoagulants within the trial. Edoxaban regimens were associated with significantly lower rates of bleeding and mortality from cardiovascular causes compared to warfarin [18]. The rates of life-threatening bleeding, intracranial bleeding, and major bleeding plus clinically relevant non-major bleeding were significantly lower in the edoxaban group. However, the annualised rate of major gastrointestinal bleeding was higher with high dose edoxaban than with warfarin (1.51% vs 1.23%), but the gastrointestinal bleeding rate was lowest with low dose edoxaban (0.82%). Giugliano et al. stated that the rate of myocardial infarction was not altered with edoxaban, and there was no increase in the risk of stroke or bleeding when patients in the edoxaban groups made the transition to open-label anticoagulant therapy at the end of the study [20].

DOACs and specific patient characteristics

DOACs appeared to be equally or more effective and safer than Warfarin in preventing systemic embolism irrespective of the patients' comorbidities [6], [16], [17], [18], [20]. Subsequently, ''real world'' studies showed that the risks of mortality, any bleeding, or major bleeding were significantly lower for apixaban and dabigatran etexilate compared with warfarin [18], [21]. We are in agreement with Shields et al. that direct comparison of the results from large, international, multicenter randomized control trials of DOACs versus warfarin for NVAF should be interpreted with caution due to differences in the mean CHADS2 score, TTR and rates of stroke and systemic embolism and hemorrhage in the warfarin group of the trials [15], [22].

In patients with NVAF with a significant risk of stroke, DOACs were reported as highly effective at preventing strokes compared to VKAs, and these provide a major improvement in the management of NVAF patients [23]. DOACs showed to have a more favourable safety profile and side effects, particularly for intracranial bleeding. Since the introduction of DOACs, there has been reported an increase in newly diagnosed patients with NVAF at risk of stroke who are receiving guideline-recommended therapy [4], [21].

Furthermore, due to the relatively recent introduction of these drugs, prescribers need to be aware of their characteristics, cautions and contraindications. Audits on prescribing oral anticoagulants reported frequent medical errors [23]. We agree with Heidbuchel et al., that the choice of the most appropriate DOAC for a patient should be based on the pharmacokinetics, pharmacodynamics and the integration of the clinical data concerning the patient's characteristics [14]. Recommendations from EHRA (European Heart Rhythm Association) suggested that patients with a history or high risk of gastrointestinal bleeding may have a lower risk of bleeding complications with apixaban and low dose edoxaban compared with dabigatran etexilate, rivaroxaban or high dose edoxaban [14]. Moreover, there was reported some evidence that patients with a high risk for ischemic stroke may benefit from dabigatran etexilate 150 mg twice daily [24].

Regarding patient-centeredness, evidence was reported that patients adhere better to once daily medications compared with those medications taken twice daily. [25] Patient's compliance was an important factor in the management of NVAF and data suggested in GARFIELD AF that patient refusal (11.2% for high-risk patients) has been the main patient factor affecting the rates of anticoagulation [4]. In patients without a contraindication to DOAC therapy, the selection among the agents was left primarily to physician and patient decision.

Wilke et al. reviewed the preferences of AF patients towards anticoagulation and showed that stroke risk reduction and limited bleeding risk were the most important attributes for an NVAF patient when deciding about oral anticoagulation [26]. NVAF patients were willing to accept higher bleeding risks if a certain threshold in reduced stroke risk could be reached [7], [26]. Steinberg et al. considered that involving the patient in the decision making when selecting a DOAC was vital for optimal management in NVAF [27]. Therefore this article encourages physicians to counsel patients about the risks and benefits of treatment and work out which is the best oral anticoagulant agent based on their characteristics (Table 3).

Conclusion

Based on the results in phase III randomised control trials discussed in this article, DOACs have shown similar efficacy but better safety patterns when compared with warfarin for NVAF management. To safely use anticoagulants, physicians should take into account patient-specific factors and shared decision making when prescribing an oral anticoagulant.

Our ''go-to'' table provides a supportive tool for physicians in preventing medical errors when managing patients on oral anticoagulants. Finally, research should be continued in clinical trials particularly for the specific populations.

Implications for Research

In this systematic review was summarised the important facts from the RCTs on oral anticoagulants. Also, a prescription tool was designed to aid family doctors/ prescribers in choosing the right agent for the right patient.

Limitations

Although we comprehensively reviewed and summarised the literature, our search was not exhaustive, and new data are emerging rapidly.

Acknowledgements

This study has received no financial or material support and is based on previously conducted studies and not involving any new studies of human or animal subjects.

References

1. Camm AJ, Lip GY, De Caterina R et al. 2012 focused update of the ESC guidelines for the management of atrial fibrillation: an update of the 2010 ESC guidelines for the management of atrial fibrillation. Eur Heart J. 2012; 33:2719-47. https://doi.org/10.1093/eurheartj/ehs253 PMid:22922413

2. Colilla S, Crow A, Petkun W et al. Estimates of current and future incidence and prevalence of atrial fibrillation in the U.S. adult population. Am J Cardiol. 2013; 112:1142-1147. https://doi.org/10.1016/j.amjcard.2013.05.063 PMid:23831166

3. Krijthe BP, Kunst A, Benjamin EJ et al. Projections on the number of individuals with atrial fibrillation in the European Union, from 2000 to 2060. Eur Heart J. 2013; 34: 2746 - 2751. https://doi.org/10.1093/eurheartj/eht280 PMid:23900699 PMCid:PMC3858024

4. Apenteng PN, Gao H, Hobbs FDR et al. Temporal trends in antithrombotic treatment of real-world UK patients with newly diagnosed atrial fibrillation: findings from the GARFIELD-AF registry. BMJ Open. 2018; 8(1): e018905. https://doi.org/10.1136/bmjopen-2017-018905 PMid:29331969 PMCid:PMC5781154

5. National Institute for Health and Clinical Excellence (NICE). Nice Clinical Guideline 180; Atrial Fibrillation: the management of atrial fibrillation, 2014. https://www.nice.org.uk/guidance/cg180

6. Larsen TB, Skjoth F, Nielsen PB et al. Comparative effectiveness and safety of non-vitamin K antagonist oral anticoagulants and warfarin in patients with atrial fibrillation: propensity weighted nationwide cohort study. BMJ. 2016; 353:i3189. https://doi.org/10.1136/bmj.i3189 PMid:27312796 PMCid:PMC4910696

7. Decision Aid NICE. Available at: https://www.nice.org.uk/guidance/cg180/resources/cg180-atrial-fibrillation-update-patient-decision-aid-243734797

8. Summary of Product Characteristics. Pradaxa®. Accessed Jan 2019. www.medicines.org.uk

9. Summary of Product Characteristics. Xarelto®. Accessed Jan 2019. www.medicines.org.uk

10. Summary of Product Characteristics. Eliquis®. Accessed Jan 2019. www.medicines.org.uk

11. Summary of Product Characteristics. Lixiana®. Accessed Jan 2019. www.medicines.org.uk

12. Comparison between NOACs. Available at http://www.hartlepoolandstocktonccg.nhs.uk/wp-content/uploads/2013/11/RDTC-NOAC-comparison-final-version.pdf

13. Comparison between NOACs. Available at: http:/www.derbyshiremedicinesmanagement.nhs.uk/assets/Clinical_Guidelines/Formulary_by_BNF_chapter_prescribing_guidelines/BNF_chapter_2/Atrial_fibrillation.pdf

14. Heidbuchel H, Verhamme P, Alings M, et al. Updated European Heart Rhythm Association practical guide on the use of non-vitamin K antagonist anticoagulants in patients with non-valvular atrial fibrillation. Europace. 2015; 17:1467-507. https://doi.org/10.1093/europace/euv309 PMid:26324838

15. Shields AM, Lip GY. Choosing the right drug to fit the patient when selecting oral anticoagulation for stroke prevention in atrial fibrillation. Journal of internal medicine. 2015; 278(1):1-8. https://doi.org/10.1111/joim.12360 PMid:25758241

16. Connolly SJ, Ezekowitz MD, Yusuf S, Eikelboom J, Oldgren J, Parekh A, Pogue J, Reilly PA, Themeles E, Varrone J, Wang S. Dabigatran versus warfarin in patients with atrial fibrillation. New England Journal of Medicine. 2009; 361(12):1139-51. https://doi.org/10.1056/NEJMoa0905561 PMid:19717844

17. Patel MR, Mahaffey KW, Garg J, Pan G, Singer DE, Hacke W, Breithardt G, Halperin JL, Hankey GJ, Piccini JP, Becker RC. Rivaroxaban versus warfarin in nonvalvular atrial fibrillation. New England Journal of Medicine. 2011; 365(10):883-91. https://doi.org/10.1056/NEJMoa1009638 PMid:21830957

18. Lip GY, Mitchell SA, Liu XI, et al. Relative efficacy and safety of non-Vitamin K oral anticoagulants for non-valvular atrial fibrillation: Network meta-analysis comparing apixaban, dabigatran, rivaroxaban and edoxaban in three patient subgroups. Int J Cardiol. 2016; 204:88-94. https://doi.org/10.1016/j.ijcard.2015.11.084 PMid:26655548

19. Granger CB, Alexander JH, McMurray JJ, Lopes RD, Hylek EM, Hanna M, Al-Khalidi HR, Ansell J, Atar D, Avezum A, Bahit MC. Apixaban versus warfarin in patients with atrial fibrillation. New England Journal of Medicine. 2011; 365(11):981-92. https://doi.org/10.1056/NEJMoa1107039 PMid:21870978

20. Giugliano RP, Ruff CT, Braunwald E, Murphy SA, Wiviott SD, Halperin JL, Waldo AL, Ezekowitz MD, Weitz JI, Špinar J, Ruzyllo W. Edoxaban versus warfarin in patients with atrial fibrillation. New England Journal of Medicine. 2013; 369(22):2093-104. https://doi.org/10.1056/NEJMoa1310907 PMid:24251359

