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Study of Hemolysin Gene "*aspHS*" and Its Phenotype in *Aspergillus Fumigatus*

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

Abstract

AIM: The main goal of this study was to analysis the "*aspHS*" gene and its phenotype in *A. fumigatus.*

METHODS: Fifty-three *A. fumigatus* strains, including environmental, clinical and reference isolates, were used in this research. PCR was carried out based on Asp-hemolysin gene sequence. Two restriction enzymes *TagI* and *NcoI* were employed for digestion of PCR products.

RESULTS: PCR products of 180 and 450 bp were generated for all *A. fumigatus* isolates. Digestion of the *aspHS* gene 180 bp amplicons with *TagI* and 450 *bp amplicons with TagI and NcoI produced the expected bands for most isolates. Hemolysin production of A*. fumigatus isolates was evaluated on sheep blood agar (SBA).

CONCLUSION: In conclusion, our results provide evidence hemolysin activity and analysis of *aspHS* gene of *A. fumigatus*. These data may be useful in early diagnosis of *A. fumigatus* infections.

Introduction

An aspergillosis is a group of infections because of opportunistic infection caused by different species of *Aspergillus*. Among this disease, invasive aspergillosis (IA) is a severe nosocomial infection winch usually has a high mortality rate. *Aspergillus fumigatus* is most common etiological cause of IA, followed by *A. flavus*, *A. niger*, *A. terreus* [1], [2]. Asp-hemolysin is a hemolytic and cytolytic toxin from *A. fumigatus* [3].

Asp-hemolysin gene has been cloned, and sequence of the gene reported [4]. The primary sequencing of Asp-hemolysin gene product was predicted from cDNA. It has 131 amino acid residues and a molecular mass of 14 275.

The hemolysin has negatively charged domains. It enables the *A. fumigatus* to disrupt blood cells and can be identified in infected patients. The produced hemolysin by *A. fumigatus* promotes infection with *Aspergillus* species and also other opportunistic infections [5], [6], [7].

A. fumigatus possess a special combination of dissimilar virulence-related factors, creation it the most important global filamentous fungi pathogen. Nevertheless, although the hemolysin has toxicity effects, it appears not to be a major virulence factor but a compound which can increase the effects of other toxic pathogenicity factors [5], [6]. Hemolysin is lethal to chickens and mice, and it also is lytic for

erythrocytes of humans, sheep and rabbits. This toxin has cytotoxic effects on macrophages and endothelial cells in vitro [8], [9].

Produced toxins seem with the fungus to defend itself from killers and competitors in environment. Moreover, these toxins could contribute to pathogenesis *A. fumigatus* because they are able directly invade the host tissue [10], [11].

The main goal of the current study was to compare hemolysin phenotype and genotype features among a variety of *A. fumigatus* isolates.

Material and Methods

Isolates of A. fumigatus

Fifty-three *A. fumigatus* isolates were used in this study. Four reference strains, including *A. fumigatus* IBRC-M 30033, IBRC-M 30040, IBRC-M 30048 and PTCC 5009, and 10 clinical and 39 environmental isolates of *A. fumigatus* were included. Eight clinical isolates were kindly provided by Dr Mojtaba Taghizadeh (Mazandaran University of Medical Sciences, Mazandaran, Iran). The environmental isolates were obtained from soil or air samples collected in Ahvaz, Iran.

The isolates were incubated on Sabouraud dextrose agar (Merck KGaA, Darmstadt, Germany) at 37°C. *A. fumigatus* isolates were identified morphologically. The isolates subcultured three times to obtain a pure culture and stained with lactophenol aniline blue. Vesicles, conidiophores, phialides and conidial arrangement were searched with a light microscope for morphological identification.

Hemolysin production of A. fumigatus isolates

Two microliters of spore suspension 5 × 10^6 from each *A. fumigatus* was inoculated on sheep blood agar (SBA). The Petri dishes were incubated at 37°C for 3 days, triplicate for each isolate. The presence of clear hemolysis in the medium indicated the evidence of hemolysin and recorded as a positive [12].

DNA extraction

One ml thick spore suspension from each *A. fumigatus* isolate was inoculated to an Erlenmeyer flask containing 100 ml yeast extract peptone dextrose medium (Merck KGaA) and incubated in an incubator shaker at 200 rpm under agitation for 48 h at 37°C for mycelia growth. The mycelia were harvested with filters, washed with 0.5 M EDTA and sterile dH2O and ground into a fine powder using liquid nitrogen with a pestle and mortar. One hundred mg powdered mycelium was transferred into a 1.5 ml sterile microtube containing 400 µl lysis buffer (100 mM Tris-HCl, pH 8.0, 30 mM EDTA, pH 8.0, 5% SDS w/v). After the incubation of the microtubes at 100°C for 20 min, 150 µl of 3 M acetate potassium was added. This suspension was kept at -20°C for 10 min and spun at 14,000 x g and 4°C for 10 min. After transferring of the supernatant to a 1.5 ml Eppendorf tube, 250 µl phenol-chloroform-isoamyl alcohol (25:24:1, v / v) was added, and the solution was shortly was vortexed and centrifuged for 10 min at 14,000 x g. The upper solution was transferred to a 1.5 ml tube, and 250 µl of chloroform-isoamyl alcohol (24:1) was added. The microtubes were then briefly vortexed and spun at 4°C and 14,000 x g for 10 min. The upper aqueous was transferred to a new microtube, and an equal volume of ice-cold 2-propanols was added. The solution was maintained at -20°C for 10 min and centrifuged at 14,000 x g for 10 min.

The supernatant was removed, and the pellet was washed with 300 µl ethanol 70%. After removal of the ethanol, DNA pellet was air-dried and dissolved in 50 µl dH2O.

PCR amplification

The fragments of the Asp-hemolysin gene were amplified by using primer sets: F-Asphs (5'-TGGTACAAGGACGGTGACAA-3') and R-Asphs (5'-GTCCCAGTGGACTCTTCCAA-3') for amplification of an 180 bp DNA [13] and Afhem1 (5' – GCATCGGTCCAAGCTTACGCA -3') and Afhm2 (5'— TTAACAGTTGCCAATGGCACC-3'') for amplification of an ~450 bp DNA [14]. Set up the PCR reactions for desired fragments to a final volume of 50 µl, containing reaction buffer, 2.2 mM MgCl2, 200 µM of each dNTP, 2.5 unit of Taq DNA polymerase (CinnaGen, Karaj, Iran), a 25 ng DNA template and 50 pmol of each primer.

Amplification conditions used were: For 180 bp fragment; Initial denaturation at 95°C for 10 min, 35 cycles of denaturation at 95°C for 1 min, annealing at 52°C for 2 min and extension at 72°C for 1 min. For 450 bp fragment; 35 cycles of denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec, extension at 72°C for 1 min, with a final extension at 72°C for 7 min [14]. Amplicons were analysed by 1% agarose gel electrophoresis in a Tris base, acetic acid and EDTA (TAE) buffer, and stained with ethidium bromide.

RFLP analysis

Restriction enzyme pattern of the 180 bp and 450 bp sequences were predicted for restriction endonucleases with Restriction Mapper Version3 software. *Tag*I (Fermentas; Thermo Fisher Scientific,

Inc., Waltham, MA, USA) for analysis of 180 bp fragment was used. *Nco*I (Fermentas; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and *Tag*I were performed for 450 bp fragment of Asp-hemolysin.

The reaction for each restriction enzyme was carried out in a total volume of 20 µl containing 10 units of the enzyme, 2 µl of the related buffer, 5 µl of the PCR product and Ultrapure water (CinnaGen, Karaj, Iran) to generate the 20-µl volume. Digested PCR products were subjected to electrophoresis on a 1.8% agarose gel in TAE buffer, and stained with ethidium bromide.

Sequencing

Several amplicons for each fragment were submitted for direct sequencing (Bioneer Corporation, Daejeon, South Korea). The sequences were searched for in the NCBI database (http://www.ncbi.nlm.nih.gov/). The sequences had 99-100% identity with *A. fumigatus* Asp-hemolysin gene deposited in the NCBI database. The package MEGA5 software (http://www.megasoftware.net) was applied for alignment of sequences.

Results

Hemolysin production test for A. fumigatus isolates

A total of 53 *A. fumigatus* isolates were screened for hemolysin production. The screening was performed with SBA. All 53 *A. fumigatus* isolates (100%) had able to produce hemolysin (Figure 1).

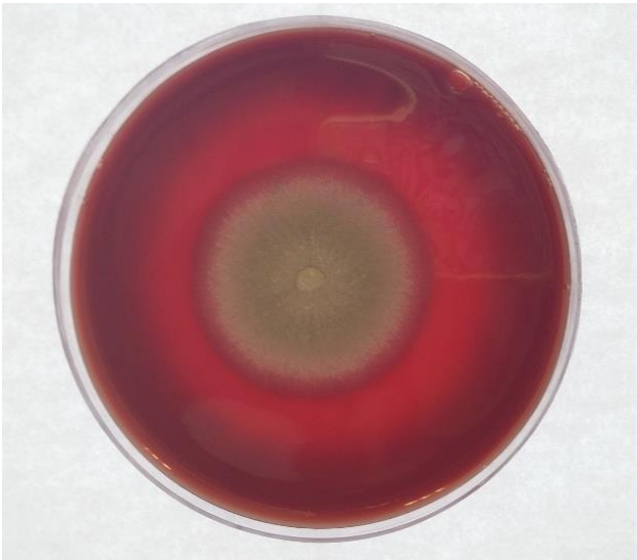

Figure 1: Hemolysin production of A. fumigatus on sheep blood agar (SBA) after 3 days at 37°C

A. fumigatus isolates gave the clear zone in a different ratio. The zone of the hemolysin production of isolates was ranged between 6-7.6 mm in diameter (Table 1).

Table 1: Ability of A. fumigatus isolates in hemolysin production on sheep blood agar at 37°C for 3 days

Sample type	6-6.5 mm		6.6-7 mm		7.1-7.6 mm		Total	
	No.	%	No.	%	No.	%	No.	%
Reference	0	0	1	25	3	75	4	100
Clinical	4	40	2	20	4	40	10	100
Environmental	3	8	24	61	12	31	39	100
Total	7	13	27	51	19	36	53	100

Thirty-six per cent of isolates has produced the zone of 7.1-7.6 mm. From this, the highest activity was belonging to reference isolates with 75% followed by clinical and environmental isolates with 40% and 30% subsequently.

Molecular variation analysis of 180 bp of aspHS gene

Using primers F-Asphs and R-Asphs, a 180 bp was amplified for all tested *A. fumigatus* isolates (Figure 2).

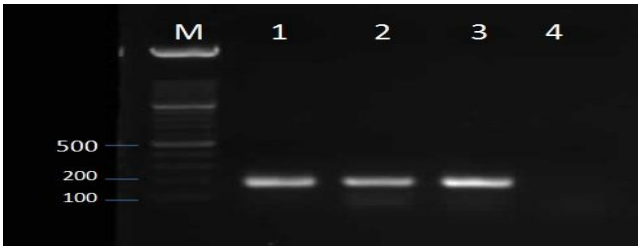

Figure 2: Agarose gel electrophoresis of 180 bp aspHS gene products of the A. fumigatus species with primers F-Asphs and R-Asphs. M, 100 bp ladder; lane 1, IBRC-M30033; lane2, IBRC-30040; Lane3, IBRC-30048; Lane4, no template control

Digestion of the *aspHS* gene amplicons with *Tag*I produced the two expected 115 and 65 bp fragments for 51 isolates. The isolates E1 and E2 showed different pattern after digestion with *Tag*I (Figure 3).

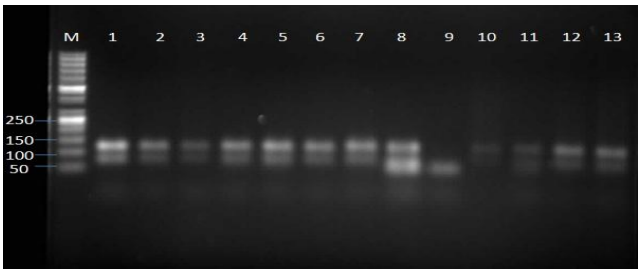

Figure 3: Restriction fragment pattern of 180 bp A. fumigatus aspHS gene digested with TagI. Lane M, 50 bp ladder; lane 1, E25; lane 2, E26; lane 3,E27; lane 4, E31; lane5, E35; lane 6, E37; lane 7, E45; lane 8,E1; lane 9, E2; lane 10, E3; lane11,E4; lane 12, E5; lane 13, E6

Molecular variation analysis of 450 bp of aspHS gene

PCR amplification of the *aspHS* gene with primers AFhem1 and AFhem2 resulted in a 450-bp band for all 53 *A. fumigatus* isolates (Figure 4).

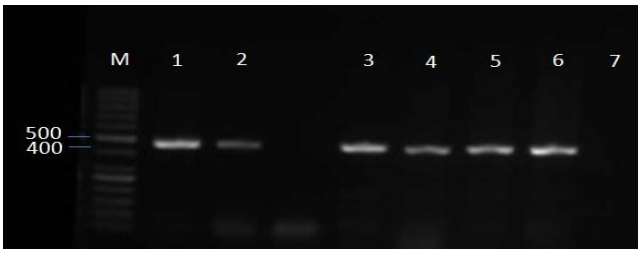

Figure 4: Agarose gel electrophoresis of 450 bp aspHS gene products of the A. fumigatus species with primers AFhem1 and AFhem2. Lane M, 50bp ladder; lane 1, PTCC5009; lane 2, IBRC-M30048; lane 3,E5; Lane 4, E9; lane 5, E10; lane 6, E11; lane 7, Negative control

Digestion of the *aspHS* gene products with *Tag*I produced the 3 expected 50, 110 and 290 bp fragments for all 53 isolates. The isolates E1 and E2 showed a different pattern after digestion with *Tag*I (Figure 5).

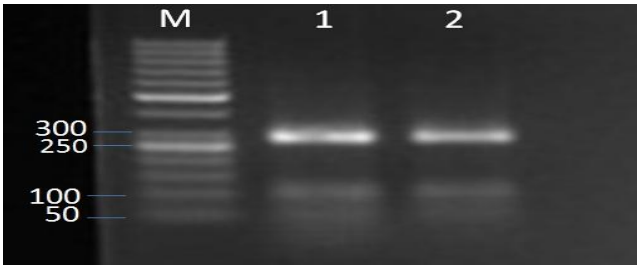

Figure 5: Restriction fragment pattern of 450 bp A. fumigatus aspHS gene digested with TagI. Lane M, 50 bp ladder; lane 1, IBRC-M30033; Lane 2, IBRC-M30040

Digestion with *NcoI* produced the 2 expected 100, 350 bp bands for 52 isolates. The isolates E2 showed different pattern after digestion with *NcoI* (Figure 6).