21. Camm AJ, Accetta G, Ambrosio G, et al. Evolving antithrombotic treatment patterns for patients with newly diagnosed atrial fibrillation. Heart. 2017; 103(4):307-314. https://doi.org/10.1136/heartjnl-2016-309832 PMid:27647168 PMCid:PMC5293840

22. Bisson A, Angoulvant D, Philippart R, et al. Non-Vitamin K Oral Anticoagulants for Stroke Prevention in Special Populations with Atrial Fibrillation. Adv Ther. 2017; 34(6):1283-1290. https://doi.org/10.1007/s12325-017-0550-7 PMid:28493056 PMCid:PMC5487882

23. Lip GY, Pan X, Kamble S, et al. Major bleeding risk among non-valvular atrial fibrillation patients initiated on apixaban, dabigatran, rivaroxaban or warfarin: a "real-world" observational study in the United States. Int J Clin Pract. 2016; 70(9):752-63. https://doi.org/10.1111/ijcp.12863 PMid:27550177

PMCid:PMC5129572

24. Schaefer JK, McBane RD, Wysokinski WE, et al. How to choose appropriate direct oral anticoagulant for patient with nonvalvular atrial fibrillation. Ann Haematol. 2016; 95:437-449. https://doi.org/10.1007/s00277-015-2566-x PMid:26658769 PMCid:PMC4742513

25. Granger CB, Alexander JH, McMurray JJ, et al. ARISTOTLE Committees and Investigators. Apixaban versus warfarin in patients with atrial fibrillation. N Engl J Med. 2011; 365(11):981-992. https://doi.org/10.1056/NEJMoa1107039 PMid:21870978

26. Wilke T, Bauer S, Mueller S, et al. Patient Preferences for Oral Anticoagulation Therapy in Atrial Fibrillation: A Systematic Literature Review. Patient. 2017; 10(1):17-37. https://doi.org/10.1007/s40271-016-0185-9 PMid:27461276 PMCid:PMC5250672

27. Steinberg BA. How I use anticoagulation in atrial fibrillation. Blood. 2016; 128:2891-2898. https://doi.org/10.1182/blood-2016-07-693614 PMid:27780804

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Apr 15; 7(7):1233-1240.
https://doi.org/10.3889/oamjms.2019.176
eISSN: 1857-9655
Review Article

Outer Ear Infections in Iran: A Review

Keyvan Kiakojuri[1], Mojtaba Taghizadeh Armaki[2,3], Ramazan Rajabnia[2,4], Abazar Pournajaf[2, 4], Mohsen Karami[2,3], Aynaz Khademian[4], Saeid Mahdavi Omran[2,3*]

[1]Department of Ear, Nose, and Throat, School of Medicine, Roohani Hospital, Babol University of Medical Sciences, Babol, Iran; [2]Infectious Diseases and Tropical Medicine Research Center, Health Research Center, Babol University of Medical Sciences, Babol, Iran; [3]Department of Medical Parasitology and Mycology, School of Medicine, Babol University of Medical Sciences, Babol, Iran; [4]Department of Microbiology, School of Medicine, Babol University of Medical Sciences, Babol, Iran

Citation: Kiakojuri K, Armaki MT, Rajabnia R, Pournajaf A, Karami M, Khademian A, Omran SM. Outer ear Infections in Iran: A Review. Open Access Maced J Med Sci. 2019 Apr 15; 7(7):1233-1240. https://doi.org/10.3889/oamjms.2019.176

Keywords: Outer Ear; Fungal infection; Bacterial infection; Bacterial-fungal infections

***Correspondence:** Saeid Mahdavi Omran. Infectious Diseases and Tropical Medicine Research Center, Health Research Center, Babol University of Medical Sciences, Babol, Iran; Department of Medical Parasitology and Mycology, School of Medicine, Babol University of Medical Sciences, Babol, Iran. E-mail: s.mahdavi@mubabol.ac.ir

Received: 14-Jan-2019; **Revised:** 06-Apr-2019; **Accepted:** 07-Apr-2019; **OnlineFirst:** 14-Apr-2019

Funding: This work was financially supported by a grant number 9338721 by Babol University of Medical Sciences, Babol, Iran

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

Abstract

BACKGROUND: Otitis externa is the fungal and bacterial infection of the outer ear.

AIM: We aimed to investigate the published papers about the outer ear infections in Iran and suggest standardised investigations and treatments.

METHODS: We used different electronic databases like PubMed, Scopus, Web of Science, Iranmedex, Google Scholar, and Magiran with specific keywords.

RESULTS: We obtained forty published full-text articles for review of data. Our results indicated the women were more infected than men. The ages of patients were < 1-81 years. As clinically symptoms, itching and Feel the ear fairy were the most common presenting complaints in most cases. Most infections were the pure bacterial and fungal origin, respectively. However, some of the studies were mixed fungal-bacterial infections — Pseudomonas spp. And Aspergillus niger were the most common bacteria and fungi isolates respectively in Iranian patents.

CONCLUSION: Fungal and bacterial specific cultures may be recommended, and anti-fungal drugs may be added, to treatment regimens in patients with otitis externa to reduce the clinical symptoms.

Introduction

Outer Ear Infection (Otitis Externa) refers to any infection of the auricle and ear canal, which is presented with no rupture and originated from microorganisms and accounts for about 5% to 20% of the total cases referring to the ear, nose, and throat clinics [1]. This type of infection is usually classified into two acute and chronic diseases [2]. Outer ear infection is one of the most common types of ear infections that physicians are faced with daily. The needs of information about the symptoms and causes of outer ear infections, this review study was performed for relevant clinical and laboratory investigations carried out in Iran. In the current study, all articles on humans' outer ear bacterial and fungal infections, bacterial-fungal infections, and drug sensitivity tests, which were conducted in Iran and published in authentic Iranian and non-Iranian journals, were reviewed. All published articles were obtained in electronic databases like PubMed, Scopus, Web of Science, Iranmedex, Google Scholar, and Magiran. The search keywords included outer ear, ear infection, fungus, bacteria, bacterial-fungal infection, and Iran.

Exclusion Criteria

In each study, there are some factors causing the exclusion of the patients. In studies on the outer ear infections, some intervening factors including the use of antibiotics (antibacterial or antifungal, according to the type of examination or infection), eardrum rupture, negative result of direct tests, middle

ear lesions without outer ear infection and surgery history (except for the successful myringoplasty and tympanoplasty surgeries), as well as the patients' lack of willingness to participate in the study were considered as exclusion criteria, and those meeting these criteria were excluded from the outer ear infections studies [3], [4], [5].

Patients' Age

Surveys conducted in different regions of Iran in the field of outer ear infections have shown that such infections are observed in all ages and the age of participants in such studies ranged from 1-81 years. Children are among those being infected with ear infections. In a study, all age groups ranging from children below one to teenagers aged 18 years with a mean age of 2.4 ± 7.5 years old suffered from ear infections, most of whom were below the age of 6 years [3], [4], [5]. Meanwhile, the average age of the patients was 10.5 years old in another study [6]. In adults, bacterial infections are more common when they are 20-29 years [7], [8] and 30-40 years old [9]. A majority of the patients were within the fourth decade of their lives [4], [10], [11]. The lowest frequency was observed for the patients aged between 50 and 59 years [7].

Otomycosis has also been observed at different ages so that the individuals aged 7-81 fall into this category [5], [8], [12]. However, the oldest patients had different years of age in different studies. The most affected ages included 10-20 years [13], 20-29 years with the average age of 35.17 years [14], 30-34 years with the average age of 30.7 years [8], 35-44 years with an average age of 43.21 years [15], 30-50 years with an average age of 17-35 years [16] and 38-48 years [5].

Patients' Gender

There is a possibility of outer ear infections in both genders; however, various studies show different frequencies for both genders. In most studies, women were more likely than men to suffer from such infections: 54.31% [4] 67.3% [8], 74.2% [17] and 89.2% [7] (Table 1).

Of course, in an investigation on children with outer ear infections, it was revealed that a larger number of boys (60.7%) were affected than girls (39.3%) [3]. Fungal infections of outer ear were also observed in both genders; however, some studies showed that the infection was more common with a range of 50.29 to 82.9% among females than males [5], [8], [12], [14], [15], [16], [18], [19], [20], [21], [22], [23]. In some studies, males were more likely to be involved with fungal ear infections than females, so that the incidence rate in males varied from 53.96%-69.23% [12], [13], [24], [25], [26]. A review study, though, showed that the overall ratio of outer ear fungal infections was 1/53 in males to females [27].

Table 1: The frequency of sex in otitis externa in Iran

Total	Gender		Ratio	Reference
	Male (%)	Female (%)	(m/F)	
75	33 (44)	42 (56)	0.79	Akhi MT, 2001 [9]
70	12 (17.14)	58 (82.56)	0.21	Kochack Alavi SK, 2004 [7]
20	12 (60)	8 (40)	1.5	Afshari MA, 2005 [26]
15	4 (26.7)	11 (73.3)	0.36	Zareii Mahmoodabadi A, 2006 [19]
174	86 (49.4)	88 (50.6)	0.97	Balouchi M, 2006 [18]
70	12 (17.1)	58 (82.9)	0.21	Bineshian F, 2006 [14]
25	15 (60)	10 (40)	1.5	Pakshir K, 2008 [35]
52	36 (69.23)	16 (30.77)	2.25	Yegane Moghadam A, 2009 [24]
26	12 (46.7)	14 (53.3)	0.86	Zareii Mahmoodabadi A, 2010 [20]
120	36 (30)	84 (70)	0.43	Rajabnia R, 2010 [29]
246	157 (67.79)	89 (32.21)	1.76	Hajjertababar M 2010 [31]
118	61 (51.69)	57 (48.31)	1.07	Barati B, 2011 [12]
54	16 (29.63)	38 (70.37)	0.42	Berjis N, 2012 [21]
293	131 (44.7)	162 (55.3)	0.81	Saki N, 2013 [22]
114	67 (58.77)	47 (41.23)	1.43	Nowrozi H, 2014 [16]
100	38 (38)	62 (62)	0.61	Nemati S, 2014 [5]
49	34 (53.96)	15 (46.04)	2.27	Ebrahimzadeh A, 2014 [13]
6	25 (44.64)	31 (55.36)	0.81	Kiakojuri K, 2015 (JJM) [15]
129	76 (58.91)	53 (41.09)	1.43	Kazemi A, 2015 [25]
184	90 (48.91)	94 (51.09)	0.96	Kiakojuri K, 2015 [3]
1990	953 (47.89)	1037 (52.11)	0.92	--

Place of Residence

Although the place of residence can play an effective role in the infection rate due to the different economic and social conditions, surveys show that the frequency of villagers and urban residents' referral for the diagnosis and treatment of ear infections depends on various conditions, including attention to their illness, access to health care facilities, and the presence of specialists with private clinics. In some surveys, urban residents were more likely to refer to the clinics for the sake of ear infections, with a rate of 77.59%-86.3% in urban areas and 17.5%-22.41% in rural areas (3, 4, 24, 28, 29). Of course, the other studies indicated that the villagers (82.81%) were more affected by this disease than the urban population (17.19%) [11].