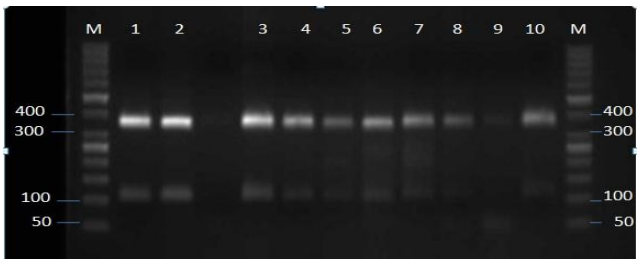

Figure 6: Restriction fragment pattern of 450 bp A. fumigatus aspHS gene digested with NcoI. Lane M, 50bp ladder; lane 1, E25; lane 2, E26; lane 3,E27; lane 4, E31; lane5, E35; lane 6m E37; lane 7, E45; lane 8, E1; lane 9:,E2; lane 10:E3

Sequencing

The PCR products several isolates were sequenced and aligned with references in the NCBI database. The sequences had 100% similarity with *A. fumigatus aspHS* gene sequences deposited in the NCBI database.

Discussion

An important reason for the high mortality connected with IA is its difficulty for early diagnosis. Asp-hemolysin is produced by *A. fumigatus*. It is a hemolytic and cytolytic toxin.The Asp-hemolysin gene is more greatly expressed *in vivo* compared to *in vitro* [15], [16] and also it has lately been described as a main *in vitro* -secreted protein [17]. Asp-hemolysin molecule has hemolytic activity on erythrocytes of rabbit and sheep and also causing in vitro cytotoxic effects on endothelial cells and macrophages in vitro. This molecule can be distinguished during infection in vivo [18].

A. fumigatus is the most important etiological agent of IA. Therefore early recognition of this species is very vital for at-risk patients.

Hemolysin created from A. fumigatus isolates from various sources, clinical and environmental.

One recent research the levels of expression of certain genes such as *gliP*, *aspHS*, *asp f 1*, and *dmaW* were found out by real-time RT-PCR analysis and higher expression was detected in vivo comparing to in vitro [15]. Thes results from these researches suggest overexpression of Asp-hemolysin during infection.

Hemolysin cytotoxicity possibly because of the capability of the hemolysin to inducing the DNA damage and creating mutations in an animal model and cell cultures. Different hemolysin can induce genotoxicity of dietary carcinogens *in vitro* considering that the level of induction was powerfully dependent to species [19], [20].

In our study, all *A. fumigatus* isolates exhibited hemolytic activity. We demonstrated that the hemolytic activity of *A. fumigatus* was significantly higher in clinical isolates compared to environmental isolates in sheep blood SDA. Thus, the hemolytic activity could employ an essential role for infections in *A.fumigatus*.

In the present study, with primers, F-Asphs and R-Asphs, an 180 bp fragment of *aspHS* gene were amplified for all tested *A. fumigatus* isolates. Two environmental isolates showed different pattern from other strains after cut with *Tag*I. Using primers AFhem1 and AFhem2, an 450 bp band fragment of *aspHS* gene was obtained for all 53 *A. fumigatus* isolates. Only one isolated demonstrated different pattern after cut with *NcoI.*

In conclusion, our results provide evidence

hemolysin activity and analysis of *aspHS* gene of *A. fumigatus*. These data may be useful in early diagnosis of *A. fumigatus* infections.

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References

1. Patterson TF, Kirkpatrick WR, White M, Hiemenz JW, Wingard JR, Dupont B, Rinaldi MG, Stevens DA, Graybill JR. Invasive aspergillosis. Disease spectrum, treatment practices, and outcomes. I3 Aspergillus Study Group. Medicine (Baltimore). 2000; 79:250-60. https://doi.org/10.1097/00005792-200007000-00006 PMid:10941354

2. Zmeili OS, Soubani AO. Pulmonary aspergillosis: a clinical update. QJM. 2007; 100:317-34. https://doi.org/10.1093/qjmed/hcm035 PMid:17525130

3. Yokota K, Shimada H, Kamaguchi A, Sakaguchi O. Studies on the toxin of Aspergillus fumigatus. VII. Purification and some properities of hemolytic toxin (asp-hemolysin) from culture filtrates and mycelia. Microbiol Immunol. 1977; 21:11-22. https://doi.org/10.1111/j.1348-0421.1977.tb02803.x PMid:16196

4. Ebina K, Sakagami H, Yokota K, Kondo H. Cloning and nucleotide sequence of cDNA encoding Asp-hemolysin from Aspergillus fumigatus. Biochim Biophys Acta. 1994; 1219:148-50. https://doi.org/10.1016/0167-4781(94)90258-5

5. Fukuchi Y, Kumagai T, Ebina K, Yokota K. Apolipoprotein B inhibits the hemolytic activity of asp-hemolysin from Aspergillus fumigatus. Biol Pharm Bull. 1996; 19:547-50. https://doi.org/10.1248/bpb.19.547 PMid:8860955

6. Malicev E, Chowdhury HH, Macek P, Sepcic K. Effect of ostreolysin, an Asp-hemolysin isoform, on human chondrocytes and osteoblasts, and possible role of Asp-hemolysin in pathogenesis. Med Mycol. 2007; 45:123-30. https://doi.org/10.1080/13693780601039615 PMid:17365648

7. Karkowska-Kuleta J, Rapala-Kozik M, Kozik A. Fungi pathogenic to humans: molecular bases of virulence of Candida albicans, Cryptococcus neoformans and Aspergillus fumigatus. Acta Biochim Pol. 2009; 56:211-24. https://doi.org/10.18388/abp.2009_2452 PMid:19543556

8. Jeong H, Hwang J, Lee H, Hammond PT, Choi J, Hong J. In vitro blood cell viability profiling of polymers used in molecular assembly. Sci Rep. 2017; 7:9481. https://doi.org/10.1038/s41598-017-10169-5 PMid:28842713 PMCid:PMC5573391

9. Kumagai T, Nagata T, Kudo Y, Fukuchi Y, Ebina K, Yokota K. Cytotoxic activity and cytokine gene induction of Asp-hemolysin to murine macrophages. Nihon Ishinkin Gakkai Zasshi. 1999; 40: 217-222. https://doi.org/10.3314/jjmm.40.217 PMid:10536308

10. Rementeria A, López-Molina N, Ludwig A, Vivanco AB, Bikandi J, Pontón J, Garaizar J. Genes and molecules involved in Aspergillus fumigatus virulence. Rev Iberoam Micol. 2005; 22:1-23. https://doi.org/10.1016/S1130-1406(05)70001-2

11. Bennett JW, Klich M. Mycotoxins. Clin Microbiol Rev. 2003; 16:497-516. https://doi.org/10.1128/CMR.16.3.497-516.2003 PMid:12857779 PMCid:PMC164220

12. Donohue M, Wei W, Wu J, Zawia NH, Hud N, De Jesus V, Schmechel D, Hettick JM, Beezhold DH, Vesper S. Characterization of nigerlysin, hemolysin produced by Aspergillus niger, and effect on mouse neuronal cells in vitro. Toxicology. 2006; 219:150-5. https://doi.org/10.1016/j.tox.2005.11.013 PMid:16338047

13. Abad-Diaz-De-Cerio A, Fernandez-Molina JV, Ramirez-Garcia A, Sendino J, Hernando FL, Pemán J, Garaizar J, Rementeria A. The aspHS gene as a new target for detecting Aspergillus fumigatus during infections by quantitative real-time PCR. Med Mycol. 2013; 51:545-54. https://doi.org/10.3109/13693786.2012.756989 PMid:23336696

14. Kumagai T, Kudo Y, Fukuchi Y, Ebina K, Yokota K. Expression of a Synthetic Gene Encoding the Asp-Hemolysin from Aspergillus fumigatus in Escherichia coli. Biol. Pharm. Bull. 2002; 25:115-117. https://doi.org/10.1248/bpb.25.115 PMid:11824538

15. Gravelat FN, Doedt T, Chiang LY, Liu H, Filler SG, Patterson TF, Sheppard DC. In vivo analysis of Aspergillus fumigatus developmental gene expression determined by real-time reverse transcription-PCR. Infect Immun. 2008; 76:3632-9. https://doi.org/10.1128/IAI.01483-07 PMid:18490465 PMCid:PMC2493243

16. Muszkieta L, Beauvais A, Pähtz V, Gibbons JG, Anton Leberre V, Beau R, Shibuya K, Rokas A, Francois JM, Kniemeyer O, Brakhage AA, Latgé JP. Investigation of Aspergillus fumigatus biofilm formation by various "omics" approaches. Front Microbiol. 2013; 4:13. https://doi.org/10.3389/fmicb.2013.00013 PMid:23407341 PMCid:PMC3569664

17. Wartenberg D, Lapp K, Jacobsen ID, Dahse HM, Kniemeyer O, Heinekamp T, Brakhage AA. Secretome analysis of Aspergillus fumigatus reveals Asp-hemolysin as a major secreted protein. Int J Med Microbiol. 2011; 301:602-11. https://doi.org/10.1016/j.ijmm.2011.04.016 PMid:21658997

18. Abad A, Fernández-Molina JV, Bikandi J, Ramírez A, Margareto J, Sendino J, Hernando FL, Pontón J, Garaizar J, Rementeria A. What makes Aspergillus fumigatus a successful pathogen? Genes and molecules involved in invasive aspergillosis. Rev Iberoam Micol. 2010; 27:155-82. https://doi.org/10.1016/j.riam.2010.10.003 PMid:20974273

19. Berne S, Sepčić K, Anderluh G, Turk T, Macek P, Poklar Ulrih N. Effect of pH on the pore forming activity and conformational stability of ostreolysin, a lipid raft-binding protein from the edible mushroom Pleurotus ostreatus. Biochemistry. 2005; 44:11137-47. https://doi.org/10.1021/bi051013y PMid:16101298

20. Das S, Lindemann C, Young BC, Muller J, Österreich B, Ternette N2, Winkler AC, Paprotka K, Reinhardt R, Förstner KU, Allen E, Flaxman A, Yamaguchi Y, Rollier CS, van Diemen P, Blättner S, Remmele CW, Selle M, Dittrich M, Müller T, Vogel J, Ohlsen K, Crook DW, Massey R, Wilson DJ, Rudel T, Wyllie DH, Fraunholz MJ. Natural mutations in a Staphylococcus aureus virulence regulator attenuate cytotoxicity but permit bacteremia and abscess formation. Proc Natl Acad Sci U S A. 2016; 113:E3101-10. https://doi.org/10.1073/pnas.1520255113 PMid:27185949 PMCid:PMC4896717

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The Phytochemical Screening, Total Phenolic Contents and Antioxidant Activities *in Vitro* of White Oyster Mushroom (*Pleurotus Ostreatus*) Preparations

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Keywords: Ethanolic extract; Pleurotus ostreatus; Phytochemical; Total phenolic content; Antioxidant activity

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Abstract

BACKGROUND: The popular commercially cultivated Pleurotus ostreatus mushroom contains very high nutrients and bioactive compounds with high antioxidant activity. The ethanolic extract seems to be the most active in preparation.

AIM: This study has an aim to compare the phytochemical analysis of a fresh, dry and ethanolic extract of Pleurotus ostreatus, to measure the total phenolic content and antioxidant activities in vitro of ethanolic extracts of Pleurotus ostreatus.

METHODS: The fresh plant's materials (FPM), dry plants materials (DPM), ethanolic extracts were macerated with 70% (EE70) and 96% ethanol (EE96) of Pleurotus ostreatus which were used for phytochemical analysis, and EE96 was used for antioxidant activity in vitro. The phytochemical analysis was conducted using the Dragendorf and Meyer, FeCl3 test, Salkowsky method, Lieberman method, amyl alcohol, foam test and the NaOH reagent. The total phenol test was carried out using the Follin-Ciocalteu method. The antioxidant activity was tested using the ABTS and H2O2 essay.

RESULTS: The phytochemical screening showed that the flavonoid, phenolic compounds, tannin, saponin, alkaloids, and steroids were detected in the FPM, DPM, EE70 and also the EE96. The alkaloid, however, was not identified by the Meyer Reagent in the FPM and DPM. The DPM and EE70 seemed to have the highest amount of saponin based on the foam that was formed. Meanwhile, steroids and flavonoids were detected at a higher level in the EE96, based on the strength of visible colour. However, triterpenoid and quinones could not be identified. In the total phenol test, there was an amount of 6.67 µg phenol in a 1 mg extract sample which was equivalent to 1 mg of Gallic Acid. The EE96 has an IC50 of 108.07 µg/mL for ABTS and an IC50 reduction of 229.17 µg/mL. The process of Pleurotus ostreatus drying did not reduce the content of active substances. The polar active substances seem to be more soluble in the EE70 than the EE96.

CONCLUSION: The higher the bioactive substances in the preparation, the more significant the bio-therapeutic effects. Ethanolic extract of Pleurotus ostreatus has a phenol content and a good antioxidant action.

Introduction

Forty per cent of the population in Indonesia are using traditional medicine, and 70% of them are in rural areas. Indonesia is rich in medical plant resources, although the utilisation is estimated to be unoptimal. Research on plants that are efficacious in medicine needs to be developed as of now. The use of natural ingredients in medicine is expected to provide fewer side effects than the use of synthetic or chemical materials, without reducing the efficacy in medicine [1], [2]. One plant that has long been known to have efficacy for treatment is white oyster mushroom (Pleurotus ostreatus Jacq: Fr Kumm). The Pleurotus ostreatus is one of the commercial mushrooms that are very popular commercially, easily cultivated and is an important food source. White oyster mushrooms contain high nutrients and various other secondary metabolites that have pharmacological effects [3], [4].

One potential pharmacological effect of Pleurotus ostreatus is the antioxidant potential. Previous studies have shown that Pleurotus ostreatus

have high antioxidant abilities. White oyster mushroom extractions have protective effects on the liver, kidneys, brain and lungs [5], [6], [7]. The literature shows that Pleurotus ostreatus contains many active substances that are useful in applying therapeutic effects, including phenolic components, flavonoids, terpenoids, polysaccharides, lectins, steroids, glycoproteins, several lipid components, and ergothioneine (ET), vitamin C, beta-carotene, and selenium. The ethanol extract of Pleurotus ostreatus is expected to be an exogenous antioxidant that can prevent and inhibit oxidative stress due to free radicals in the body, while being able to synergize with the body's endogenous antioxidants [5], [6], [7], [8], [9], [10].