Patients' Occupation

The occupation of the affected is less likely to affect the ear infections; however, it was found that the housewives (37.7%) are more likely to suffer from the outer ear infections than those having other occupations. The most affected individuals are self-employed (18.83%) and clerks (18.1%) [4]. A survey on children also revealed that 50% of the affected were students and the rest were below school age [3]. In one study, all the infected were farmers [25].

Predisposing Factors

In Bacterial infection

In case of underlying illnesses and predisposing factors of outer ear bacterial infections, it has been noted that the use of ear cleaners (89.1%), ear manipulation (95.6%), swimming at sea (9.1%), and swimming in pools (5/5%) are the most important factors predisposing this disease [8]. Also, ear infection history can also play a role in this regard

[29].

Although the role of the seasons has been less concerned in these studies, a study has shown that the disease was more frequent in the autumn (57.3%) than in other seasons. Meanwhile, the lowest infection rate was observed in the spring (16%), though, no justification is reported in this case [9].

In Fungal infection

The factors influencing the fungal infection include the use of antibiotics and steroid (21.91%-88.5%), swimming (5.08-16%), a history of swimming at sea (10.3%) and bathing (66.6%) [5], [18]. Also, the use of ear cleaners (84.6%), ear manipulation (87.2%), and trauma can also be noted in this regard [8], [12], [30].

The season can also be introduced as a predisposing factor so that the heat, which is a factor affecting the growth of the fungi, can be a contributing factor in some cases. It has been suggested that the number of the affected is higher in summer than other seasons (30.4%-45.61%), even though, the rate of infection in the autumn was high (57.3%) as well. On the other hand, the lowest rates are observed in the spring and winter [9], [12], [16], [22].

Clinical Finding

Outer ear infections have different signs and symptoms depending on the severity of the disease and its causes. In bacterial infections, the most common symptoms are swelling (82-93.96%), discharge (64%-86.21%) and hearing loss (57.66% - 90.51%). Furthermore, itching, scaling, and a feeling of fullness in ears were also other symptoms of outer ear bacterial infections [3], [4], [11], [31]. Pain is one of the symptoms of outer ear bacterial infection, and it has been shown that 64.8% of ear pain is related to outer ear infection [32]. In general, pain is seen in patients with outer ear infections (74.3%-85.9%) [3], [23]. In these infections, the pus, and discharge, as well as cerumen (in chronic forms) are in bright colours (white, yellow) and dark colours (light brown-dark brown) and this can partly reflect the pathogens [11], [15], [20], [33].

Symptoms of outer ear fungal infection, although similar to bacterial infections, are low in severity and prevalent in different individuals with various symptoms [14]; however, the most common symptoms are swelling, discharge, itching, tinnitus, and hearing loss [16], [23].

The presence of lump (69.2%), itching (65%), and pain (55%), a feeling of fullness in ears (46%), pus (40%), and auditory impairment (33%) was among the reasons of referring to the clinics [12]. In the clinical examinations, the absence of fever (89.7%), ear sensitivity (71.8%), the presence of a fungal lump in Otoscopy (69.2%) and epithelium scaling (59%) are also diagnosed by specialists [8]. A totally, the itching had the highest rate (Figure 1).

Sampling

To have a definite diagnosis of the disease, the samples of pus, lump, skin, or anything in the auditory canal should be extracted by ear, nose and throat specialists. This can be performed under a microscope using a sterile swab, speculum, suction or curette, depending on the available facilities, the type of lesion, and the physician's expertise [3], [4], [6], [12], [14], [15], [34], [35].

Direct examination and culture

The samples should be tested within the least possible time in the laboratory. The speed at which the samples are inoculated in a culture medium to grow bacteria or fungi according to a physician's request and its spread on the lam for direct testing would bring more accurate findings and more appropriate treatment. The direct result of direct culture and testing plays the most critical role regarding the bacterial or fungal pathogens, respectively.

In the samples spread on the lam using the hot-stained method, the presence of bacteria in various forms or true or false mycelium show the presence of Otitis Externa, which is also consistent with the culture results [3], [13], [15], [16], [19], [31]. The samples suspected to outer ear fungal infections are usually lightened by potassium hydroxide (10%) and examined microbiologically [8], [13], [16], [25].

Culture

Suitable culture media for initial isolation and identification of bacterial or fungal factors causing otitis are different and essential for the diagnosis of bacterial infection. Various environments have been used as the primary and specific media for isolating and identifying bacteria. Specific media for bacterial growth include the blood agar (Sheep Blood), EMB, Thioglycolate, Mac Conkey's Agar, Chocolate Agar (CHOC), and Eosin Methylene Blue Agar [5], [8], [12], [14], [30], [31].

Usually, the culture media are incubated for 18 to 24 h or a maximum of 48 h at 37°C for the bacterial growth [17], [29], [31]. In all cases, sterile conditions should be observed to prevent environmental contamination [35]. Considering that the diagnosis of the bacterial ear infection depends on the positive culture results [4]; therefore, in order to determine the identity of the bacteria observed on the plates, it is necessary to detect them in differential media such as TSI, Cimon Citrate agar, STM, MRVP, Urea agar or Lysine Decar Boxylase [4], [8].

Meanwhile, enzymatic methods and other methods including catalase test, mannitol fermentation, Optochin, oxidase, liquid citrate, and ONPG are also used for the final confirmation of bacteria [3], [8], [31].

Such media as Suburo Dextrose Agar (S), Chloramphenicol (Sc) or Cyclohexamide (Scc) have been used for the growth of fungal agents and initial isolation of these factors [4], [5], [8], [13], [14], [34]. Occasionally, penicillin and streptomycin antibiotics have been used instead of chloramphenicol [35]. These media are kept for 1 to 4 weeks at 25°C, 25°C-27°C, or 30°C, aiming the growth of fungal elements [4], [12], [34].

In addition, the fungi were prepared using different methods such as chopped samples, Lactophenol Cotton Blue staining for initial identification of fungi, sugar adsorption using API 20C AUX kit, chromosomal method for identification of yeasts [14], production of serum germinal tubes (stored at 37°C), generation of vesicles (Chlamydoconidia) using Corn Meal Agar along with Polysorbate 80 and chrome agar for the diagnosis of *Candida albicans* [14], [35], Malt Extract Agar, Yeast Malt Agar, and culturing on lam for string fungi [8], [12], [13], [16], [22], [29].

Microbial Pathogens

In most studies, bacteria are considered as the main cause of outer ear infection, and the fungus is the secondary reasons. In some studies, however, the involvement of both bacteria and fungus is also reported. The number of isolated organisms is different in different studies, and the number of species is reported to be up to 23 bacterial species [9] and 17 fungal species [29].

Bacterial etiological agents

Bacteria, which, after direct testing of pus and discharge, are reported in most studies as the main cause of outer ear infections, consist of many variations. In a majority of studies, *Pseudomonas aeruginosa* (25.8%-79.3%) and *Staphylococcus aureus* (12.4%-63.46%) were among the most common bacteria; however, other bacteria extracted from the outer ear infections include gram-negative bacilli (49.99%), coagulase-negative *staphylococci* (CoNS) (12.5%-37.96%), *Enterobacte-riaceae* (24.08%), *Bacillus* species (18.9%-23.2%), Gram-positive cocci (20.45%), *diphtheriae* (17.24%), *Pseudomonas* (11.3%) and *streptococcus* (10.71%) species [4], [6], [7], [8], [9], [14], [17], [28], [31]. Aerobic bacteria play a significant role in outer ear infections, and they have also been identified as agents of outer ear infections over recent years. In a study of 75 patients with an outer ear infection, 9 species (10.23%) of obligatory anaerobic gram-negative bacilli, including 2 species of Bacteroides and 7 species of fusiform, and 54 species (61.36%) of optional anaerobic species were isolated [9]. In most studies showed *Pseudomonas* spp., *Bacillus* spp., CoNS and *S. aureus* were predominant etiological agents of bacteria in these studies (Table 2).

Mix Bacterial infection

Based on laboratory results, although the main cause of the disease is usually an organism (bacteria or fungus), more than one organism is isolated from the outer ear auditory canal in some studies, in which the decision to determine the major pathogen and the choice of appropriate treatment is to some extent difficult. In of Kiakjouri's et al., study in Babol, 35% of individuals with outer ear infection had several bacteria [28].

Table 2: The distribution of etiological agents of otitis externa in Iran

Number	Total (%)	Number of cases (%)	Number of Species	The most fungal agents N (%)			Number of Species	The most bacterial agents N (%)			Reference
1	52	52	ND	*Candida* spp. 26 (50)	*Aspergillus* spp. 10 (19.23)	*Penicillium* spp. 4 (7.69)	ND*	-	-	-	[33]
2	75	75	14	*Yeast*	Mycelia	-	16	*Pseudomonas spp.* 25 (31.65)	*Entrobacteriacea* spp. 19 (24.08)	*S. aurous* 13 (16.45)	[9]
3	101	78	-	*A. niger* 10 (12.82)	*A. fumigatus* 5 (6.41)	*Nigrospora* spp. 4 (5.13)	6	*Pseudomonas spp.* 28 (35.89)	*Prodentia* spp. 5 (6.41)	*S aurous* 4 (5.13)	[8]
4	70	73	15	-	-	-	8	*P. aeruginosa* 35 (47.9)	*Entrobacteriacea* spp. 16 (21.4)	*S aurous* 14 (19.2)	[7]
5	35	20	-	*A. niger* 14 (43.7)	*C. albicans* 5 (15.6)	*A. fumigatus* 4 (12.5)	-	-	-	-	[26]
6	174	120	7	*A. flavus* 48 (40)	*A. niger* 44 (36.7)	*C. albicans* 8 (6.6)	-	-	-	-	[18]
7	70	8	9	*C. parapsilosis* 5 (62.5)	*C. glabrata* 2 (25)	*C. krusei* 1 (12.5)	-	-	-	-	[14]
8	15	15	3	*A. niger* 8 (53.33)	*A. flavus* 2 (13.3)	*A. fumigatus* 1 (6.67)	-	-	-	-	[19]
9	92	92	7	-	-	-	3	Coagulase negative *S.* 42 (45.2)	*S aurous* 9 (9.5)	*S. pneumonia* 4 (4.7)	[37]
10	55	123	4	*A. niger* 48 (39)	*Candida* spp. 45 (36.6)	*A. flavus* 21 (17.1)	-	-	-	-	[23]
11	243	25	10	*Penicillium* spp. 8 (32)	*Candida* spp. 5 (20)	*Alternaria* spp. 2 (8)	-	-	-	-	[35]
12	120	17	5	*A. niger* 8 (47.1)	*A. flavus* 3 (17.6)	*Rhizopus* spp. 3 (5.8)	-	-	-	-	[34]
13	91	52	8	*A. niger* 32 (61.9)	*C. albicans* 7 (13.5)	*A. fumigatus* 3 (5.8)	-	-	-	-	[24]
14	120	116	-	-	-	-	3	*S. aurous* 24 (20.8)	*Bacillus* spp. 22 (18.19)	*Pseudomonas* spp. 13 (11.3)	[36]
15	179	-	-	-	-	-	-	-	-	-	[31]
16	120	154	17	*A. flavus* 19 (12.4)	*Cladosporium* 18 (11.7)	Mycelia sterile 18 (11.7)	-	-	-	-	[29]
17	57	26	8	*A. niger* 8 (30.8)	*A. flavus* 6 (23.1)	*C. albicans* 3 (11.5)	-	-	-	-	[20]
18	60	60	-	-	-	-	-	-	-	-	[28]
19	66	-	-	-	-	-	-	-	-	-	[6]
20	171	118	6	*A. flavus* 53 (44.52)	*A. niger* 45 (38.14)	*C. albicans* 9 (7.63)	-	-	-	-	[12]
21	54	54	7	*C. albicans* 13 (24.1)	*A. niger* 13 (24.1)	*A. flavus* 11 (20.4)	-	-	-	-	[21]
22	100	13	-	*A. niger* 8 (8)	*A. flavus* 3 (3)	*A. fumigatus* 2 (2)	-	-	-	-	[38]
23	124	88	ND	-	-	-	ND	*S. aurous* 25 (29)	*P. aeruginosa* 23 (25.8)	-	[17]
24	77	-	-	-	-	-	-	-	-	-	[32]
25	293	-	ND	*A. niger* 197 (67.2)	*A. flavus* 38 (13)	*C. albicans* 34 (11.6)	-	-	-	-	[22]
26	63	49	17	*Aspergillus* spp. 15 (30.61)	T. mentagrophytes 11 (24.5)	*Mucor* spp. 8 (16.33)	-	-	-	-	[13]
27	200	114	4	*A. niger* 102 (89.4)	*A. fumigatus* 6 (5.3)	*C. albicans* 5 (4.4)	-	-	-	-	[16]
28	100	43	4	*A. niger* 21 (49)	*C. albicans* 14 (23)	*A. fumigatus* 7 (16)	-	-	-	-	[5]
29	50	-	-	-	-	-	-	-	-	-	[40]
30	140	129	11	*A. niger* 73 (62.9)	*A. flavus* 24 (20.7)	*A. fumigatus* 11 (9.5)	-	-	-	-	[25]
31	56	33	4	*A. niger* 20 (35.71)	*A. flavus* 6 (10.71)	*C. albicans* 4 (7.14)	10	*Bacillus* spp. 13 (21.23)	*Streptococcus* spp. 6 (10.7)	*Pseudomonas* spp. 6 (10.71)	[3]
32	184	184	11	*A. niger* 39 (21.2)	*Penicillium* spp. 24 (13.04)	*Candida* spp. 19 (10.33)	7	*Bacillus* spp. 36 (19.58)	Coagulase negative *S.* 28 (15.22)	*Streptococcus* spp. 14 (7.6)	[10]
33	56	54	10	*A. niger* 31 (55.36)	*C. albicans* 8 (14.9)	*A. fumigatus* 3 (5.35)	-	-	-	-	[15]
34	116	101	-	-	-	-	8	*Bacillus* spp. 26 (22.4)	Coagulase negative *S.* 23 (19.43)	*Diphteroid* spp. 20 (17.24)	[4]
Total	3280	2086	-	806 (38.64)	344 (16.49)	185 (8.87)	-	254 (12.18)	151 (7.24)	88 (4.21)	-

ND*: Not Determined.

This rate was 15.9% in another study, which contained anaerobic and aerobic bacteria as well as the fungi [9]. In other studies, the presence of two or more bacteria was found to be between 11.29%-14.74% [6], [17].

Non-pathogenic Bacteria

All organisms isolated from the ear were not pathogenic, and some of them were as normal flora. This is confirmed in some studies. In a study on the isolated bacteria from the outer ear canal, 60.4% and 93.1% of the outer ear infection in the ear samples of patients and healthy people were respectively non-pathogenic bacteria, and the rest were pathogenic ones [36].

Bacteria in the healthy individuals' ears

The healthy individuals' ears can also contain different microorganisms as a result of their direct exposure to the surroundings, manipulation of the ear, and proximity to their surrounding organs. Studies have shown that the coagulase-negative staphylococci with a frequency of 45.2%-66.5% are the most common bacterial agent in cancer patients and non-cancer individuals. Due to the high presence of *S.aureus* in cancer patients (11.9%-15.7%), it is inferred that cancer patients are at higher risk of outer ear bacterial infection [37].

On the other hand, the ear cerumen of the healthy individuals also consists of different bacteria, the most common of which are *S. epidermidis* (38.7%), *diphtheria* (22.4%), *Bacillus* (19.58%), coagulase-negative *Staphylococci* (15.22%) and *Streptococcus* (7.61%) [10], [36]. Further studies confirmed the presence of bacteria such as coagulase-negative *Staphylococci* and *diphtheria* [6].

Fungi etiological agents

Although the main resources on ear disease have noted that only 10% of the outer ear infections are caused by fungi [35], this ratio has undergone a major change over recent years so that some surveys have reported the range of this rate from 11.4% [14], 14.3% [3], 19.64% [15] and 33.25% [22] to 43% [5]. In a study of 171 patients, 69% had fungal organisms, and 25.15% had bacterial infection [12]. The number and species of fungi generating otomycosis have also changed over these two decades. The number of fungal species isolated from otomycosis has also reached 17 in various studies [29]. In a majority of studies, saprophytes are the main cause of otomycosis (57.33%) [13]; meanwhile, the *Aspergillus* is the main source (41.66%) of this disease [12], [13]. On most studies, *A. niger* has been the main cause of otomycosis, with a rate of above 50% (55.36%-40.48%) [5], [16], [19], [22], [24], [26], [38] (Table 2).

In some other studies, the role of this species has been reported to be non-significant in otomycosis (9.9%-39%) [8], [10], [12], [15], [23]. Over recent years, other species of *Aspergillus*, including *A. flavus*, have been identified as important factors in otomycosis [3], [18]. These studies have implied that this species has caused 4%-23.1% of otomycosis cases and acts the same as *A. niger* [3], [12], [20], [22], [23], [29]. Of the other species of *Aspergillus*, the role of which has been highlighted in otomycosis, is *A. fumigatus*, varying from 5.3% to 20.5% in different studies [16], [22], [25]. In the past decades, the impact of other saprophytes has been low in otomycosis; however, with a decreased role of *A. niger* and increased role of other species of *Aspergillus* in recent years, other saprophytes, such as *Penicillium* spp (2-13%) [10], [22], *Cladosporium* spp (11.7%) [29] and *Alternaria* spp (11.11%) [13] play a role in otomycosis.

Fungi without a horizontal blade, such as *Mucor* spp and *Rhizopus* spp, have played a significant role in otomycosis, as documented in some studies [13], [34]. Yeasts are considered as the second group of fungi in otomycosis, whose role is reported to vary from 8.9% to 24.1% [8], [13]. They are even reported in some studies as the major agents of otomycosis [21]. Of the various species that play a critical role in otomycosis [29], the *Candida albicans* have had the central role in most studies. This species in various studies have been caused otomycosis in 7.14%-14.29% of the cases [3], [12], [16], [22]. Other species such as *C. tropicalis* (50%) [8], *C. glabrata*, and *C. parapsilosis* [14], and the like have been introduced as main factors causing otomycosis [15], [23]. The third category of fungi causing otomycosis is dermatophytes, which play a less vital role in this disease. In a study, its frequency was found to be 17.32%, with *Trichophyton mentagrophyte*s and *Trichophyton violaseum*, as well as *Epidermophyton floccosum*, being the main drivers of this infection [13].

Mix fungal infection

Fewer studies have investigated the role of several fungi in otomycosis. Studies have shown that only one fungal agent is mostly involved in otomycosis [13], [29]; however, the presences of two fungi (14%) and more than 2 fungi (42.5%) have been reported in some cases [3], [13], [29].