The preparation of the ethanolic extract of Pleurotus ostreatus is aiming to separate the active substances from other materials that are not needed by using certain solvents. Factors that can influence the quality of extract formed include the selection of plants that are used, the procedure for making extractions and the selection of solvents used. Plants used for making extractions can be fresh plants or in a dry form, but it is worried that the drying process will affect the content of the active ingredients inside. Drying techniques are often done traditionally or by using open dryers. The selection of solvents in the extraction should have the following properties. Namely, low toxicity, easily evaporated at low temperatures, increasing the speed of extractions, can be preservative, will not make the extraction complex or split. The choice of solvents in creating the extraction will affect the active substance to be dissolved. Ethanol can cause more filtered polyphenols than water extractions. This solvent is more effectively used on cell walls, or unipolar seeds and ethanol can activate the polyphenol oxidation enzyme which can degrade polyphenols. To observe the qualitative, the active substances contained in plant samples and herbal preparations should be carried out with a pre-clinical phytochemical screening [11], [12]. Measurement of total phenolic levels in the extraction will also show the estimation of phenol levels in extractions which are very important for their antioxidant potential [13], [14].

The antioxidant potential of the ethanolic extract of Pleurotus ostreatus can be measured using various methods both in vitro and in vivo. In vitro antioxidant tests are very diverse, the results of the phytochemical analysis and in vitro antioxidant tests can qualitatively be the basis for confirming the antioxidant effects of the white oyster mushroom ethanol extraction in vivo. It is hoped that the results of the vitro test will be in line with the results of the Vivo test, the substances or extracts that are thought to have antioxidant activity in vitro will also show good antioxidant potential in vivo [15].

Based on the data above, this study will compare the active content of Pleurotus ostreatus in fresh plant materials (FPM), dry plant material (DPM), ethanol extraction using 70% ethanol (EE70) and ethanol extraction using 96% ethanol (EE96). This study will also assess the antioxidant activity of the ethanolic extraction of Pleurotus ostreatus using the two in vitro methods.

Material and Method

Manufacture of White Oyster Mushroom Extraction

Pleurotus ostreatus are obtained from mushroom cultivation centres in Cisarua village, West Bandung regency, Indonesia. Fresh plants materials of Pleurotus ostreatus are brought in the morning after being harvested, then washed using running water and cleaned from contaminating material until the material is gone. The mushrooms are then dried using an oven at 50°C for 2-3 days. The dried mushrooms that are obtained are about 8% of the fresh sample. The dried Simplicia is mashed and then forms a dry powder of the white oyster mushroom (Pleurotus ostreatus). The dried oyster mushroom powder is then macerated, each with 70% ethanol and 96% ethanol, with a ratio of 1:10. The liquid extraction obtained was then filtered with a Whatman No. filter paper at 40 and then evaporated with a rotary evaporator at 50°C and put into the oven at 40°C to obtain a concentrated extraction. Fresh plant samples, dry powder, 70% ethanol extraction and 96% ethanol extractions were then subjected to phytochemical screening [1], [12], [16], [17].

Alkaloid Test

Dragendroff Test: The sample (100 mg) was dissolved in 10 mL of solvent, then filtered into a filtration. The filtration (2 mL) was added with an HCL (acid) and a few drops of the Dragendroff reagent. The formation of brownish-red deposits shows the presence of alkaloids [11], [18].

Mayer Test: Samples (100 mg) were dissolved in 10 mL of solvent, then filtered to form a filtration. The filtration (2 mL) was added with an HCL (acid) and a few drops of the Meyer reagent. The formation of brownish-yellow deposits indicates the presence of alkaloids. (Tiwari et al., 2011) [11], [18].

Triterpenoid and Steroid Tests

Liebermann-Burchard Test: 50 mg of the sample was extracted with chloroform and then filtered. 2 ml of filtrate formed which were then added with 1-2 ml of acetic anhydride and 2 drops of concentrated H2SO4 from the side of the tube. The formed colour is red, then blue and then a green color which shows sterols [18].

Salkowski's Test: 50 mg of the sample was extracted with chloroform then filtered. The filtrate obtained was added with a few drops of concentrated sulfuric acid and then shaken. The chloroform layer shows the red and yellow colour of the acid layer indicating steroids. A brownish-red colour indicates a triterpenoid [12], [19].

Saponin Test

Foam test/Froth method: The extraction was dissolved in 20 ml in distilled water (dist. H_2O) so that all the samples were submerged, and boiled for 2-3 minutes. The solution is then cooled and shaken. The appearance of foam for ± 10-15 minutes shows a sign of saponin [11], [16].

Flavonoid Tests

Ammonium Test: The extraction is heated with 10 ml of ethyl acetate in boiling water for 3 minutes. The results are then filtered to form a filtration. The filtration is then mixed with 1 ml of dilute ammonia solution (1%), and then shaken. These two layers will separate. The absence of yellow on the ammonia layer indicates flavonoids [19].

Shinoda Tests: 2-3 ml of filtrate extraction is then added with magnesium metal. Then the HCL concentrate is added. The appearance of a magenta colour signifies the presence of flavonoids [12], [19].

Kinnon

NaOH Test: A 5 ml filtrate was added a few drops of NaOH. The reddish-blue colour that appears indicates a quinone [17].

Tanin test and phenolic compound/ polyphenolic

Fe (III) Chloride Test: Samples (100 mg) was dissolved in 10 mL of solvent, then filtered. The filtration (2 mL) is added with 1 mL FeCl3 3%. The presence of a green to slightly blackish deposits indicates the presence of tannins and polyphenols [12], [18], [19].

Gelatin Test: Extraction 0.1 g of the sample added with 10 ml of water, then boiled for several minutes. Then filtered and the filtration was added with 2 mL of a 1% gelatin solution containing NaCl. If a white precipitate is formed, it indicates the presence of tannins and phenolic components [11], [12].

Measurement of Total Phenolic Content

The total phenol test was implemented using the Follin-Ciocalteu method. The polyphenols in plant extractions will react with the Folin-Ciocalteu reagent and form a blue complex which can be quantitatively measured using visible-light spectrophotometry. The formation of a blue chromophore is caused by the forming of a phosphotungstic-phosphomolybdenum complex, and the maximum absorption depends on the alkaline solution and the phenolic concentration (the higher the phenol content, the deeper the blue colour) inside. The reactions that occur are accurate and specific for some phenolics [20], [21].

Samples of ethanolic extractions of P. ostreatus include an amount of 15 µl in each well. Then, 75 µl of Folin-Ciocalteu 10% and 7.5 µl of Sodium Carbonate amount at 60 µl. On the blank, 150 µl of DMSO sample solvent is added. Then incubated at a time of 10 minutes at 500°C. The absorbance was measured at λ = 760 nm on the spectrophotometer. The total phenol concentration will be calculated based on the calibration curve obtained using various concentrations of gallic acid and can be seen from the number of mg in gallic acid equivalents (GAEs) per gram of the dry sample [20], [21].

Measurement of Antioxidant Activity in Vitro

ABTS ({2, 2 '- azinobis- (3-ethyl-benzothiazoline-6-sulphonic acid)}) essay: The ABTS assay is a test of antioxidant potential using a spectrophotometer to measure the blue colour that appears when a sample of white oyster mushroom ethanol extraction is added to a blue-green chromophore ABTS + (2, 2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid). Herbal samples will reduce ABTS + to be oxidised ABTS and cause decolourisation. A Cation ABTS is made by adding a solid manganese dioxide (80 mg) to 5mM of a liquid stock solution of ABTS [15], [22].

A 2 µl extraction sample was inserted into the 96-well plate, then added with a 198 µl ABTS work reagent into the well containing the sample (well sample). At well blank, an amount of 200 µl sample solvent (DMSO) is added. In the well control, 200 µl of ABTS work reagent is added, then incubated in a plate for 6 minutes at 37°C. The absorbance is measured using a microplate reader at λ = 745 nm.

$$\%\ \text{Reducing activity} = \frac{\text{Control Absorption} - \text{Sample Absorption}}{\text{Control Absorption}} \times 100\%$$

The percentage of the ABTS radical inhibition (%) was determined by the absorbance ratio of ABTS • + in the sample compared to the absorbance of the control. The result is in the form of an Inhibitory Concentration (IC50); the lower IC50 value will show a stronger ABTS scavenging capacity. Measurements were made with a repetition of three times. [15], [22], [23].

Hydrogen Peroxide Scavenging (H_2O_2) Test

The examination of hydrogen peroxide scavenging (H_2O_2) assay is a method for assessing the size of the ability of the white oyster mushroom ethanol extraction in capturing hydrogen peroxides. The capture of H_2O_2 was measured with the method described by Mukhopadhyay et al. (2016) with a slight modification [24].

The trapping activity of hydrogen peroxide (H_2O_2) was measured with the ferrous ammonium sulphate and phenanthroline reaction methods with minor modifications. The ferrous ammonium sulphate which reacts with phenanthroline will form an orange Fe2+ -tri-phenanthroline complex. H_2O_2 will inhibit the formation of the complex so that antioxidants that trap H_2O_2 will cause the formation of orange Fe2+ -tri-phenanthroline complex [24].

Mixtures of control solutions, samples and blank samples that were added with H_2O_2 are included in the 96-well plate, then incubated for 5 minutes in a dark room with room temperature. Then each sample and the blank mixture was added with 1, 10-phenanthroline as much as 75 µL, and re-incubated for 10 minutes in a dark room with room temperature. The absorbance is measured using a wavelength of 510 nm [24].

The percentage of trapping actions is calculated using the formula**:**

$$\% \text{ scavenging of } H_2O_2 = \frac{\text{A Sample}}{\text{A Control}} \times 100$$

A: Absorbance

Results

A. The results of the phytochemical analysis of white oyster mushrooms

Table 1 shows that the active ingredient was contained in the preparation of fresh white oyster mushrooms, dry preparations, 70% ethanol extractions and 96% ethanol extractions are alkaloids, steroids, flavonoids, saponins, tannins and phenolic components. Quinone and triterpenoids were not found in all samples. The Meyer alkaloid method was not found in fresh samples and dry samples but was found in the extraction preparation. Flavonoids were not found by the Shinoda test method, but were found with ammonium test. Flavonoids and steroids are more commonly found in the 96% ethanol extraction than the others.

Table 1: Comparison of phytochemical preparations for white oyster mushrooms

Phytochemical analysis		Sample			
Coumpoud/ class	Methode/ Test	Fresh Plant Materials (FPM)	Dry Plant materials (DPM)	Ethanolic extraction mixed with 70% ethanol (EE70)	Ethanolic extraction mixed with 96% ethanol (EE96)
Alkaloid	Dragendroff	±	+	+	+
	Meyer	-	-	+	+
Steroid	Sawkosky	+	+	+	++
	Lieberman	+	+	+	+
Triterpenoid	Lieberman	-	-	-	-
Flavonoid	Ammonium Test	±	+	+	++
	Shinoda Test	-	-	-	-
Saponin	Foam Test	+	++	++	±
Quinone	NaOH	-	-	-	-
Tanin	Gelatin	+	+	+	+
	FeCl3	-	±	-	-
Phenolic	Gelatin test	+	+	+	+
coumpouds	FeCl3	+	+	+	+

Information: (± → weak, + → strong, ++ → very strong).

B. Total phenolic content of ethanolic extractions of Pleurotus ostreatus (EE96)

In Table 2, it can be seen that in the ethanol extraction of white oyster mushrooms were at a total phenol of 6.67 µg phenol in 1 mg extract sample which was equivalent to 1 mg Gallic Acid. (6.67 µg phenol/1 mg GAE/extraction) or equivalent to 667 mg GAE/100 gr in the sample.

Table 2: Results of the Analysis of Total Phenol of Oyster Mushroom Extractions

Final	Total Phenol			Average	Total Phenol (µg sample extraction/mg GAE)			Average	SD	RSD
Conc. (µg/ml)	1	2	3		1	2	3			
2000	10.64	10.81	10.89	10.78	5.32	5.41	5.45	5.39	0.06	1.18
1000	6.56	6.81	6.80	6.72	6.56	6.81	6.80	6.72	0.14	2.12
500	3.74	4.09	4.01	3.95	7.49	8.19	8.02	7.90	0.37	4.64

C. The antioxidant activity of white oyster mushroom extractions by the ABTS method

The concentration of the extractions of white oyster mushroom ethanol tested in the ABTS test were: 400; 200; 100; 50; 25; 12.5; 6.25 (µg/mL) and the largest reduction activity is at a dose of 400 µg/mL with an ABTS reduction activity of 90.98%. Reduction activities of various concentrations showed significantly different results with ($p < 0.05$) (Table 3).

Table 3: ABTS Reduction Activities of EE96 (Average Tukey HSD Post HocTest Results)

Concentrations (µg/mL)	Average ABTS Reduction Activity (%)
	EE96
400	90.98 ± 1.04^{g}
200	77.22 ± 0.08^{f}
100	52.13 ± 1.37^{e}
50	35.96 ± 2.14^{d}
25	22.54 ± 1.45^{c}
12.5	12.09 ± 0.31^{a}
6.25	15.43 ± 0.02^{b}

Data were presented as a mean ± standard deviation. Different letters in the same column are significant at $P < 0.05$ (Tukey HSD post hoc test).

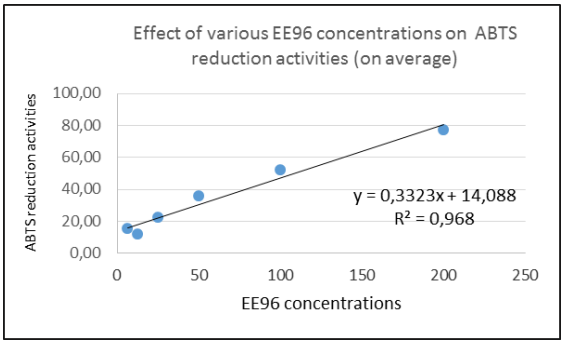

Figure 1: Dosage curve for inhibition presets of EE96 with a correlation coefficient (R2) of 0.98

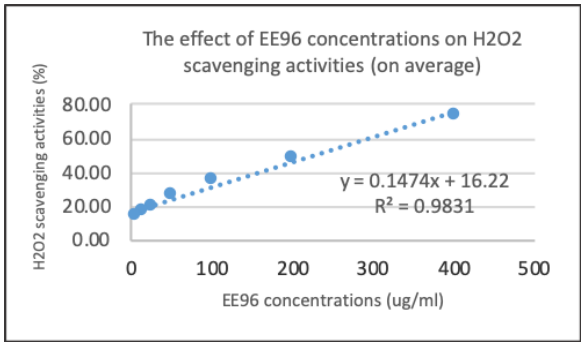

Figure 2: The dose-response curve for the percentage of the EE96 roaming activity with a correlation coefficient (R2) of 0.98

The ABTS reduction activity of the white oyster mushroom ethanol extraction showed a dose-response curve with an average equation of Y = 0.3323x + 14.088 and obtained a 50% inhibitory concentration of 108.07 ± 1.28 µg/mL (Fig. 1, Table 3 and 4).

Table 3: IC50 value of ABTS reduction from EE96

Sample of EE96	Equation	R2	IC50 (µg/mL)	IC50 (µg/mL)
1 test	Y = 0.3339x + 14.084	0.97	107.57	108.07 ± 1.28
2nd test	Y = 0.3331x + 14.323	0.96	107.11	
3nd test	Y = 0.3300x + 13.857	0.98	109.32	
Average	Y = 0.3323x + 14.088	0.97	108.07	

The IC50 value of ABTS reduction from EE96 and data analysis of ABTS reduction activities from EE96 are shown in Table 3 and Table 4.

Tabel 4: Data Analysis of ABTS Reduction Activities from EE96

Sample	Final Conc. (µg/ml)	Reduction of ABTS (%) 1	2	3	Average	SD	RSD	IC50 Rata - rata - R2	IC50 Ul. 1 - R2	IC50 Ul. 2 - R2	IC50 Ul. 3 - R2	Rata-rata IC50
EE96	400	92.10	90.05	90.78	90.98	1.04	1.14	108.07	107.57	107.11	109.52	108.07
	200	77.28	77.13	77.26	77.22	0.08	0.10					
	100	52.99	52.84	50.55	52.13	1.37	2.63					108.07 ± 1.28
	50	35.78	38.19	33.92	35.96	2.14	5.96					
	25	22.13	21.35	24.16	22.54	1.45	6.43					
	12.5	12.37	12.13	11.75	12.09	0.31	2.58					
	6.25	15.41	15.46	15.43	15.43	0.02	0.14	0.97	0.97	0.96	0.98	1.28

D. The antioxidant activity of the ethanol extraction of Pleurotus ostreatus (EE96) by the H_2O_2 essay method

The concentrations of the extraction of white oyster mushroom ethanol tested in the H2O2 test were: 400; 200; 100; 50; 25; 12.5; 6.25 (µg/mL) and the greatest reduction activity is at a dose of 400 µg/mL with a combustion activity of 73.01% H_2O_2 (Table 5). Reduction activities of various concentrations showed significantly different results with a $p < 0.05$.

Table 5: H_2O_2 Capture of Ethanolic extractions of Pleurotus ostreatus (EE96)

Concentrations (µg/mL)	Average Scavenging activity of H_2O_2 (%) EE96
400	73.01 ± 2.58^{f}
200	47.88 ± 1.03^{e}
100	34.54 ± 2.59^{d}
50	26.25 ± 1.70^{c}
25	18.96 ± 2.37^{b}
12.5	16.26 ± 0.10^{ab}
6.25	13.61 ± 0.20^{a}

*) Data is presented in the form of a mean ± standard deviation; Lowercase differences in the same column show the significance of the data of P < 0.05 (Tukey HSD Post hoc Test).

The H_2O_2 roaming activity of the ethanolic extract of Pleurotus ostreatus showed a dosage response curve with an average equation of Y = 0.1474x + 16.220 and obtained an inhibitory value of 50 concentrations at 229.31 ± 3.38 µg/mL (Fig. 2, Table 6 and 7).

Table 6: Analysis of H_2O_2 Roaming Activities for EE96

Sample	Final Conc. (µg/ml)	Roaming activities of H2O2 (%) 1	2	3	Average	SD	RSD	IC50 Rata -rata - R2	IC50 Ul. 1 - R2	IC50 Ul. 2 - R2	IC50 Ul. 3 - R2	Rata-rata IC50
EE96	400	70.55	72.79	75.69	73.01	2.58	3.53	229.17	231.40	231.12	225.41	229.31
	200	48.20	48.71	46.73	47.88	1.03	2.16					
	100	37.45	32.48	33.68	34.54	2.59	7.51					229.31 ± 3.38
	50	27.64	26.75	24.36	26.25	1.70	6.47					
	25	21.25	16.51	19.11	18.96	2.37	12.52					
	12.5	16.31	16.33	16.15	16.26	0.10	0.59					
	6.25	13.68	13.38	13.76	13.61	0.20	1.49	0.98	0.96	0.98	0.99	3.38

Analysis of H_2O_2 roaming activities for EE96 and IC50 value of capturing of H_2O_2 from EE96 are shown in Table 6 and Table 7.

Table 7: IC50 Value of Capturing of H_2O_2 from EE96

Sample of EE96	Equation	R^2	IC_{50} (µg/mL)	IC_{50} (µg/mL)
1 Test	Y = 0.1391x + 17.812	0.96	231.40	229.31±3.38
2nd Test	Y = 0.1493x + 15.494	0.98	231.12	
3nd Test	Y = 0.1537x + 15.354	0.99	225.41	
Average	Y = 0.1474x + 16.220	0.98	229.17	

Discussion

Medicinal plants contain various chemical compounds or active substances that have therapeutic properties. The active substances that are inside medicinal plants depend on the plant species, the time and method of harvesting, the drying process and the production of medicinal plants [25], [26], [27]. Pleurotus ostreatus is a medicinal plant that has many active metabolites which can be used as antioxidants, anti-inflammatory, anti-cholesterol and developed into an anti-cancer. One of the medicinal preparations of white oyster mushrooms which are currently being developed is the ethanolic extract of Pleurotus ostreatus [4], [5], [6], [7].

The quality of herbal preparations such as the ethanol extraction of P. ostreatus is strongly influenced by the drying process, the selection of solvents used and the ratio of solvents and this solved ones. Ethanol is a universal solvent that is widely used in making extractions because it can dissolve polar and nonpolar substances. To improve the ability of ethanol in drawing polar active substances, it can be done by adding the composition of the water in the substances so that the percentage of ethanol drops, so in this study, there is a comparison between the use of 70% ethanol and 96%. Ethanol can dissolve active substances such as tannins, phenols, saponins, proanthocyanidins, reduce sugars, flavonoids, terpenoids, glycosides. More steroids are found in water and methanol solvents [11].

The result of the phytochemical screening of P. ostreatus herbal preparations showed that the drying process performance did not have much effect on the content of the active ingredient in white oyster mushrooms. Temperatures that are too high when drying can damage the structure of the active substance [26], [27]. In flavonoids and phenolics the levels of fresh preparations appear to be less strong compared to the dry preparations or in extractions, this is probably due to higher levels of freshwater in the preparation so that the levels of the active ingredients appear less.

The active ingredient contained in the preparation of FPM, DPM, EE70 and EE96 are alkaloids, steroids, flavonoids, saponins, tannins and phenolic components. Alkaloids can also be found in ether, methanol or water solvents but cannot be found in hexane. Alkaloids are active metabolic which have antimicrobial effects by inhibiting DNA topoisomerase. More flavonoids observed in the Ammonium test method were found in (EE96), but the literature mentioned that this active substance would have appeared more in water solvents and could appear in methanol solvents. In this study, the Shinoda test gave negative results for flavonoids, and this is probably since there are currently more than 4000 flavonoid compounds identified and the possibility of each test will identify different compounds. The concentration of flavonoids will be reversed more in the 70% ethanol compared to pure ethanol because it increases the polarity. Flavonoid compounds are polyphenolic components that are widely found in plants and have a variety of biological activities including antimutagenic and anticancer effects [11], [26], [27].

Phenol is found in the ethanol extractions of white oyster mushrooms; this is because phenol will show better positive results in alcohol than in water. Phenol is the main compound that is thought to be related to the antioxidant effects of white oyster mushrooms. Its antioxidant ability is strongly related to the hydroxyl content contained in it. Previous research has shown that phenol is proven to prevent damage to the liver, lungs and kidneys. The phenolic component is the main component that affects its antioxidant activity [3], [4], [5], [6], [7]. Phenol can act as a reducing agent for hydrogen donors and singlet oxygen quenchers and has a potential metal chelation effect [7], [28].

The phenolic component in Pleurotus ostreatus contains various types, including vanillic acid, myricetin, naringin, homogentic acid, 5-O-caffeoylquinic acid, chrysin, routine, gentisic acid, gallic acid, protocatechuic acid. caffeic acid, tannic acid, syringic acid, cinnamic acid and p-coumaric acid. Antioxidant properties found in many fungi are generally in the form of phenolic acids and flavonoids, followed by tocopherols, ascorbic acid and carotenoids. Phenolic compounds or polyphenols are thought to be able to inhibit TNF-α gene expression and inhibit their production [29], [30]. The tannin in this study shows positive results. Flavonoids and tannins are part of the polyphenolic component. Tests for tannins show positive results from all four samples. A high tannin intake of phenolpropyranoids can reduce the risk of coronary heart disease [18].

The results of this study showed that in the ethanol extract of 70% ethanolic extraction of P. ostreatus (EE70) there was a total phenol of 6.67 µg phenol in 1 mg of the extraction sample which was equivalent to 1 mg Gallic Acid. (6.67 µg phenol/1 mg GAE/extraction) or equivalent to 667 mg GAE/100 gr sample. This result when compared with the total phenol content of chloroform extraction from the clary sage (Salvia sclarea), an aromatic plant originating from southern Europe, does look smaller, which is the 28.91 µg in the 1 mg extraction. In the extraction of the acetone clary sage (Salvia sclarea), there is a total phenol of 35.24 µg. But in the same study also showed that it turned out that the total phenol level did not illustrate the correlation with the antioxidant strength calculated using the thiocyanate method [20].

In the research of Settharaksa, 2014, the comparison of the total phenol levels saw differences in the total phenol of the Trigasornmas formula extractions in several solvents. Formula trigasornmas are of the coral plant (Jatropha multifida L.), stamen lotus (Nelumbo nucifera Gaertn), and bael fruit (Aegle marmelos (L.) Corr). All ingredients were bought from traditional pharmacies in Bangkok, Thailand. The results showed that water extractions had a higher total phenol content than other compounds at around 1.955.23 ± 60.87 mg GAE/100 g samples. These results differed significantly ($P < 0.05$) with ethyl acetate, methanol, dichloromethane, ethanol and hexane. This research confirms that in this study water seems to be a better solvent than ethanol or acetone. This is probably due to the majority of the polyphenols dissolving in water. Solvents with different polarity can affect dissolved phenol levels [13].

Other studies have shown that the total phenol of P. citrinopileatus can be cultivated with

100% Castanea media. The Sativa is quite high at 2.529 ± 0.010 mg GAE/g, whereas Ostreatus which is bred at 50% C. Sativa + 50% P. Orientalis sawdust shows a lower total of phenol levels at 1.232 ± 0.060 mg GAE/g. This result was lower than some wild herbs containing a total phenol of 2.83-25.38mg GAE/g [14]. Other studies using Ostreatus P. samples developed in Malaysia showed water extractions of Ostreatus P. contained a total phenol of 798.55 mg GAE/100g. In this study, the antioxidant extraction of water from P. ostreatus correlated with the total phenolic content [31]. When compared with the research on the previous status, there are indeed few differences, and the results are relatively smaller, this can be due to the differences in the substrate for logs when cultivated, the harvest, characteristics of the species itself and herbal preparations made.

The ABTS reduction activity of the white oyster mushroom ethanol extraction in this study showed a dosage response curve with an average equation of Y = 0.3323x + 14,088 and obtained a 50% inhibitory concentration of 108.07 ± 1.28 µg/mL. Other studies have shown that ethanolic extractions from the aerial parts of the medicinal plant Coronopus didy have the potential of obtaining a strong ABTS with an IC50 (4.32 × 102 µg/mL) and this value is higher than the DPPH scavenging activity with an IC50 7.80 × 102 µg/mL]. This shows that the components taken with an ethanol solvent have a strong ABTS of radical-scavengers [23].

The ABTS reduction activity of Ethanol extractions of Sativa, vanillin, and the coumaric acid oriza showed that the IC50 sativa oriza extraction was the highest (145.67 µg/mL) when compared to vanillin (4.96 µg/mL) and the coumaric acid (1.67 µg/mL). These results show that the oriza sativa ethanol extraction showed a weak antioxidant activity compared to the other two pure components [32]. The ethanol extract of white oyster mushrooms measured using the same method showed lower IC 50 results than the sativa oriza, so the antioxidant potential seemed to be more this is higher compared to the sativa oriza extraction, but it is indeed lower when compared the to vanillin or asan coumarik [32].

Cultivated antioxidant Pleurotus ostreatus studies in Malaysia showed the potential of ABTS•+ scavenging from P. ostreatus (87.29 ± 0.54%) and this result was significantly higher when compared to the asm ascorbate at the concentrations of 1000 and 1500 µM (49.88 ± 0.17% and 73.11 ± 0.22%), also with the BHA in 1000 and 1500 µM concentrations (48.64 ± 0.36% and 65.84 ± 0.17%, respectively). The correlation coefficient (r) between the antioxidant activity, DPPH scavenging ability (%), and TPC was 0.915, the correlation coefficient between ABTS + scavenging ability (%) and TPC was 0.767, and the correlation coefficient between r FRAP and TPC was 0.981. This shows that the phenol content in this study contributes to the antioxidant potential of P. ostreatus extractions from Malaysia [14]. In this study the P. ostreatus showed a reduction potential of 90.98% at a concentration of 400 µg/L, this shows that the ability of antioxidants is quite well when compared to similar species originating from Malaysia.

In this study, the antioxidant activity was also assessed using an H2O2 essay. Hydrogen peroxide is an important biological compound produced in the process of aerobic metabolism, and its production is increased in the conditions of infection, exercise, stress, radiation and other inductions. Humans can be exposed to H2o2 from the environment at around 0.28 mg/kg body weight/day. Hydrogen peroxide can be inhaled or absorbed through the eyes or skin. This compound is not toxic but can turn into toxic compounds and produce more toxic compounds, such as hydroxyl through Fenton reactions, or become hypochlorous acid because of the enzyme myeloperoxidase (MPO). Increased production of H2O2 will ultimately increase ROS and cause redox imbalances resulting in oxidative stress. This oxidative stress can be inhibited by antioxidants, and the antioxidant ability to scavenge ROS can vary [24], [25], [26], [27], [28], [29], [30], [31], [32].