Isolation of fungi from healthy individuals

Because of the ears' connection with their surroundings, the fungi have also been isolated from cultivating ear cerumen of healthy individuals (without infection). These fungi include *A. niger*, *Penicillium*, and *Candida* [10], *A. Niger*, *A. flavus* and *Rhizopus*

spp [34], Sterile mycelium, *Cladosporium* spp [29] (8%), *Penicillium* spp, *Cladosporium* spp and *Candida* spp [35], *Cladosporium* spp, *Alternaria* spp and *Penicillium* spp [28] and *Aspergillus* spp, *Mucor* spp and *Rhodoturula* spp [13].

Bacterial-fungal infections

In some cases, the ear infection may also be caused by both fungi and bacteria (regardless of the primary role of each in causing infection). This can be diagnosed based on the positive results of direct testing and culturing for fungi and the positive culture result for bacteria. Such infections are of importance, especially in the field of treatment, and cause problems of ear infections [39]. This rate varies from 7.24% [17] to 15.2% [8] in these studies.

Lack of growth

The outer ear canal samples do not always have a growing organism in the common media since clinical symptoms may indicate bacterial or fungal ear infection with no growing organisms in appropriate bacterial or fungal media. This, while demonstrating the value of the culture results for the initiation of the treatment, indicates that some of the outer ear canal lesions are caused by other infectious agents such as viruses and non-infectious agents. The study showed that the percentage of these infections (the positive result of direct testing and culture for the fungus and the positive culture results for bacteria) in the laboratory was 69% [12]. The percentage of the organism non-growth in different fungal or bacterial culture media varied from 3.537%-29.03% [3], [4], [5], [8], [10], [17], [28], [29] In some studies, the culture of infection-free cerumen also lacked growth, which varied from 16.25%-89.71% [29], [35].

The sensitivity of bacteria to medicine

The announcement of laboratory results of direct testing and culturing samples from outer ear infections are required to initiate the treatment of these patients; however, determining the sensitivity or resistance of these organisms to antibacterial antibiotics completes this cycle to help these patients more. Accordingly, a variety of methods are used to determine the sensitivity of the bacteria to drugs, the most common of which is gel diffusion using the Kirby-Bauer discs [6], [7], [17], [30], [37]. The bacteria isolated from the ear canal show different rates of sensitivity to antibiotics. In a study employing Hinton agar diffusion using paper plates, it was found that coagulase-negative *Staphylococci* isolated from the auditory canal of the hospitalised cancer patients are as much as 25% and 85% resistant to vancomycin and penicillin-G, respectively. In non-hospitalized cancer patients, the rates were 45% and 80%, respectively. On the other hand, all isolates of *S. aureus* were resistant to vancomycin and penicillin-g. Instead, none of the isolates of *S. pneumonia* isolated from hospitalised cancer patients was resistant to tetracycline [37]. Another study suggested that most bacteria isolated from patients (94.52%) with outer ear infection were sensitive to ciprofloxacin and gentamicin (89%); however, the highest resistance was observed for amoxicillin (93.6%) and cloxacillin (78.8%). Sensitivity to ciprofloxacin was observed in 100% of *P. aeruginosa* and *S. aureus*. Furthermore, all isolates of *S.epidermidis* were sensitive to ciprofloxacin and gentamicin [7]. The highest sensitivity of *S. aureus* to medicine was observed for vancomycin (100%), gentamicin (92.3%), and trimethoprim (76.2%), even though, the highest observed resistance was observed for penicillin (100%), gluxacillin (84.6%) and sulfamethoxazole (76.2%) [30]. On the other hand, *S. aureus* was sensitive to vancomycin [6]. *Pseudomonas* spp was sensitive to vancomycin and ceftriaxone and resistant to cotrimoxazole [17].

Antifungal susceptibility testing

Given the difference in fungal sensitivity to antifungal drugs, it is advisable to begin the treatment of these patients by assessing the sensitivity or resistance of these organisms to antifungal drugs. Different methods are used to determine the sensitivity of fungi to medicine. The best method used in most studies is to determine the minimum inhibitory concentration (MIC) in a liquid medium (broth microdilution). Also, the Disk Gel Diffusion sensitivity identification method is also used in some other studies. In a sensitivity study, 15 fungal species from clinical specimens of otomycosis were tested against nystatin, clotrimazole, and miconazole in dilutions of 100 units per disc, 10 and 50 micrograms per disc, respectively. Also, the sensitivity of these fungi to different dilutions of sorbitan from 0.125-2 micrograms per disc was studied. The results showed that *Aspergillus* spp with the whole diameters between 21-32 mm were sensitive to the above drugs [40]. Also, it has been shown that the sensitivity of *A. niger* was greater to clotrimazole (95.4%) than fluconazole (90.47%); however, their difference was not significant [5].

Conclusion

The present study was a review of 9 studies investigating bacterial pathogens and 25 studies on fungal pathogens (eight of which had both bacterial and fungal pathogens). It was shown although outer ear infections are mainly caused by bacterial pathogens; the contribution of fungi to the growth of these infections is increasing, as it is evident in the

present review study. This is almost similar to the findings of other countries. On the other hand, the diversity of organisms (bacterial or fungal) is also changing, and new species are introduced as factors causing outer ear infections. Furthermore, some fungi that have played a minor role in otomycosis are isolated from these patients. Due to these changes, it seems that the methods of treatment for these patients should be modified and the use of antifungal drugs with the anti-bacterial antibiotics, as one method to improve or eliminate the problems of these patients, should be included in the treatment protocol. In addition to the improved recovery, this would lead to a reduced number of referrals and decreased costs.

References

1. Ong YK, Chee G. Infection of the external ear. Ann Acad Med Singapore. 2005; 34(4):330-4. PMid:15937574

2. Hirsch BE. Infections of the external ear. Am J Otolaryngol. 1992; 13(3):145-55. https://doi.org/10.1016/0196-0709(92)90115-A

3. Kiakojuri K, Nazari M, Rajabnia R, Khafri S, Mahdavi Omran S. The evaluation of the causes of otitis externa in children referring to the ENT clinic of Ayatollah Rohani Hospital of Babol, 2013-14. J Babol Univ Med Sci. 2015; 17(5):25-30.

4. Kiakojuri K, Mahdavi Omran S, Jalili B, Hajiahmadi M, Bagheri M, Ferdousi Shahandashti E, et al. Bacterial otitis externa in patients attending an ENT clinic in Babol, North of Iran. Jundishapur J Microbiol. 2016; 9(2):e23093. https://doi.org/10.5812/jjm.23093 PMid:27127584 PMCid:PMC4841979

5. Nemati SH, Hassanzadeh R, Khajeh Jahromi S, Delkhosh Nasrollah Abadi A. Otomycosis in the north of Iran: common pathogens and resistance to antifungal agents. Eur Arch Otorhinolaryngol. 2014; 271(5):953-7. https://doi.org/10.1007/s00405-013-2486-0 PMid:23595615

6. Mehdinejad M, Khosravi AD, and Mahmoudabadi AZ. Study of bacterial flora in children,s with hearing aid earmoulds in Ahvaz, Iran. Pak J Biol Sci. 2010; 13(5):245-8. https://doi.org/10.3923/pjbs.2010.245.248 PMid:20464948

7. Kochak Alavi SK, Irajian GH, Beheshti AS, Bineshian F, Hajighorbani AH. The freguency of bacterial agents in otitis externa from Semnan seneitivity test (2000-2003). Semnan J Med Sci. 2004; 6(2):135-9.

8. Shokohi T, Ahanjan M, Kasiri A. The microbiology and mycology survey of otitis externa in Bu Ali Sina ear nose and throut clinic, Sari. J Mazandaran Univ Med Sci. 2001; 11(32):1-9.

9. Akhi MT, Ahmadian A, Nejadkazem M, Ramazanzadeh R. Study on an aerobic bacteria isolated from otitis externa and some predisposing factors (Tabriz. 1998). Tabriz J Med Sci. 2001; 35(52):5-10.

10. Kiakojori K, Gooran AK, Rajabnia R, Mahdavi Omran S. A study on the effectiveness of boericke alcohol and miconazole ointment for the prevention of outer ear infections after suction clearane. J Babol Univ Med Sci. 2015; 17(4):29-35.

11. Mahdavi Omran S, Jalili B, Rajabnia R, and Kiakojuri K. clinical and demographical findings of otitis externa in adult patients who referred to Roohani Hospital, Babol, Iran. Int J Curr Microbiol App Sci. 2015; 4(5):133-9.

12. Barati B, Okhovvat SAR, Goljanian A, Omrani MR. Otomycosis in central Iran: a clinical and mycological study. Iran Red Crescent Med J. 2011; 13(12):873-6. PMid:22737432 PMCid:PMC3371907

13. Ebrahimzadeh A, Mousavi M. Fungal flora in external ear canal in chronic otitis media. J Gorgan Univ Med Sci. 2014; 16(3):122-6.

14. Bineshian F, Irajian GH, Koochak- Alavi SK, Fredonian MR. A study on the frequency of fungal agents in otitis externa in Semnan. Iran J Pathol. 2006; 1(4):141-4.

15. Kiakojuri K, Rajabnia R, Jalili B, Khafri S, Mahdavi Omran S. Otomycosis in adolescent patients referred to the therapeutic centers in Babol city, Iran. Jundishapur J Microbiol. 2015; 8(5):e17138. https://doi.org/10.5812/jjm.8(5)2015.17138

16. Nowrozi H, Doustdar Arabi F, Ghaffarnejad Mehraban H, Tavakoli A, Ghooshchi GH. Mycological and clinical study of otomycosis in Tehran, Iran. Bull Env Pharmaco Life Sci. 2014; 3(2):29-31.

17. Golshiri A, Mokhtaree MR, Bahramabadi R, Shabani Z, Sayadi A, Abbasi A. Prevalence of microbial agents and pattern of antimicrobial resistance in patients with acute otitis externa in Rafsanjan in 2011. Com Health J. 2014; 7(4):10-7.

18. Balouchi M, Berjis N, Okhovat AR. The frequency of fungul infections of externa ear. Isfahan Med J. 2006; 24(82):72-5.

19. Zarei Mahmoudabadi A. Mycological studies in 15 cases of otomycosis. Pak J Med sci. 2006; 22(4):486-8.

20. Zarei Mahmoudabadi A, Masoomi SA, Mohammadi H. Clinical and mycological studies of otomycosis. Pak J Med Sci. 2010; 26(1):187-90.

21. Berjis N, Okhovat SA, Soleimani Koujani Z, Baradaran SH. Comparing the therapeutic effect of clotrimazole and tolnaftate in treating variety of fungal species producing otomycosis in Alzahra and Kashani Hospitals, Iran. J Isfahan Med Sch. 2012; 29(164):1831-41.

22. Saki N, Rafiei A, Nikakhlagh S, Amirrajab N, Saki S. Prevalence of otomycosis in khouzestan province, south- west Iran. J Laryngol Otol. 2013; 127(1):25-7. https://doi.org/10.1017/S0022215112002277 PMid:23164073

23. Kiakojuri K, Hasanjani Roshan MR, Sepidgar SAA. Suction clearance and 2% topical miconazole versus the same combination with acidic drops in the treatment of otomycosis Southeast Asian J Trop Med Public Health. 2007; 38(4):749-53. PMid:17883017

24. Yeganeh Moghadam A, Asadi MA, Dehghani R, Hooshyar H. The Prevalence of otomycasis in Kashan, Iran, during 2001-2003. Jundishapour J Microbiol. 2009; 2(1):18-21.

25. Kazemi A, Majidinia M, Jaafari A, Ayatollahi Mousavi SA, Zarei Mahmoudabadi A, and Alikhah H. Etiologic agents of otomycosis in the north- western area of Iran. Jundishapur J Microbiol. 2015; 8(9):e21776. https://doi.org/10.5812/jjm.21776 PMid:26495108 PMCid:PMC4609173

26. Afshari MA, Kachuei R, Ajal Loueial M. The frequencey of otomycosis in pateints who reffered to ENT clinic, Baghiatolah Hospital. Teb Nezami. 2005; 7(2):121-4.

27. Gharaghani M, Seifi Z, Zarei Mahmoudabadi A. Otomycosis in Iran: a review. Mycopathologia. 2015; 179(5-6):415-24. https://doi.org/10.1007/s11046-015-9864-7 PMid:25633436

28. Kiakojuri K, Mahdavi Omran S, Rajabnia R, Asgarzadeh SH. Inhibitory effect of cerumen of fungi with potential of otomycosis Iran J of Otorhinolaryngol. 2010; 22(59):31-8.

29. Rajabnia R, Mahdavi Omran S, Majidian AR, Aghajanpour SM. Comparison of fungal flora in patients with acute otitis externa and healthy subjects. J Babol Univ Med Sci. 2010; 12(3):36-7.

30. Akhi M, Ahmadian A, Nejadkazem M, Ramazanzadeh R. Study on aerobic bacteria isolated from otitis externa and their sensitivity. Tabriz J Med Sci. 2001; 35(51):5-9.

31. Hajjartabar M. Pseudamonas aeruginosa isolated from otitis externa associated with recreational waters in some public swimming pools in Tehran Iran J Clin Infet Dise. 2010; 5(3):142-51.

32. Taziki MH, Bahnampour N. Causes of primary otalgia. J Gorgan Uni Med Sci. 2013; 15(3):110-3.

33. Sheikh MS, Qazi BY, Rameen B. Otomycosis in Khozestan Ind J Otolaryngol Head Neck Surg. 1993; 45(2):73-7.

34. Zarei Mahmoudabadi A, Abshirini H, Rahimi R. Fungal flora of hearing aid moulds and ear canal in hearing aid wearers in school children in Ahvaz, Iran(2008). Jundishapur J Microbiol. 2009; 2(1):22-4.

35. Pakshir K, Sabayan B, Javan H, Karamifar K. Mycoflora of human external auditory canal in Shiraz, southern Iran. Iran Red Crescent Med J. 2008; 10(1):27-9.

36. Kiakojori K, Mahdavi Omran S, Majidian AR, Ferdosi Shahandashti E, Daroonkolaii A, Rajabnia R. Comparing cerumen bacterial flora in acute otitis externa patients and healthy controls. Iran J Otorhinolaryngol. 2010; 22(60):93-6.

37. Kalantar E, Mosaei M, Ekrami A, Pedram M. Isolation and antimicrobial susceptibility of bacteria from external ear canal of cancer patients at Shafa Cancer Hospital- Ahvaz. J Center Res Ther. 2006; 2(1):17-9. https://doi.org/10.4103/0973-1482.19769

38. Mikaeili A, Nazari S. Aspergillus and otomycosis in Kermanshah, Iran. 5th Advances against Aspergillosis, 26-28 January 2012, Istanbul: Turkey, 2012.

39. Bauer C, Jenkins H. Otologic symptoms and syndromes. Cummings Otolaryngology: Head & Neck Surgery 6th ed Philadelphia, PA: Elsevier Saunders, 2015.

40. Zarei Mahmoudabadi A, Seifi Z, Gharaghani M. Lamisil, a potent alternative antifungal drug for otomycosis. Current Medical Mycology. 2015; 1(1):18-21. https://doi.org/10.18869/acadpub.cmm.1.1.18 PMid:28680976 PMCid:PMC5490317

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Apr 15; 7(7):1241-1245.
https://doi.org/10.3889/oamjms.2019.264
eISSN: 1857-9655
Review Article

Hypertension after Kidney Transplantation: Clinical Significance and Therapeutical Aspects

Galina Severova-Andreevska[1*], Ilina Danilovska[1], Aleksandar Sikole[1], Zivko Popov[2,3], Ninoslav Ivanovski[3,4]

[1]University Clinic of Nephrology, Medical Faculty, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia; [2]Macedonian Academy for Science and Arts, Skopje, Republic of Macedonia; [3]Zan Mitrev Clinic, Skopje, Republic of Macedonia; [4]Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia

Citation: Severova-Andreevska G, Danilovska I, Sikole A, Popov Z, Ivanovski N. Hypertension after Kidney Transplantation: Clinical Significance and Therapeutical Aspects. Open Access Maced J Med Sci. 2019 Apr 15; 7(7):1241-1245. https://doi.org/10.3889/oamjms.2019.264

Keywords: Kidney transplant recipients; Arterial hypertension; Antihypertensive drugs; Review article

***Correspondence:** Galina Severova-Andreevska. University Clinic of Nephrology, Medical Faculty, University Ss "Cyril and Methodius" of Skopje, Skopje, Republic of Macedonia. E-mail: galinaseverova@yahoo.com

Received: 02-Feb-2019; **Revised:** 02-Apr-2019; **Accepted:** 03-Apr-2019; **Online first:** 14-Apr-2019

Funding: This research did not receive any financial support

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

Abstract

Most of the kidney transplanted patients develop arterial hypertension after renal transplantation. Together with very well-known and usual risk factors, post-transplant hypertension contributes to the whole cardiovascular morbidity and mortality in the kidney transplant population. The reasons of post-transplant hypertension are factors related to donors and recipients, immunosuppressive therapy like Calcineurin Inhibitors (CNI) and surgery procedures (stenosis and kinking of the renal artery and ureteral obstruction). According to Eighth National Committee (JNC 8) recommendations, blood pressure > 140/90 mmHg is considered as hypertension. The usual antihypertensive drugs used for the control of hypertension are Calcium channel blockers (CCB), Angiotensin-converting enzyme (ACE) inhibitors, Angiotensin –II receptor blockers (ARB), B- blockers and diuretics. Follow the KDIGO guidelines the target blood pressure < 140/90 mmHg for patients without proteinuria and < 125/75 mmHg in patients with proteinuria is recommended. Better control of post-transplant hypertension improves the long-term graft and patient's survival.

Introduction

Post-transplant cardiovascular disease

Kidney transplantation is the optimal treatment of patients with ESRD who would otherwise require dialysis. Despite the fact that kidney transplantation reduces the risk of both, cardiovascular morbidity and mortality remain a leading cause of death with functioning graft among kidney transplant recipients (KTR) [1], [2], [3], [4] Among the traditional and non-traditional risk factors, post-transplant hypertension (PTxH) remains one of the major contributors to the post-transplant cardiovascular morbidity (PTCVM) and mortality and the most common causes of chronic graft dysfunction and kidney transplant failure [5], [6], [7], [8]. Cardiovascular pathology is responsible for approximately 40% of deaths among KTR [9]. The annual risk of fatal and non-fatal cardiovascular events in KTR is 3-5% which is 50-fold higher than in the general population. Both, classical and non-classical cardiovascular factors equally contribute to the increased incidence of PTCVM [10], [11], [12].

Hypertension and Post-Transplant Hypertension

According to 8-the report of Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure (JNC 8) the new blood pressure classification was introduced bearing in mind the target organ damage and the presence of other factors of comorbidity like Chronic Kidney Disease (CKD), Diabetes, BMI, Hyperlipidemia etc. Thus, Hypertension is defined if office BP is ≥ 140/90 and ambulatory BP ≥ 130/90 in normal persons under the age of 60. The prevalence of PTxH among kidney

recipients is between 55-90% [13], [14]. Significant contributions to the clinical outcome of hypertensive KTR are age, BMI, time after the surgery, gender, presence of chronic allograft nephropathy, cellular and antibody-mediated rejection episodes, use of immunosuppressant, etc.

Regarding hypertension in CKD and transplant patients, KDIGO recommendations are more strict: The BP should be kept below or equal of 130/85 and 125/75 if the patients are proteinuric [15], [16]. The introduction and acceptance of ambulatory blood pressure (ABP) control in the follow up of hypertension in general population as well as in KTR enables more detailed classification of hypertension and therefore a new patient stratification. In KTR both, office and ambulatory BP control should be performed in every day clinical practice. Out of the hypertension classification according to the JNC 8, recently different types of hypertension were clinically introduced as: Masked Hypertension (MH), White Coat Hypertension (WCH), Sustained Hypertension (SH), Isolated Nocturnal Hypertension (INH), Isolated Diurnal Hypertension (IDH), Awake and Asleep hypertension, all with `confirmed clinical significance [17], [18], [19], [20] The most investigated types of hypertension among kidney transplant recipients and CKD patients are WCH and MH. WCH is defined as well-controlled home hypertension but poorly controlled clinic hypertension. MH is the reverse phenomenon, poorly controlled BP at home but normal in the clinic.