In the test of antioxidant activity, oyster mushroom extractions have an antioxidant activity by combining H2O2. The oyster mushroom extraction has an IC50 229.17 µg/mL. In the previous study P. florida and C. indica are at 200-1000 µg/ml with the results of the radical hydroxyl radical scavenging with IC50 values for P. florida and C. indica were 220.70 ± 6.0 µg/ml and 148.23 ± 1.01 µg/ml [34]. These results show that the ability of Pleurotus ostreatus in the capture of H2O2 is almost the same as P fluoride, both of which are in the oyster mushroom group.

In the research of Mukhopadhyay, 2016 there is a comparison of some of the abilities of H2O2 extractions with the same method for several antioxidants, which showed the scavenging activity of H202 (IC Value) as the following. Malicidal acid 53.68 ± 2.91 µg/mL, pyrogallol 47.35 ± 3.01 µg/mL, Ascorbic acid 426.80 ± 24.82 µg/mL, Sodium pyruvate > 1000 µg/mL, Mannitol Dimethyl Sulphoxide (DMSO) No effect, Sodium azide no effect, Uric acid Peroxynitrite scavenger no effect, Etodolac2699 ± 479.50 mM, Indomethacin > 5000 mM. Galic acid, pyrogallol and ascorbic acid are known as common antioxidants, while sodium pyruvate is a specific antioxidant as an H2O2 scavenger. Sodium azide is a single oxygen scavenger, and mannitol and DMSO are OH scavengers. Based on these studies, the ability of the white oyster mushroom in scavenging is very well, even when compared with the ascorbic acid [24].

Antioxidant tests for H_2O_2 activity used by Fernando used an L-Ascorbic acid 10.00.14, C. sinensis (black tea) 91.96-2.51, Gallic acid 7.82-0.19, Tannic acid 8.17 0.10 mg/ml). The H2O2 activity test used both colourimetric but different reagents. Fernando's research used H2O2, phenol and 4-aminoantipyrine which reacted with the horseradish

peroxidase (HRP). This reaction uses a chromogen quinone imine which was assessed using a wavelength of 504 nm [35]. In this study, the antioxidant activity of the white oyster mushroom ethanol extraction was seen using the TEAC and H2O2 essay methods because these two methods were used less frequently than the in vitro antioxidant activity test method. The most commonly used methods are the a-diphenyl-bpicrylhydrazyl DPPH, hydroxyl and Superoxide dismutase (SOD) and b-carotene linolate [35].

Ethanol extractions are one of the most frequently used solvents for extracting, which is thought to have an antioxidant activity which is other than water and methanol solvents. All three are good polarity solvents which can dissolve polar active substances such as phenols and flavonoids. The use of water is rather limited because it requires special techniques when evaporating, while the use of methanol is limited due to its toxic effects [33].

Plants have excellent antioxidant potential because they contain enzymatic antioxidants as well as non-antioxidant enzymes. Antioxidant enzymes, for example, are SOD, catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase. Non-enzyme antioxidants contain antioxidants with small molecular weights such as ascorbic acid, phenol, glutathione, carotenoids, flavonoids or large, heavy molecules such as tannins. This is probably due to two reasons; the first is genetically indeed are the basic ability for plants to produce phytochemicals that can reduce the physiological processes, or as protection against oxidative stress environments such as plant parasites and microbes [33].

Some secondary metabolites are routinely formed, but some are formed in response to biytic and abiotic stress. Accumulation of phenolic compounds is because changes in the phenylpropanoid metabolism have been widely observed in several stressful conditions. In plants, phenolics can act as antioxidants by donating electrocution to guaiacol-type peroxidases to detoxify the production of H_2O_2 under stressful conditions. Phenolics also have protection capabilities against UV radiation through its ability to scavenge [36].

Biotic stress, such as wounds in plants, can induce phenolic metabolism and increase phenolic synthesis. Tanin is also thought to have a protective effect resembling phenol. Alkaloids can also generally protect plants against antimicrobials or attack herbivores and UV radiation. In the in vitro test, the alkaloid antioxidant activity was reported to be moderate to undetermined. Terpenoids are a secondary metabolite whose group is broad, consisting of 40,000 components. Monoterpenes, sesquiterpenes and diterpenes have been shown to have antioxidant activity. Tetraterpenes and carotenoids are also reported to have an antioxidant activity both in vitro and in vivo, some types of carotenoids such as beta-carotene show a prooxidant effect at high concentrations and high oxygen pressure [36].

So far phenols are known as the most important secondary metabolite based on the assessment of antioxidant activity, both in vitro and in vivo. Plant phenolic components are generally classified into five groups, namely phenolic acids, flavonoids, lignans, stilbenes and tannins. Phenolic compounds generally have one or more aromatic rings with one or more hydroxyl groups. Allegedly phenolic antioxidant capacity will increase by the addition of free hydroxyl groups and conjugations from the side chains of their aromatic rings. Flavonoids and phenolic acids are the largest groups of plants that are biosynthesized from the derivatives of acetate and shikimate pathways, as well as the shikimate pathway of phenylalanine or tyrosine [36].

Phytochemicals from these two groups were found to have an excellent antioxidant activity both in vitro and in vivo. It is also known that these metabolites will interact with physiological antioxidants such as ascorbic acid or tocopherol and will synergise the biological effects of both. Flavonoids and phenylpropanoids will cause oxidation with the help of the peroxidase enzyme and can act as H2O2 scavengers. In various studies, it was seen that the antioxidant potential of plants containing phenol, generally related to electron donation, reduced the ability in the metal ion chelating [36].

In conclusion, the process of drying Pleurotus Ostreatus was not reduced in the content of active substances. The phytochemical screening is obtained from all the samples. The polar active substances seem to be more soluble in the EE70 than in the EE96. The higher the bio-substances in preparation, the more significant the bio-therapeutic effects. White oyster mushrooms contain high levels of total phenol and high antioxidant activity of ABTS and a promising capture of H2O2, because of that this plant is very convincing to develop as a source of a medicinal plant for therapy.

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References

1. Hamda SS, Khanuja SPS, Longo G, Rakesh DD. Extraction technologies for medicinal and aromatic plan. Trieste: ICS UNIDO; 2008.

2. WHO. Legal Status of Traditional Medicine and Complementary / Alternative Medicine: A Worldwide Review. 2001.

3. Singh V, Vyas D, Pandey R, Sheik IA. Pleurotus ostreatus produces antioxidant and anti arthritis activity in wistar albino rats. World J Pharm Phar Sci. 2015; 4(05):1230-46.

4. Elsayed EA, Enshasy HE, Wadaan MAM, Aziz R. Revies Article. Mushrooms: A potensial natural source of anti-inflammatory compounds for medical applications. Hindawi. 2014; 2014:1-15. https://doi.org/10.1155/2014/805841 PMid:25505823 PMCid:PMC4258329

5. Jayakumar T, Thomas PA, Geraldine P. In-vitro antioxidant activities of an ethanolic extract of the oyster mushroom, Pleurotus ostreatus. Innov Food Sci Emerg. 2009; 10:228-34. https://doi.org/10.1016/j.ifset.2008.07.002

6. Jayakumara T, Sakthwel M, Thomas PA, Geraldine P. Pleurotus ostreatus an oyster mushroom, decereases the oxidative strees induced by carbon tetrachloride in rat kidneys, heart and brain. Chem-biol interact. 2008; 176(2-3):108 - 20. https://doi.org/10.1016/j.cbi.2008.08.006 PMid:18786523

7. Rahimah SB, Sastramihardja H, Sitorus T. Efek antioksidan jamur tiram putih pada kadar malondialdehid dan kepadatan permukaan sel paru tikus yang terpapar asap rokok. MKB. 2010; 42(4):195-202. https://doi.org/10.15395/mkb.v42n4.36

8. Roumyana D, Petrova, Abraham Z, Reznick, Solomon P. Wasser, et al. Fungal metabolites modulating nf-κb activity: an approach to cancer therapy and chemoprevention (review). Oncol Rep. 2008; 19(2):299-308. https://doi.org/10.3892/or.19.2.299 PMid:18202775

9. Jayakumar T, Thomas PA, Ramesh E, Pitchairaj. An extract of the mushroom, Pleurotus ostreatus, bolsters the glutathione redox system in various organs of aged rats. J Med Food. 2010; 13(4):1-8. https://doi.org/10.1089/jmf.2009.1130 PMid:20673055

10. Reis FS, Barros L, Martins A, Ferreira ICFR. Chemical composition and nutritional value of the most widely appreciated mushrooms: an inter-species comparative study. Food ChemToxicol. 2012; 50(2):191-7. https://doi.org/10.1016/j.fct.2011.10.056 PMid:22056333

11. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: a review. International pharmacerica sciencia. 2011; 1:1.

12. Sutomo, Arnida, Rizki MI, Triyasmono L., Nugroho A., Mintowati E, Salamiah. Skrining Fitokimia dan Uji Kualitatif Aktivitas Antioksidan Tumbuhan Asal Daerah Rantau Kabupaten Tapin Kalimantan Selatan. Jurnal Pharmascience. 2016; 3(1):66 - 74.

13. Settharaksa S., Madaka F., Chakree K., Charoenchai L., Total phenolic and flavonoid contents and antioxidant properties of thai traditional herbal international Journal of Pharmacy and Pharmaceutical Sciences. 2014; 6(9).

14. Yildiz S, Yilmaz A, Can Z, Kiliç C, Yildiz UC. Total phenolic, flavonoid, tannin contents and antioxidant properties of Pleurotus ostreatus and Pleurotus citrinopileatus cultivated on various sawdust. GIDA. 2017; 42(3):315-23. https://doi.org/10.15237/gida.GD16099

15. Widowati W, Darsono L, Suherman J, Yelliantty Y, Maesaroh,M. High Performance Liquid Chromatography (HPLC) analysis, antioxidant, antiaggregation of mangosteen peel extract (Garcinia mangostana L.). Int J BioSci Biochem Bioinformatics. 2014; 4(6):458-466. https://doi.org/10.17706/ijbbb.2014.4.6.458-466

16. Lusiana L. Potensi Antioksidasi Ekstrak Etanol Jamur Tiram Putih (Pleurotus Ostreatus). GRADIEN: Jurnal Ilmiah MIPA. 2015; 11(1):1066-9.

17. Kumar RS, Venkateshwar C, Samuel G, Rao RG. Phytochemical Screening of some compounds from plant leaf extracts of Holoptelea integrifolia (Planch.) and Celestrus emarginata (Grah.). 2013; 2(8):65-70.

18. Joseph BS, Kumbhare PH, Kale MC. Preliminary phytochemical screening of selected Medicinal Plants. Int. Res. J. of Science & Engineering. 2013; 1(2):55-62

19. Sheel R, Nisha K, Kumar J. Preliminary Phytochemical Screening of Methanolic Extract of Clerodendron infortunatum. Journal of Applied Chemistry (IOSR-JAC). 2014; 7(1):10-13. https://doi.org/10.9790/5736-07121013

20. Gülçin I, Uğuz MT, Oktay M, Beydemir Ş, Küfrevioğlu Öl. Evaluation of the antioxidant and antimicrobial activities of clary sage (Salvia sclarea L.). Turkish Journal of Agriculture and Forestry. 2004; 28(1):25-33.

21. Schofield P, Mbugua DM, Pell AN. Analysis of condensed tannins: a review. Animal feed science and technology. 2001: 91(1-2):21-40. https://doi.org/10.1016/S0377-8401(01)00228-0

22. Widowati W, Janeva WB, Nadya S, Amalia A, Arumwardana S, Kusuma HSW, Arinta Y. Antioxidant and Antiaging Activities of Jasminum sambac Extract, and its Compounds, J Reports Pharmaceutic Sci 2018; 7(3):270-285. https://doi.org/10.20307/nps.2017.23.3.192

23. Noreen H, Semmar N, Farman M, McCullagh JSO. Measurement of total phenolic content and antioxidant activity of aerial parts of medicinal plant Coronopus didymus. Asian Pacific Journal of Tropical Medicine. 2017; 10(80):792-801. https://doi.org/10.1016/j.apjtm.2017.07.024 PMid:28942828

24. Mukhopadhyay D, Dasgupta P, Roy DS, Palchoudhuri S, Chatterjee I, Ali S, Dastidar SG. A Sensitive In vitro Spectrophotometric Hydrogen Peroxide Scavenging Assay using 1, 10-Phenanthroline. Free Radicals & Antioxidants. 2016; 6(1). https://doi.org/10.5530/fra.2016.1.15

25. Ramawat KG, Merrilon JM. Bioactives molecule and Medicinal Plants. Springer, 2008:22-18. https://doi.org/10.1007/978-3-540-74603-4 PMid:19189646

26. Doughari JH. Phytochemicals: extraction methods, basic structures and mode of action as potential chemotherapeutic agents. InPhytochemicals-A global perspective of their role in nutrition and health. InTechOpen. 2012.

27. Ahmad I, Aqil F, Owais M, editors. Modern phytomedicine: Turning medicinal plants into drugs. John Wiley & Sons, 2006. https://doi.org/10.1002/9783527609987

28. Boonsong S, Klaypradit W, Wilaipun P. Antioxidant activities of extracts from five edible mushrooms using different extractants. Agriculture and natural resources. 2016; 50:89-97. https://doi.org/10.1016/j.anres.2015.07.002

29. Sigh V, Pandey R, Vyas D. Antioxidant potentiality of Pleurotus ostreatus (MTCC142) cultivated on different agro wastes. Asian Journal of Plant Science and Research. 2015; 5(6):22-7.

30. Paul AT, Gohil VM, Bhutani KK. Modulating TNF-α signaling with natural products. Drugs discov today. 2006; 11(15-16):725-32. https://doi.org/10.1016/j.drudis.2006.06.002 PMid:16846800

31. Yim H, Chye FY, Tan CT, Ng YC, Ho CW. Antioxidant Activities and Total Phenolic Content of Aqueous Extract of Pleurotus ostreatus (Cultivated Oyster Mushroom). Mal J Nutr. 2010; 16(2):281-91.

32. Widowati W, Fauziah N, Heridman H, Afni M, Afifah E, Kusuma HSW, Nufus H, Arumwardana S and Rihibiha DD. Antioxidant and anti aging assays of Oryza sativa extracts, vanillin and coumaric acid. J Nat Remed. 2016; 16(3):88-99. https://doi.org/10.18311/jnr/2016/7220

33. Nur Alam M, Bristi NJ, Rafiquzzaman M. Review on in vivo and in vitro methods evaluation of antioxidant activity. Saudi Pharmaceutical Journal. 2013; 21:143-152. https://doi.org/10.1016/j.jsps.2012.05.002 PMid:24936134 PMCid:PMC4052538

34. Prabu MA, Kumuthakalavallia R. Antioxidant activity of oyster mushroom (Pleurotus florida [Mont.] singer) and milky mushroom (Calocybe indica P and C). Int J Curr Pharmaceut Res. 2016; 8(3):1-4.