The prevalence of MH among KTR is between 23 and 58% with a confirmed harmful effect on graft and patients survival. WCH with a percentage of 4-20% is less important, but still require awareness [20], [21], [22]. The appearance of resistant hypertension (7% of PTxH) is also a confirmed entity which should be treated successfully with carefully chosen antihypertensive drugs [23], [24], [25]. All types of hypertension defined by ABP monitoring are largely investigated in renal transplant recipients and are parts of the efforts to control PTxH on a recommended level according to KDIGO criteria. Table 1 presents a different classification of Hypertension which is also used in KTR.

TABLE 1: Definition, types and classification of hypertension

Types	Values
CBP – clinical blood pressure	Average BP ≥ 140/90
ABP – Ambulatory blood pressure	Average BP ≥ 130/90
Awake ABP	BP ≥ 135/85
Sleep ABP	BP ≥ 120/70
Normotension	CBP and ABP within the normal range
Masked Hypertension	CBP – within normotensive range ABP – within hypertensive range
White coat Hypertension	CBP – within hypertensive range ABP – within normotensive range
Sustained Hypertension	CBP – within hypertensive range ABP – within normotensive range
Nighttime Hypertension (Nocturnal)	Awake ABP – within normotensive range Sleep ABP –within hypertensive
Daytime Hypertension (Diurnal)	Awake ABP – within hypertensive range Sleep ABP – within normotensive range
All-day Hypertension	Awake ABP – within hypertensive range Sleep ABP – within hypertensive range

CBP – office control of BP; AMB – 24 h – ambulatory BP control.

Pathogenesis of post-transplant hypertension

The pathogenesis of hypertension during the post-transplant period is multifactorial which include traditional and non-traditional risk factors. Some of them are related to transplant surgery, underlying kidney disease, use of immunosuppression, chronic or acute graft rejections. The majority of these factors are reversible, and some are preventable. They are presented in Table 2.

TABLE 2: Causes of Post-Transplant Hypertension

Factors	Examples
Immunosuppression	Cyclosporine
	Tacrolimus
	Glucocorticoid
Graft disease	Delayed graft function
	Chronic allograft nephropathy
	De novo and recurrent glomerular disease
	Acute rejection
Recipient factors	Underlying kidney disease
	Essential hypertension
	Native kidney presence
	Excessive weight gain
	Secondary Hyperparathyroidism
Donor factors	Preexisting donor hypertension
	Advanced donor age
	Subarachnoidal haemorrhage
	Use of right kidney
	Female gender
Surgery	Transplant renal artery stenosis
	Use of right kidney
	Cold and warm ischemia time
	Renal artery and vein anastomosis time

Regarding the mechanism of PTxH it seems that both, volume load and vasoconstriction are present. In the case of renal artery stenosis and CNI dependent hypertension, the vasoconstriction dominates due to the increase of renin-angiotensin system, up-regulation of endothelin-1 and reduction of the bioavailability of nitric oxide. Both cyclosporine and tacrolimus may cause a salt-sensitive form of hypertension with the consecutive fluid overload [26], [27], [28].

Among donor dependent risk factors are already well-confirmed age, history of hypertension, underlying disease (diabetes) vascular disease and genetic predisposition. The use of steroids facilitates sodium and volume retention and contributes to insulin resistance and diabetes.

The positive experience of renal denervation of native kidneys in renal transplant recipients, even in several cases, emphases the possible role of sympathetic overactivity [29], [30]. The appearance of renal transplant artery stenosis put for sure RA system in the game which could be solved with the appropriate therapeutic procedure. In the cases of recurrent or de-novo glomerulonephritis, classical mechanisms of renal hypertension are involved [31].

Long term consequences

Increased cardiovascular morbidity and mortality

Like in general population many studies confirmed a PTxH as a strong risk factor for ischemic heart disease, congestive heart failure, coronary artery disease, increased arterial stiffness and stroke. The most clinically significant consequence of hypertension is left ventricular hypertrophy, left atrial enlargement and diastolic dysfunction which are responsible for a variety of cardiovascular events after kidney transplantation. Some authors find a strong correlation between left ventricular hypertrophy and post-transplant isolated nocturnal hypertension while 24-hour ABP hypertension and altered Night-Day BP profile are independently associated with Carotid Intima/Media thickness [32], [33].

Chronic Graft Dysfunction

Together with rejection, PTxH is one of the major factors which is responsible for reduced long-term graft and patients survival. A single centre study on long-term kidney transplant survival rate, KTR with diastolic BP of 89-99 mm Hg had statistically decreased GFR compared to recipients with lower blood pressure. Also, recipients with lower blood pressure in the first year have better graft survival. The study of Mange et al. demonstrated the increased risk of graft failure for every 10 mm Hg in SBP and 10 mm Hg in diastolic BP [34]. Higher blood pressure correlates with greater rates of progression of decreased renal function.

Chronic allograft nephropathy, renamed by Banff classification with interstitial fibrosis and tubular atrophy (IF-TA), is associated with gradual deterioration in graft function, relevant proteinuria and new or worsening hypertension in the absence of any other worsening factors. Non-HLA antibody-mediated rejection, with an antibody targeting angiotensin II type receptors, develops hypertension secondary to vascular rejection. According to the Collaborative Transplant Study PTxH is an independent risk factor for chronic allograft nephropathy and graft failure [35]. Increasing systolic BP was associated with decreased graft survival at any level of diastolic pressure.

Regarding the association with host alloimmune response and acute and chronic rejection, post-transplant hypertension has been confirmed among the rejection of free kidney transplant recipients. However, it could be not excluded that PTH has a deleterious effect of graft small vessels with an increasing of immunogenicity of impaired graft tissue. In addition, some experimental studies confirmed that hypertension increases expression of growth factors and MHC II in chronic allograft nephropathy. PTxH is strongly associated with chronic allograft nephropathy and vice-versa and presents a significant non-immunological risk factor for late graft failure. Altered day-night BP profile, reverse dipper pattern are also associated with early inflammation and constitutes an independent predictor of graft failure [36], [37], [38], [39], [40].

Treatment

Lifestyle

The usual recommendations of the Hypertension Associations worldwide may also be applied to kidney transplant recipients. Slightly reduction of salt intake, 30-60 min of moderate daily physical or aerobic activity, maintenance of body mass index between 18-25 kg/m2 and keeping the waist circumference under 102 for men and <88 for women. Light dietetic measures could also be recommended as a low-fat diet, moderate alcohol consumption and more vegetables and fruit in everyday nutrition [27].

Treatment target and pharmacological management

Bearing in mind all the consequences of non-controlled BP in KTR, achievement of target BP level is strongly recommended. The pharmacological treatment should start when office BP is more than 140/90 or ABP more than 130/80 as well as in any case of isolated nocturnal, masked and sustained hypertension or altered day-night time BP profile. The start should be with thiazide diuretics with the addition of Calcium Channel Blockers (CCB), Angiotensin-II Receptor Blockers (ARB) and Angiotensin-II Converting Enzyme (ACE) inhibitors according to the KDIGO and JNC 8 recommendations [13], [15]. But the regulation and reaching of target BP level are far of an easy task. An average analyses of BP in kidney transplant recipients confirm that despite the whole spectrum of antihypertensives and careful BP measurements, the majority of the patients are still uncontrolled. Whenever we should start our pharmacological approach to the PTxH, hypertensive effects of CNI (especially Cyclosporine A) has to be taken into consideration. The use of other immunosuppressive protocols including mTOR inhibitors or Belatacept instead of CNI may provide better control and beneficial effects on long term graft and patients survival [27].

Invasive procedures

In patients with proven transplant renal artery stenosis, Percutaneous transluminal angioplasty (PTA) and surgery could be applied if the degree of a

stenotic lesion is more than 80%. However, it should be careful in decision because of polar infarcts, haematoma, intimal flaps, thrombosis and anastomotic re-stenosis [29], [30].

Renal denervation of the native kidneys is an interesting and probably promising therapeutic procedure which can be effective in some individual cases. A few reports confirmed a beneficial effect of this procedure, but it is still far of any definitive conclusion. Interestingly the effect was on nocturnal, and ABP was some of the non-dipper patients became dipper [31].

In the recent report from Dallas (USA), Lerman et al. performed laparoscopic bilateral nephrectomy in 5 cases of resistant hypertension in kidney and pancreas transplant recipients. Mean arterial pressure improved in the next six months after the surgery and renal function remained stable. Despite the beneficial results of this small report, there is no sufficient data to recommend this aggressive procedure. The future controlled studies should confirm the justification of surgical approach as a therapeutic measure [41].

Conclusion

The high prevalence of arterial hypertension in KTR contributes to chronic graft damage and significantly decreased graft and long-term patient survival. Despite the evidence of the adverse effects of hypertension, BP control has been poor despite the use of different combinations of antihypertensive drugs. Adequate diagnosis methods should be permanently implemented using classical office and home BP readings. Twenty-four hour ABPM allows very valuable information on circadian rhythm and nocturnal blood pressure. PTH remains a very important issue in clinical follow up of kidney transplant recipients.