35. Fernando CD, Soysa P., Optimized enzymatic colorimetric assay for determination of hydrogen peroxide (H2O2) scavenging activity of plant extracts. MethodsX. 2015; 18(2):283-91. https://doi.org/10.1016/j.mex.2015.05.001 PMid:26285798 PMCid:PMC4500160

36. Kasote DM, Katyare SS, Hegde MV, Bae H. Significance of antioxidant potential of plants and its relevance to therapeutic applications. International journal of biological sciences. 2015; 11(8):982. https://doi.org/10.7150/ijbs.12096 PMid:26157352 PMCid:PMC4495415

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Clinical Science

Magnetic Resonance Imaging Features of Common Posterior Fossa Brain Tumors in Children: A Preliminary Vietnamese Study

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Abstract

BACKGROUND: Magnetic Resonance Imaging (MRI) nowadays plays an important role in the evaluation of posterior fossa brain tumours in children for appropriate diagnosis, treatment planning, and follow-up.

AIM: To assess the MRI features of common posterior fossa brain tumours including medulloblastomas, ependymomas, and pilocytic astrocytomas along with the postoperative parameters to contribute the local knowledge to the neuroradiology and neurosurgery fields.

METHODS: The study was performed at Children's Hospital 02 from January 2016 to June 2019. In this study, all pediatric patients adopted MRI to evaluate the posterior fossa brain tumours' characteristics and then underwent surgery to eradicate the posterior fossa tumours. We retrospectively compared the baseline parameters, MRI parameters, and postoperative parameters among medulloblastomas, ependymomas, and pilocytic astrocytomas.

RESULTS: There were 62 patients (27 medulloblastomas, 20 ependymomas, and 15 pilocytic astrocytomas) in this research. The main structure of medulloblastomas and ependymomas was predominantly solid, whereas the main structure of pilocytic astrocytomas was superiorly cystic ($p < 0.05$). Ependymoma tended to extend tumour through foramina of Luschka and Magendie ($p < 0.05$). Medulloblastomas chiefly showed iso intensity on T2W and FLAIR images meanwhile ependymomas and pilocytic astrocytomas predominantly appeared hyperintensity on T2W and FLAIR images. Medulloblastomas and ependymomas were mostly high intensity on DWI, and low intensity on ADC whereas pilocytic astrocytomas were usually low intensity on DWI and high intensity on ADC. After injecting CE, pilocytic astrocytomas showed a mixed intensity whereas the signal intensity of medulloblastoma and ependymoma on T1CE was generally strong. There were positive correlations between FH diameter and estimated blood loss ($r = 0.289$, $p < 0.05$); and surgical time ($r = 0.312$, $p < 0.05$).

CONCLUSION: MRI plays a crucial role in demonstrating the features of posterior fossa brain tumours for appropriate diagnosis of medulloblastomas, ependymomas, and pilocytic astrocytomas. Medulloblastomas are problematic tumours and the clinicians should also take into consideration in cases of larger feet-to-head diameter of tumours to ensure the efficacy and safety surgery for patients.

Introduction

Viet Nam, the second most crowded nation positioned in South East Asia, is economically classified as a developing country in the world. Ho Chi Minh city is the largest city located in the south of the country with nearly 10 million people living there, of whom about 20-25% were under 15-year-old with the male to female ratio at birth is 1.122 [1], [2]. Intra-axial cranial tumours are the second commonest neoplasm following leukaemia in children occurring in 2.4 to 4 per 100,000 people. In children, primary intra-axial supratentorial brain tumours account for 30-40% [3]. Whereas, infratentorial brain tumours occupy approximately 60 – 70% of all brain tumours [4].

In a previous study, the result showed that the incidence of brain cancer in Vietnam is 2.2 per 100000 for male and 1.4 per 100000 for female. Meanwhile, the percentage of brain cancer in male and female children was 18.1% and 17.2%, respectively [1]. In reports issued by Central Brain Tumor Registry of the United States, the findings revealed that brain tumours have an incidence of 5.47 per 100,000 children under 14-year-old. It is critical that the most common tumours, leading the cause of cancer-related death were posterior fossa brain tumours (medulloblastomas, ependymomas, and pilocytic astrocytomas) [5], [6], [7], [8].

The incidence of pediatric brain tumours in Vietnam is lower than that of other countries due to some following reasons [2]. Vietnamese radiology was

legitimately established after Victory Dien Bien Phu by 1954, which was the end of the war against French invasion. Then, Vietnam was suffered from the war against the United States until 1975. Thus, radiology has just developed for nearly 45 years. Even though MRI nowadays plays an important role in the evaluation of brain tumours for appropriate diagnosis, treatment planning and follow-up, approximately 80% of the Vietnamese cities and central hospitals owned only 51 magnetic resonance imaging (MRI) systems by 2009 [9].

Therefore, it is an apparent fact that there was an under-assessment brain tumour for children in Vietnam due to the insufficiency of non-invasive and innovative MRI modality [2]. Also, there are only nine hospitals in Vietnam where the appropriate evaluation and surgery for patients with brain tumours can be carried out efficaciously (4 in Ha Noi, 1 in Da Nang and 4 in Ho Chi Minh city) [10]. Currently, there is no systematic MRI study about pediatric posterior fossa brain tumours in Vietnam. Hence, in this study, we aimed to assess the MRI features of common posterior fossa brain tumours in children including medulloblastomas, ependymomas and pilocytic astrocytomas along with the postoperative parameters to contribute the local knowledge to the neuroradiology and neurosurgery fields.

Material and Methods

Ethical consideration

Institutional Review Board of Children's Hospital 02 approved this retrospective study (745 / ND2-CDT).

Patient population

The study was carried out in the Department of Radiology and Department of Neurosurgery, Children's Hospital 02 from January 2016 to June 2019. In this research, all pediatric patients adopted MRI to evaluate the tumours' characteristics before treatment. Then, patients underwent surgery to eradicate the posterior fossa tumours. Histopathological samples were analysed by histopathologist who had 8-year experience in interpreting brain tumours.

MRI protocol

Before 2019, MRI 1.5T scanners were utilised (Essenza, Siemens, Erlangen, Germany and Optima, GE Healthcare, Milwaukee, The United States of America). By 2019, Scanning was additionally performed by 1.5T Multiva, Philips Medical Systems, Best, the Netherlands. All MRI images were interpreted by two certificated MRI radiologists (NMD and MTLB) with over 10-year experience. It is noted that MRI protocols were the same and fully approved by both radiology and neurosurgery departments including non-contrast sagittal T1-weighted imaging (T1W); axial T2-weighted imaging (T2W), coronal T2-Fluid-Attenuated Inversion Recovery (FLAIR), axial gradient-recalled echo T2*WI, axial Diffusion-weighted imaging (DWI) with apparent diffusion coefficient (ADC) and axial T1-weighted with contrast enhancement (CE) (T1CE) (0.1 ml/kg-Gadovist, Bayer, Germany or 0.2ml/kg-Dotarem, Guerbet, France).

Parameters

Baseline parameters included the age, sex, symptoms, tumour location, tumour diameters (anterior-posterior (AP); right-left (RL); and feet-head (FH)), tumour characteristics (main structure, components, dilated ventricle, peritumoral oedema, and tumour extension). MRI parameters were comprised of signal intensity (SI) of T1W, T2W, FLAIR, DWI, ADC, and T1CE.

Postoperative parameters included tumour histology, estimated blood loss, surgical time, time at intensive care unit, and total hospital admission time.

Data collection and Statistics

Data were collected and stored on a spreadsheet (Excel 2010; Microsoft, Redmond, Washington). Data were analysed by SPSS version 23 (IBM, Armonk, New York). Continuous variables were introduced as mean ± standard deviation and range meanwhile nominal variables were presented as percentage or number of cases. Nominal variables were compared by performing Fisher's exact test. Continuous variables were compared by exploiting the Anova with or without Post-Hoc test if appropriate. Pearson correlation test is used to investigate the relationship between two continuous variables. The observed differences were statistically significant if the p-value was less than 0.05 ($p < 0.05$).

Results

Baseline parameters

As shown in Table 1, there are 62 pediatric patients (male/female = 1.07/1) with mean age: 6.73 years ± 3.50 (1-15). Medulloblastomas were more dominant in male than in female meanwhile ependymomas and pilocytic astrocytomas were more common in female than in male.

Table 1: Baseline characteristics

Baseline parameters	Overall n = 62	Medulloblastoma n = 27	Ependymoma n = 20	Pilocytic astrocytoma n = 15	P value
Age (years)	6.73 ± 3.50 (1-15)	7.79 ± 2.96 (2-15)	4.50 ± 2.85 (1-11)	7.60 ± 3.94 (2-15)	0.002*[α]
Gender					0.019*[β]
Female	30 (48.4%)	8	11	11	
Male	32 (51.6%)	19	9	4	
Symptoms					0.201
Headache	33 (53.2%)	14	10	9	
Vomiting	13 (21%)	10	2	1	
Muscle weakness	6 (9.7%)	1	3	2	
Ataxia	3 (4.8%)	1	1	1	
Unconsciousness	3 (4.8%)	1	2	0	
Epilepsy	1 (1.6%)	0	1	0	
Faint	1 (1.6%)	0	0	1	
Sensory disorder	1 (1.6%)	0	0	1	
Dysmetria	1 (1.6%)	0	1	0	
Location					< 0.001*[β]
Fourth ventricle	41 (66.1%)	21	19	1	
Vermis	9 (14.5%)	2	1	6	
Right cerebellar hemisphere	7 (11.3%)	2	0	5	
Left cerebellar hemisphere	5 (8.1%)	2	0	3	
Diameters					
RL (mm)	48.82 ± 11.01 (21-83)	47.81 ± 9.70 (21-62)	45.00 ± 9.72 (25-63)	55.73 ± 12.29 (32-83)	0.012*[α]
AP (mm)	45.69 ± 11.67 (17-67)	44.07 ± 10.70 (20-67)	41.75 ± 10.04 (17-55)	53.83 ± 12.01 (25-67)	0.005*[α]
FH (mm)	46.63 ± 11.45 (16-68)	44.41 ± 9.98 (17-63)	46.00 ± 13.82 (16-68)	51.47 ± 9.54 (30-65)	0.153
Main structure					< 0.001*[β]
Solid	37 (59.7%)	22	14	1	
Mixed	15 (24.2%)	5	6	4	
Cyst	10 (16.1%)	0	0	10	
Components					< 0.001*[β]
Cyst inside tumor	18 (29.0%)	1	2	15	
Necrosis	17 (27.4%)	10	7	0	
Hemorrhage	8 (12.9%)	6	2	0	
Calcification	2 (3.2%)	1	1	0	
Peritumoral edema	21 (33.9%)	12	5	4	0.302
Dilated ventricle	55 (88.7%)	25	17	13	0.689
Tumor extension					0.016*[β]
Magendie foramina	7 (11.3%)	2	5	0	
Left Luschka foramina	4 (6.5%)	1	3	0	
Right Luschka foramina	3 (4.8%)	0	2	1	
Both Luschka foramina	2 (3.2%)	0	2	0	
Sylvius aqueduct	2 (3.2%)	1	1	0	

* Statistically significant; [α] Anova test; [β] Fisher's exact test.

The mean age of ependymoma was significantly lower than the two other types ($p < 0.05$). Two most common locations of tumours were fourth ventricle and vermis. The means of three diameters (AP, RL, and FH) of pilocytic astrocytomas were higher than those of two other types. The main structure of medulloblastomas and ependymomas was predominantly solid whereas the main structure of pilocytic astrocytomas was superiorly cystic ($p < 0.05$).

Necrosis and haemorrhage were observed more commonly in medulloblastomas and ependymomas; meanwhile, pilocytic astrocytomas mostly contains cyst inside the tumours ($p < 0.05$). Peritumoral oedema was significantly higher in patients with medulloblastomas than in two other types ($p < 0.05$). Ependymomas tended to extend tumours through foramina of Luschka and Magendie ($p < 0.05$).

MRI parameters

As shown in Table 2, medulloblastomas chiefly showed isointense on T2W and FLAIR images meanwhile ependymomas, and pilocytic astrocytomas predominantly appeared hyperintensity on T2W and FLAIR images. Medulloblastomas and ependymomas were mostly high intensity on DWI, and low intensity on ADC whereas pilocytic astrocytomas were usually low intensity on DWI and high intensity on ADC. After injecting CE, pilocytic astrocytomas showed a mixed intensity whereas the signal intensity of medulloblastomas and ependymomas on T1CE was generally strong.

Table 2: MRI characteristics

MRI parameters	Overall n = 62	Medulloblastoma n = 27	Ependymoma n = 20	Pilocytic astrocytoma n = 15	p
T1W					0.474
Hypointense	58 (93.5%)	25	18	15	
Mixed	4 (6.5%)	2	2	0	
T2W					< 0.001*[β]
Hyperintense	45 (72.6%)	10	20	15	
Isointense	15 (24.2%)	15	0	0	
Mixed	2 (3.2%)	2	0	0	
FLAIR					< 0.001*[β]
Hyperintense	27 (43.5%)	7	14	6	
Isointense	17 (27.4%)	16	1	0	
Mixed	13 (21%)	4	5	4	
Hypointense	5 (8.1%)	0	0	5	
DWI					< 0.001*[β]
Hyperintense	39 (62.9%)	23	16	0	
Hypointense	23 (37.1%)	4	4	15	
ADC					< 0.001*[β]
Hypointense	39 (62.9%)	23	16	0	
Hyperintense	23 (37.1%)	4	4	15	
T1CE					< 0.001*[β]
Strong	35 (56.5%)	20	14	1	
Mixed	17 (27.4%)	0	3	14	
Slight	10 (16.1%)	7	3	0	

* Statistically significant; [β] Fisher's exact test.

Postoperative parameters

As shown in Table 3, ANOVA test showed that there was no significant difference among total admission time, estimated blood loss, intensive care unit time and surgical time among three groups. Nonetheless, Post-Hoc test revealed that total hospital admission time and intensive care unit time of patients with medulloblastomas were significantly higher than those of patients with pilocytic astrocytomas ($p = 0.008$ and $p = 0.045$, respectively).

Correlation test

It is observed that there were weak positive correlations between FH diameter and estimated blood loss ($r = 0.289$, $p < 0.05$, Pearson test); and surgical time ($r = 0.312$, $p < 0.05$, Pearson test). Meanwhile, there was a moderate positive correlation between surgical time and estimated blood loss ($r = 0.485$, $p < 0.05$, Pearson test).

Discussion

In this clinical study, we focused only the three most common posterior fossa brain tumours including medulloblastomas, ependymomas, and pilocytic astrocytomas. We observed that there were

significant differences between age and gender among these groups. In a previous study, mean ages of medulloblastomas, ependymomas, and pilocytic astrocytomas were 6.2; 4.7; and 6.2, respectively. Moreover, the male to female ratios for medulloblastomas, ependymomas, and pilocytic astrocytomas were 1.3/1; 0.67/1; and 1.12/1 [11]. Meanwhile, in our study, mean ages for medulloblastomas, ependymomas, and pilocytic astrocytomas were 7.79; 4.50; and 7.60. Furthermore, the male to female ratios for medulloblastomas, ependymomas, and pilocytic astrocytomas in this present study were 2.37/1; 0.82/1; and 0.36/1. In many literature papers, medulloblastomas, accounting for 40% of posterior fossa tumors, more prevailing in male, are usually seen before 7-year-old whereas ependymomas, accounting for approximately 20% of the posterior fossa tumours in children with a slight increase of incidence in boys, have a peak incidence in younger pediatric patients from 3- to 5-year-old [12], [13]. On the other hand, pilocytic astrocytomas, making up 30% of posterior fossa tumours, appearing between the ages of 5- to 13-year-old [12], [13], [14], have an equal incidence between girls and boys [15]. Although there are small discriminations about the mean ages and gender ratio among different studies, the results of our study are in agreement with the epidemiological information [11], [12], [13].

Medulloblastomas and ependymomas, usually predominantly solid tumours, are often positioned on the midline in 75% of cases. Medulloblastomas generally progress in the fourth ventricle from the vermis meanwhile ependymomas mostly grow in the fourth ventricle, both resulting in ventricular obstruction and hydrocephalus [13], [16]. Medulloblastomas may hardly expand into the foramina of Magendie or Luschka whereas ependymomas frequently show extension through Magendie and Luschka foramina [13]. By contrast, pilocytic astrocytomas always situate in cerebellar hemisphere and superiorly have mixed appearance of the cystic tumour with mural portion. They slowly evolve and rarely emerge as a solid tumour [13]. Due to typical characteristics of solid tumours, necrosis and haemorrhage appear more common in medulloblastomas and ependymomas than pilocytic astrocytomas [12], [17]. Also, peritumoral oedema presents more generally in patients with medulloblastomas than two other types because medulloblastomas, the most malignant tumour among three types, are graded as 4. As shown in Table 1, our findings of baseline characteristics of three tumour types are absolutely in agreement with previous studies [12], [13], [15], [16], [17].

Typically, medulloblastomas are densely packed cells and hyperchromatic nuclei, which will result in hypointensity to isointense on T1W image [18]. Both hyperintensity on DWI and hypointensity on ADC coexisting with hypointense to isointense T2W are due to high cellularity of the tumour [18], [19]. Among three types, medulloblastomas are highly malignant and hypervascular; hence, the tumours absorb contrast agents vigorously. Absence of enhancement is seldom, which is noticed in only 7.5% of the tumours [12], [20]. Meanwhile ependymomas, generally manifest hypointense T1W, hyperintense T2W, and iso- to hyper-intense FLAIR. On post-contrast enhancement T1W images, tumours generally demonstrate avid enhancement. There are few tumours manifesting little or absence of post-gadolinium enhancement despite being consisted of solid tissue. DWI shows diminished diffusivity within most of ependymomas also due to high cellularity. It is reported that diffusivity of ependymomas is commonly intermediate between that of medulloblastomas and pilocytic astrocytomas [21], [22]. Pilocytic astrocytomas typically appear as a predominant cystic tumour with mural nodule. The cysts of pilocytic astrocytomas are commonly hypointense on T1W and hyperintense on T2W; and FLAIR. In few cases, tumours show hyperintensity on T1W and FLAIR when fluid is highly proteinaceous. Unrestricted diffusion is a typical feature of pilocytic astrocytomas [23], [24]. Tumours mainly show mixed enhancement because of central cysts without absorbing contrast agents, while the mural portions tend to enhance homogenously and noticeably [12]. As shown in Table 2, our MRI findings are completely in line with these studies [12], [18], [19], [20], [21], [22], [23], [24].

In some previous studies, the findings showed that the two most frequent symptoms were headache and vomiting. Also, hydrocephalus was observed in 78-86.7% of the patients [4], [25]. In this present study, two dominant symptoms were also headache and vomiting, and hydrocephalus appeared in 88.7% of patients. The two most common locations of tumours were fourth ventricle and vermis along with the means of three-dimension diameters over 4.5 cm producing intracranial hypertension, ventricular obstruction, and dilated ventricle resulting in these clinical symptoms. Hence, our findings are in agreement with these studies [4], [25].

Four parameters related to the treatment (surgical time, estimated blood loss volume, intensive care unit time, and total hospital admission time) were not generally significantly different. Medulloblastomas and ependymomas are predominantly solid, but pilocytic astrocytomas are principally cystic. Thus, the elimination of medulloblastomas and ependymomas will be more complicated than that of pilocytic astrocytomas. Nevertheless, in this present study, the means of three-dimension diameters of pilocytic astrocytomas were over 5 cm and higher than those of medulloblastomas. In a previous study, the findings showed that brain tumours with a diameter of at least 5cm are considered as giant brain tumour which generates difficulties during the surgery [25]. Thus, there are pros and cons in performing surgery to eradicate the medulloblastomas, ependymomas and pilocytic astrocytomas in this present study leading to

generally insignificant differences among those parameters. Nevertheless, Post-Hoc test revealed that total hospital admission time and intensive care unit time of patients with pilocytic astrocytomas were significantly shorter than those of patients with medulloblastomas. This difference is mainly due to medulloblastomas classified as grade-4 more malignant than pilocytic astrocytomas regarded as grade-1 the most benign tumour amongst three tumour types [13], [15] (Table 3).

Table 3: Postoperative characteristics

Postoperative parameters	Overall n = 62	Medulloblastoma n = 27	Ependymoma n = 20	Pilocytic astrocytoma n = 15	p
Surgical time (hour)	5.0 ± 1.1 (3-7)	5.07 ± 1.11 (3-7)	5.25 ± 1.07 (4-7)	4.47 ± 1.06 (3-6)	0.099
Blood loss volume (ml)	267.1 ± 173.8 (50-1000)	284.82 ± 193.00 (80-1000)	277.00 ± 152.90 (50-500)	222.00 ± 166.83 (50-700)	0.515
Intensive care unit time (day)	3.0 ± 1.6 (1-9)	3.29 ± 1.59 (2-8)	3.15 ± 1.76 (2-9)	2.67 ± 1.03 (1-5)	0.108
Hospital admission time (day)	41.3 ± 22.9 (11-136)	44.96 ± 17.54 (19-98)	44.80 ± 32.13 (11-136)	30.20 ± 12.13 (13-51)	0.096

In this present study, FH diameter had a positive correlation with estimated blood loss and surgical time. The tumours were predominantly located in the fourth ventricle and vermis (80.6%). Anatomically, the fourth ventricle is rather loose at the centre but very narrow to the above Sylvius aqueduct and below central canal of spinal cord. Therefore, the eradication of tumour tissues at the centre of fourth ventricle will be faster and more efficient than tissue nearby sophisticated structures like Sylvius aqueduct and the top of central canal of spinal cord. It is clear that the higher the diameters are, the bigger the tumours are and surgery of bigger tumours will take more time to complete than smaller tumours. We also noticed a positive correlation between surgical time and estimated blood loss. In addition, when the surgical time gets longer to eradicate as much tumour tissue as possible, the blood loss from the collapse of the supplying vascularity of tumour will enhance. In this study, tumors with strong enhancement reflected that perfusion of tumors is significantly effective. Thus, these results are appropriate.

There are some limitations in this present study. Firstly, it is a retrospective design with a small population size; therefore, we do not have abundant and variant findings. Secondly, due to the limitation of innovatively quantified workstation, it is inclined to qualitative research. Thirdly, we just concentrated on three common types of posterior fossa brain tumours in children. Further studies should be as a prospective design with larger sample size. In addition, we also suggest that further studies will introduce quantitative parameters for more objective findings. Future studies also need to focus more on other types of brain tumours to produce plentiful knowledge for clinicians.

In conclusion, MRI plays a crucial role in demonstrating the features of these tumours for appropriate diagnosis and treatment planning. In practice, based on MRI, each tumour has typical MRI features to help clinician discriminate depended on baseline characteristics and MRI characteristics. Among these tumour types, medulloblastomas are problematic brain tumours and the clinicians should take into consideration in cases of larger feet-to-head diameter of tumour to ensure the efficacy and safety surgery for patients.

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References

1. Nguyen QM, Nguyen HC, Parkin DM. Cancer incidence in Ho Chi Minh City, Viet Nam, 1995-1996. Int J Cancer. 1998; 76(4):472-9. https://doi.org/10.1002/(SICI)1097-0215(19980518)76:4<472::AID-IJC5>3.0.CO;2-O

2. Nguyen QM, Nguyen HC, Kramarova E, Parkin DM. Incidence of childhood cancer in Ho Chi Minh City, Vietnam, 1995-97. Paediatr Perinat Epidemiol. 2000; 14(3):240-7. https://doi.org/10.1046/j.1365-3016.2000.00272.x PMid:10949216

3. Chang YW, Yoon HK, Shin HJ, Roh HG, Cho JM. MR imaging of glioblastoma in children: usefulness of diffusion/perfusion-weighted MRI and MR spectroscopy. Pediatr Radiol. 2003; 33(12):836-42. https://doi.org/10.1007/s00247-003-0968-8 PMid:14564423

4. Dorner L, Fritsch MJ, Stark AM, Mehdorn HM. Posterior fossa tumors in children: how long does it take to establish the diagnosis? Childs Nerv Syst. 2007; 23(8):887-90. https://doi.org/10.1007/s00381-007-0323-8 PMid:17429658

5. Ostrom QT, Gittleman H, Xu J, Kromer C, Wolinsky Y, Kruchko C, et al. CBTRUS Statistical Report: Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2009-2013. Neuro Oncol. 2016; 18(suppl_5):v1-v75. https://doi.org/10.1093/neuonc/now207 PMid:28475809

6. Ostrom QT, de Blank PM, Kruchko C, Petersen CM, Liao P, Finlay JL, et al. Alex's Lemonade Stand Foundation Infant and Childhood Primary Brain and Central Nervous System Tumors Diagnosed in the United States in 2007-2011. Neuro Oncol. 2015; 16 Suppl 10:x1-x36. https://doi.org/10.1093/neuonc/nou327 PMid:25542864 PMCid:PMC4277295

7. Guerreiro Stucklin AS, Grotzer MA. Cerebellar tumors. Handb Clin Neurol. 2018; 155:289-99. https://doi.org/10.1016/B978-0-444-64189-2.00019-6 PMid:29891066

8. Cuellar-Baena S, Morales JM, Martinetto H, Calvar J, Sevlever G, Castellano G, et al. Comparative metabolic profiling of paediatric ependymoma, medulloblastoma and pilocytic astrocytoma. Int J Mol Med. 2010; 26(6):941-8. https://doi.org/10.3892/ijmm_00000546 PMid:21042791

9. Kiet H. State and future of radiology and nuclear medicine in Vietnam. Biomed Imaging Interv J. 2009; 5(4):e34. https://doi.org/10.2349/biij.5.4.e34 PMid:21610998 PMCid:PMC3097725

10. Trung le X, Long le X, Tu N, Long NT, Nga NT, Son TV, et al. Brain tumours in Ho Chi Minh City. J Clin Neurosci. 1998; 5(4):421-2. https://doi.org/10.1016/S0967-5868(98)90276-4

11. Panigrahy A, Krieger MD, Gonzalez-Gomez I, Liu X, McComb JG, Finlay JL, et al. Quantitative short echo time 1H-MR spectroscopy of untreated pediatric brain tumors: preoperative diagnosis and characterization. AJNR Am J Neuroradiol. 2006; 27(3):560-72.

12. Poretti A, Meoded A, Huisman TA. Neuroimaging of pediatric posterior fossa tumors including review of the literature. J Magn Reson Imaging. 2012; 35(1):32-47. https://doi.org/10.1002/jmri.22722 PMid:21989968

13. Koob M, Girard N. Cerebral tumors: specific features in children. Diagn Interv Imaging. 2014; 95(10):965-83. https://doi.org/10.1016/j.diii.2014.06.017 PMid:25150727

14. Plaza MJ, Borja MJ, Altman N, Saigal G. Conventional and advanced MRI features of pediatric intracranial tumors: posterior fossa and suprasellar tumors. AJR Am J Roentgenol. 2013; 200(5):1115-24. https://doi.org/10.2214/AJR.12.9725 PMid:23617498

15. Choudhri AF, Siddiqui A, Klimo P, Jr. Pediatric Cerebellar Tumors: Emerging Imaging Techniques and Advances in Understanding of Genetic Features. Magn Reson Imaging Clin N Am. 2016; 24(4):811-21. https://doi.org/10.1016/j.mric.2016.07.006 PMid:27742118

16. Sumer-Turanligil NC, Cetin EO, Uyanikgil Y. A contemporary review of molecular candidates for the development and treatment of childhood medulloblastoma. Childs Nerv Syst. 2013; 29(3):381-8. https://doi.org/10.1007/s00381-012-2014-3 PMid:23292496

17. Rasalkar DD, Chu WC, Paunipagar BK, Cheng FW, Li CK. Paediatric intra-axial posterior fossa tumours: pictorial review. Postgrad Med J. 2013; 89(1047):39-46. https://doi.org/10.1136/postgradmedj-2011-130075 PMid:22977284

18. Koeller KK, Rushing EJ. From the archives of the AFIP: medulloblastoma: a comprehensive review with radiologic-pathologic correlation. Radiographics. 2003; 23(6):1613-37. https://doi.org/10.1148/rg.236035168 PMid:14615567

19. Meyers SP, Kemp SS, Tarr RW. MR imaging features of medulloblastomas. AJR Am J Roentgenol. 1992; 158(4):859-65. https://doi.org/10.2214/ajr.158.4.1546606 PMid:1546606

20. Nelson M, Diebler C, Forbes WS. Paediatric medulloblastoma: atypical CT features at presentation in the SIOP II trial. Neuroradiology. 1991; 33(2):140-2. https://doi.org/10.1007/BF00588252 PMid:2046898

21. Yuh EL, Barkovich AJ, Gupta N. Imaging of ependymomas: MRI and CT. Childs Nerv Syst. 2009; 25(10):1203-13. https://doi.org/10.1007/s00381-009-0878-7 PMid:19360419 PMCid:PMC2744772

22. Rumboldt Z, Camacho DL, Lake D, Welsh CT, Castillo M. Apparent diffusion coefficients for differentiation of cerebellar tumors in children. AJNR Am J Neuroradiol. 2006; 27(6):1362-9.