References

1. Naele J and Smith A. Cardiovascular risk factors following renal transplant.World J Transplant 2015; 5:183-195. https://doi.org/10.5500/wjt.v5.i4.183 PMid:26722646 PMCid:PMC4689929

2. Kono K, Fujii H, Nakai K et al. Relationship Between Type of Hypertension and Renal Arteriosclerosis in Chronic Glomerular Disease. Kidney Blood Press Res. 2014; 41:374-383. https://doi.org/10.1159/000443440 PMid:27327274

3. Stoumpos S, Jardien A and Mark P. Cardiovascular morbidity and mortality after kidney transplantation. Transplant Int. 2015; 28:10-21. https://doi.org/10.1111/tri.12413 PMid:25081992

4. Svensson M, Jardine A, Fellstrom B et al. Prevention of cardiovascular disease after renal transplantation. CO-Transplantation. 2012; 17 (4):393-400. https://doi.org/10.1097/MOT.0b013e3283560a3b

5. Konstantopoulou AS, Konstantopoulou PS, Paapargyriou Ik et al. Masked, white coat and sustaned hypertenson : comparison of target organ damage and psychometric parameters. Journal of Human Hypertension. 2010; 24:151-157. https://doi.org/10.1038/jhh.2009.55 PMid:19571827

6. Soveri I, Holme I, Holdaas H et al. A cardiovascular risk calculator for renal transplant recipients. Transplantation. 2012; 94:57-62. https://doi.org/10.1097/TP.0b013e3182516cdc PMid:22683851

7. Tainio J, Quist E, Miettinen J et al. Blood Pressure Profiles 5-10 Years After Transplant in Pediatric Solid Organ Recipients. J Clin Hypertens. 2015; 17:154-161 https://doi.org/10.1111/jch.12465 PMid:25557075

8. Drawz PE, Alper AB, Anderson Ah et al. Masked Hypertension and Elevated Nighttime Blood Pressure in CKD: Prevalence and Association with Target Organ Damage. Clin J Am Soc Nephrol. 2016; 11(4):642-52. https://doi.org/10.2215/CJN.08530815 PMid:26912547 PMCid:PMC4822674

9. Malamaci F, Tripepi R, Leonardis D et al. Nocturnal Hypertension and Altered Night-Day BP Profile and Atherosclerosis in Renal Transplant Patients. Transplantation. 2016; 100:2201-2211. https://doi.org/10.1097/TP.0000000000001023

10. Aparicio LS, Alfie J, Barochiner J, Cuffaro PE, Rada M, Morales M, Galarza C, Waisman GD. Hypertension: the neglected complication of transplantation. ISRN Hypertension. 2013; 2013:1-10. https://doi.org/10.5402/2013/165937

11. Prasad G, Rizicka M, Burns K et al. Hypertension in dialysis and kidney transplant patients. Can J Cardiol. 2009; 25(5):309-314. https://doi.org/10.1016/S0828-282X(09)70495-7

12. Konstantopoulou AS, Konstantopoulou PS, Paapargyriou Ik et al. Masked, white coat and sustaned hypertenson: comparison of target organ damage and psychometric parameters. Journal of Human Hypertension. 2010; 24:151-157. https://doi.org/10.1038/jhh.2009.55 PMid:19571827

13. James PA, Oparil S, Carter BL, Cushman WC, Dennison-Himmelfarb C, Handler J, Lackland DT, LeFevre ML, MacKenzie TD, Ogedegbe O, Smith SC. 2014 evidence-based guideline for the management of high blood pressure in adults: report from the panel members appointed to the Eighth Joint National Committee (JNC 8). Jama. 2014; 311(5):507-20. https://doi.org/10.1001/jama.2013.284427 PMid:24352797

14. Sberro-Soussan R, Rabant R, Snanoudj R et al. Home and office blood pressure monitoring in renal transplant recipients. Journal of Transplantation 2012; 2012:1-6. https://doi.org/10.1155/2012/702316 PMid:22577515 PMCid:PMC3345274

15. Kasiske B, Zeier M, Chapman J et al. KDIGO Clinical practice guideline for the care of kidney transplant recipients: a summary. Kidney Int. 2010; 77(4):299-311. https://doi.org/10.1038/ki.2009.377 PMid:19847156

16. Hamdani G, Nehus E, Hanevold C et al. Ambulatory Blood Pressure, Left Ventricular Hypertrophy and Allograft Function in Children and Young Adults After Kidney Transplantation. Transplantation. 2017; 101:150-156. https://doi.org/10.1097/TP.0000000000001087 PMid:26895218 PMCid:PMC4990501

17. Kaul A, Sharma RK, Gupta A et al. Spectrum of Hypertension in post transplant. JAPI. 2010; 58:221-223. PMid:21046874

18. Saxena A, Sharma A. Hypertension in Post-renal Transplant Patients. Saudi J Kidney Dis Transpl. 2014; 25:22-28. https://doi.org/10.4103/1319-2442.124466 PMid:24434378

19. Castillo-Lugo J and Vergne-Marini P. Hypertension in Kidney Transplantation. Semin Nephrol 2005; 25:252-260. https://doi.org/10.1016/j.semnephrol.2005.02.009 PMid:16202698

20. Mangray M and Vella J. Hypertension after Kidney Transplant. Am J Kidney Dis. 2011; 57:331-341.

https://doi.org/10.1053/j.ajkd.2010.10.048 PMid:21251543

21. Kaul A, Sharma RK, Gupta A et al. Spectrum of Hypertension in post transplant. JAPI. 2010; 58:221-223. PMid:21046874

22. Mange KC, Cizman B, Joffe M et al. Arterial Hypertension and Renal Allograft Survival. JAMA. 2000; 283:633-638. https://doi.org/10.1001/jama.283.5.633 PMid:10665703

23. Arias M, Fernandez -Fresco G, Gago M, et al. Clinical characteristics of resistant hypertension in renal transplant patients. Nephrol Dial Transplant . 2012; 27(4):S36-S38. https://doi.org/10.1093/ndt/gfs481

24. Tantasattamo E, Ratanasrimetha P, Spanuchart I et al. Overlooked Cause of Resistant Hypertension in a New Kidney. Ann Clin Exp Hypertension. 2015; 3(2):1030.

25. Lakkis JI. Weir MR. Treatiment - resistant hypertension in the transplant recipient. Semin Nephrol. 2014; 34:560-570. https://doi.org/10.1016/j.semnephrol.2014.08.010 PMid:25416665

26. Kaufeld J, Schiffer M, Chatzikyrkou C. Pathogenesis and Manegement of Hypertension after Kidney Transplantation. Current Hypertension Reviewrs. 2012; 8:296-301. https://doi.org/10.2174/1573402111208040296

27. Ponticelli C, Cucchiari D and Graziani G. Hypertension in kidney transplant recipients. Transplant Int. 2011; 24:523-533. https://doi.org/10.1111/j.1432-2277.2011.01242.x PMid:21382101

28. Alizadeh F, Dehghani S, Rahmati M et al. Does hypertension remain after Kidney Transplantation. Acta Medica Iranica. 2015; 53:297-300. PMid:26024705

29. Hiremath S, Fergusson D, Doucette S et al. Renin Angiotensin System Blockade in Kidney Transplantation: A Systemic Review of the Evidence. Am J Transplant. 2007; 7:2350-2360. https://doi.org/10.1111/j.1600-6143.2007.01928.x PMid:17845569

30. Dragun D, Muller DN, Brasen JH, et al. Angiotenzin II type 1-receptors activating antibodies in renal- allograft rejection. N Engl J Med. 2005; 352(6):558-569. https://doi.org/10.1056/NEJMoa035717 PMid:15703421

31. Dobrowolski L, Bemelman F, ten Berge I et al. Renal Denervation of the Native Kidneys For Drug - resistant Hypertension After Kidney Transplantyation. Clin Kidney J. 2015; 8:79-81. https://doi.org/10.1093/ckj/sfu134 PMid:25713714 PMCid:PMC4310436

32. Toprak A, Koc M, Tezcan H et al. Night - time blood pressure load is associated with higher left ventricular mass index in renal transplant recipients. Journal of Human Hypertension. 2003; 17:239-244. https://doi.org/10.1038/sj.jhh.1001536 PMid:12692568

33. Demirkol O, Oruc M, Ikitimur B et al. Ambulatory Blood pressure Monitoring and Echocardiographic Findings in Renal Transplant Recipients. J Clin Hypertens. 2016; 18:766-771. https://doi.org/10.1111/jch.12755 PMid:26689296

34. Mange KC, Cizman B, Joffe M et al. Arterial Hypertension and Renal Allograft Survival. JAMA. 2000; 283:633-638. https://doi.org/10.1001/jama.283.5.633 PMid:10665703

35. Opelz G, Wujciak T, Ritz E et al. Association of chronic kidney graft failure with recipient blood pressure.Collaborative Transplant Study. Kidney Int. 1998; 53:217-222. https://doi.org/10.1046/j.1523-1755.1998.00744.x PMid:9453022

36. Modena FM, Hostetter TH, Salahudeen AK, Najarian JS, Matas AJ, Rosenberg ME. Progression of kidney disease in chronic renal transplant rejection. Transplantation. 1991; 52(2):239-244. https://doi.org/10.1097/00007890-199108000-00011 PMid:1871796

37. Fernandez-Fresnedo G, Palomar R, Escallada R et al. Hypertension and long-term renal allograft survival. Nephrol Dial Transplant. 2001; 16:105-109. https://doi.org/10.1093/ndt/16.suppl_1.105 PMid:11369835

38. Solez K, Colvin RB, Racusen LC,et al. Banff'05 meeting report: differential diagnosis of chronic allograft injury and elimination of chronic allograft nephropaty ('CAN'). Am j Transplant. 2007; 7(3):518-526. https://doi.org/10.1111/j.1600-6143.2006.01688.x PMid:17352710

39. Massy Z. Guijarro C, Wiederkehr M et al. Chronic renal allograft rejection: Immunologic and nonimmunologic risk factors. Kidney Int. 1996; 49:518-524. https://doi.org/10.1038/ki.1996.74

40. Schindler R, Tullius S, Tanriver Y et al. Hypertension increases expression of growth factors and MHC II in chronic allograft nephropathy. Kidney Int. 2003; 63:2302-2308. https://doi.org/10.1046/j.1523-1755.2003.00034.x PMid:12753322

41. Lerman M, Hinton S and Aronoff R. Bilateral native nephrectomy for refractory hypertension in kidney transplant recipients. International Journal of Surgery Case Reports. 2015; 15:127-129. https://doi.org/10.1016/j.ijscr.2015.08.001 PMid:26348394 PMCid:PMC4601950