23. D'Arco F, Khan F, Mankad K, Ganau M, Caro-Dominguez P, Bisdas S. Differential diagnosis of posterior fossa tumours in children: new insights. Pediatr Radiol. 2018; 48(13):1955-63. https://doi.org/10.1007/s00247-018-4224-7 PMid:30120502

24. Chourmouzi D, Papadopoulou E, Konstantinidis M, Syrris V, Kouskouras K, Haritanti A, et al. Manifestations of pilocytic astrocytoma: a pictorial review. Insights Imaging. 2014; 5(3):387-402. https://doi.org/10.1007/s13244-014-0328-2 PMid:24789122 PMCid:PMC4035491

25. Guo A, Suresh V, Liu X, Guo F. Clinicopathological features and microsurgical outcomes for giant pediatric intracranial tumor in 60 consecutive cases. Childs Nerv Syst. 2017; 33(3):447-55. https://doi.org/10.1007/s00381-017-3341-1 PMid:28180935

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Clinical Science

Angiographically Based Direct Implantation of the Bioresorbable Vascular Scaffold in Non-ST Segment Elevation Acute Coronary Syndrome: Feasibility and Outcome

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Abstract

BACKGROUND: Direct implantation of metallic drug-eluting stents is recommended for lesions with high thrombotic burden; however, this can't be applied to bioresorbable scaffold for which adequate lesion preparation is recommended.

AIM: We aimed at assessing the feasibility and safety of direct scaffold implantation based only on angiographic assessment in patients presented with non-ST segment elevation acute coronary syndrome.

METHODS: The study was a retrospective two-centre study conducted over patients diagnosed with NSTE-ACS presented to cardiology department at Juan Ramon Hospital, Spain and critical care department, Cairo University in the period between February 2016 to May 2017. We included patients for whom we depend only on angiographic assessment for decision making whether to directly implant the scaffold or predilate the lesion and we excluded patients for whom intracoronary imaging was used at the index procedure either for pre or post-implantation. The primary outcome of interest was the device-oriented composite endpoints (DOCE) including cardiac death, and MI attributed to the target vessel and TLR. The secondary endpoints were the broader patient-oriented composite outcome (POCE) and scaffold/stent thrombosis. POCE includes all-cause mortality, any MI and any revascularisation (including TLR, TVR and revascularisation of non- target vessel)

RESULTS: Among 46 patients with NSTE-ACS treated with BVS, we did direct implantation in 20 patients (group A), and we used pre dilatation in 26 patients (group B). The two groups have similar demographics and clinical criteria. Procedural success was obtained in all study population. Mean follow up duration was 12 months. We have total of 10% device-oriented composite endpoints in group A versus 15% in group B (p-value = 0.684). We didn't document any cardiac death in both groups. In group B we had one (3.8%) non-fatal MI while there was no MI in group A (P-value = 1). In group A we had 2 cases (10%) of TLR while in group B there were 3 cases (11.5%) TLR (P-value = 1). We have two cases (7.7%) of TVR in group B and one in group A p-value = 1. All cases were planned staged PCI. Scaffold thrombosis occurred in one case in group A (5%) and two cases in group B (7.7%) p-value = 1.

CONCLUSION: With proper lesion selection, direct BVS implantation in all-comers NSTE-ACS patients is feasible and safe even without the aid of intracoronary imaging.

Introduction

Despite that drug-eluting stents (DES) with biocompatible or biodegradable polymers have a considerably improved safety profile and considered a standard of care for patients with coronary artery disease [1], [2], bioresorbable stents, commonly referred to as scaffolds, can provide support to the vessel wall for a defined period after angioplasty but are subsequently resorbed [3].

Current recommendation for the bioresorbable vascular scaffold (BRS) implantation is plaque preparation with adequate pre dilatation [4], [5] however in the setting of large thrombus burden like patients with acute coronary syndrome (ACS), aggressive pre dilatation may result in an increased risk of distal embolization and subsequent flow

deterioration [6].

Moreover, the culprit lesion in both groups has different morphologic patterns. Lesions in ST-segment elevation myocardial infarction (STEMI) tends to be softer, more lipid-rich, with thinner cap with more thrombotic burden mainly red thrombus [7] making them an ideal substrate for the BRS which is not the case for the non-ST segment elevation ACS (NSTE-ACS). NSTE-ACS represents a challenging subset in which BRS is under-investigated.

On the other hand, precise vessel/scaffold sizing should be performed, preferably with optical coherence tomography (OCT), which also allows accurate assessment of scaffold apposition [8]. However, in the setting of all-comers ACS patients intracoronary imaging may not be available especially in low- and middle-income countries. We aimed at assessing the feasibility and safety of direct scaffold implantation based only on angiographic assessment in a high-risk group of patients (NSTE-ACS).

Methods

The current study was a retrospective two-centre study conducted over patients diagnosed with NSTE-ACS presented to cardiology department at Juan Ramon Hospital, Spain and critical care department, Cairo University in the period between February 2016 to May 2017.

We included patients for whom we depend only on angiographic assessment for decision making whether to directly implant the scaffold or predilate the lesion and we excluded patients for whom intracoronary imaging whether intravascular ultrasound (IVUS) or OCT were used at the index procedure either for pre or post-implantation.

We used the ABSORB (Abbott Vascular, Santa Clara, CA, USA), the second-generation device, BVS 1.1 which is an everolimus-eluting BRS composed of Poly-L-lactic acid (PLLA) and Poly-D, L-lactic acid (PDLLA), designed in in-phase zigzag hoops linked by bridges

When pre dilatation was attempted it was done with balloon 0.5 mm smaller or equal to scaffold device recommended. In the second group direct scaffold implantation was done. Deployment of the scaffold was done with slow increase of two atmospheres every five seconds until the scaffold is completely expanded. The pressure is maintained for 30 seconds. Post-dilatation, when attempted, was done with non-compliant balloon at high pressure (> 16 atm) and the dilatation limit was 0.5 mm above the nominal diameter.

Clinical follow-up was obtained by the clinical visit and/or through telephone contact, according to a schedule specific for each site. Major adverse cardiac events were collected at discharge and the end of the follow-up period. The primary outcome of interest was the device-oriented composite endpoints (DOCE) including cardiac death; MI attributed to the target vessel and TLR [9]. The secondary endpoints were the broader patient-oriented composite outcome (POCE) and scaffold/stent thrombosis. POCE includes all-cause mortality, any MI and any revascularisation (including TLR, TVR and revascularisation of non- target vessel) [9]. MI definitions were based on the most recent universal definition of MI [10]. All deaths were considered cardiac unless proven otherwise. Stent/scaffold thrombosis definitions were based on the Academic Research Consortium (ARC) criteria [9].

Data were coded and entered using the statistical package SPSS (Statistical Package for the Social Sciences) version 25. Data were summarised using mean, standard deviation, median, minimum and maximum 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 test [11]. For comparing categorical data, Chi-square ($\chi 2$) test was performed. Exact test was used instead when the expected frequency is less than 5 [12]. P-values less than 0.05 were considered as statistically significant.

Results

From whole patients who received at least one BVS during the mentioned period, forty-six patient were enrolled in our study. Those patients received at least one BVS depending on visual assessment of the angiography without the aid of any intracoronary imaging modality during the index procedure.

Table 1: Clinical characteristics

		Direct (20)	Predilatation (26)	P value
Patient Characteristics				
Age		49.85 ± 9.16	52.50 ± 7.40	0.602
Male sex		16 (80.0%)	17(65.4%)	0.275
Smoking		15 (75.0%)	20 (76.9%)	1
Obesity		2 (10.0%)	3 (11.5%)	1
Dyslipidemia		6 (30.0%)	9 (34.6%)	0.741
Hyperuricemia		1 (5.0%)	1 (3.8%)	1
Hypertension		5 (25.0%)0	9 (34.6%)	0.482
DM		3 (15.0%)	3 (11.5%)	1
Family history of IHD		0	1 (3.8%)	1
Clinical Presentation and management				
TIMI risk score		1.9 ± 0.91	2.0 ± 0.85	0.885
Admission diagnosis	Unstable angina	9 (45.0%)	13 (50.0%)	0.736
	NSTEMI	11 (55.0%)	13 (50.0%)	
Early invasive strategy		15 (75.0%)	21 (80.8%)	0.726
Elective strategy		5 (25.0%)	5 (19.2%)	
Single vessel disease		17 (85.0%)	20 (76.9%)	0.711
MVD		3(15.0%)	6 (23.1%)	

The enrolled patients were divided into two groups. Group A included 20 patients who received direct scaffold implantation and group B included 26

patients in which pre dilatation was done. Patients' demographics, clinical data and risk factors were nearly similar in both groups as shown in Table 1. In group A 11 patients (55%) had NSTEMI and 9 patients (45%) had unstable angina, while in group B NSTEMI represent 50% (13 patients) and UA represent 50% (13 patients), P = value 0.736. Group A has TIMI risk score of 1.9 ± 0.91 as compared to group B 2.0 ± 0.85, P-value = 0.885. Post-dilatation was done in 90% of patients in group A and 88% of group B, p-value = 1. Angiographic and procedural data are presented in Table 2.

Table 2: Angiographic and procedural data

Target vessel	LAD	16 (69.6%)	19 (57.6%)	0.453
	LCX	4 (17.4%)	6 (18.2%)	
	RCA	3 (13.0%)	8 (24.2%)	
Post-dilatation		18 (90.0%)	23 (88.5%)	1
Scaffold size, mm		3.23 ± 0.25	3.04 ± 0.33	0.018
Scaffold length, mm		18.15 ± 6.06	20.45 ± 6.25	0.140
Dissection		1 (5.0%)	4 (15.4%)	0.369
Slow flow		1 (5.0%)	0 (0.0%)	0.435
No re-flow		1 (5.0%)	0 (0.0%)	0.435
Stent thrombosis		0 (0%)	0 (0%)	----
Side-branch compromise		3 (15%)	6 (23.1%)	0.711

Procedural success was obtained in all study population. Offline QCA analysis was done for all patients and data are presented in Table 3. Immediate clinical success was achieved in all cases. There was no significant difference between procedural complications in both groups.

Table 3: QCA data

	QCA		
	Direct	Predilatation	P-value
MLD	1.15 ± 0.31	0.94 ± 0.29	0.008
RVD	3.02 ± 0.44	2.83 ± 0.46	0.171
DS %	61.74 ± 11.41	65.47 ± 10.99	0.170
Acute gain	1.25 ± 0.38	1.17 ± 0.48	0.880
Late loss	0.52 ± 0.53	-0.65 ± 3.46	0.210
Late loss index	36.57 ± 24.59	39.43 ± 48.47	0.607

Edge dissection occurred in one patient (5%) in group A and 4 patients (15.4%) in group B, p-value 0.369. Slow flow and no-reflow occurred in 2 patients in group A yet this was statistically insignificant, p-value = 0.435. Side branch compromise occurred in 3 patients (15%) in group A and in 6 patients (23%) in group B p-value = 0.945. Those who had chest pain or impaired TIMI flow were treated with either balloon dilatation in side-branch ostium or final kissing inflation. We didn't document any in-hospital major adverse events. Mean FU duration of this group was 12 months. Angiographic follow up was done in 7 patients in group A (35%) while in group B angiographic follow up was done in 14 patients (53%) P-value = 0.451. During the whole FU period, there was a lower incidence of both device-oriented and patient-oriented composite endpoints in the direct implantation group (group A), yet this was statistically insignificant (Table 4). DOCE occurred in 10% in group A and in 15% in group B (p-value = 0.684). POCE occurred in 15% in group A and 23% in group B (p-value = 0.711). We didn't document any cardiac death in both groups. In group B we had one (3.8%) non-fatal MI while there was no MI in group A (P-value = 1). In group A we had 2 cases (10%) of TLR while in group B there were 3 cases (11.5%) TLR (P-value = 1). One case of TLR in group A was due to very late definite scaffold thrombosis and was treated with DES. The other case underwent an OCT which revealed ISR with neoatherosclerosis and was treated with another scaffold. In group B one case of TLR was also due to late thrombosis and was treated with DES. OCT of the second case revealed diffuse intimal hyperplasia and was treated with scoring balloon followed by a drug coating balloon (DCB) angioplasty, and the third case has neoatherosclerosis and was treated with DES. We have two cases (7.7%) of TVR in group B and one in group A p-value = 1. All cases were planned staged PCI.

Scaffold thrombosis occurred in one case in group A (5%) and two cases in group B (7.7%) p value = 1. In group A the patient presented with UA, OCT under expanded struts which treated with aggressive post dilatation, intracoronary GPIIb-IIIa inhibitor and DES. In group B one patients with late scaffold thrombosis presented with STEMI three days after discontinuation of the Aspirin. Primary PCI was done with implantation of a DES. The other patient presented with recurrent chest pain and his OCT revealed proximal edge dissection that was treated with implantation of another scaffold.

Table 4: Outcome

	Composite end points		
	Direct	Predilatation	P value
Death	0	0	---
MI	0	1 (3.8%)	1
TLR	2 (10%)	3 (11.5%)	1
TVR	1 (5%)	2 (7.7%)	1
ST	1(5%)	2 (7.7%)	1

Discussion

In the setting of emergency PCI to an ACS patient using the BVS the operators will be faced with too difficult decisions. First, whether to predilate the lesion as recommended for this specific device or to directly implant the scaffold as preferred in lesions with high thrombotic burden. The second difficult scenario is about the appropriate sizing of the scaffold and sizing of the balloons for pre and post dilatation if intracoronary imaging is not available which is a common scenario in all-comers ACS patients due to high cost or limited availability.

There is no solid data to support decision making in this difficult scenario. Several studies showed that direct implantation of metallic DES is associated with the reduction of flow disturbances after primary PCI, better ST-segment resolution as well as better survival at 30 days and one year [13], [14], [15], [16].

However, in the BVS where pre dilatation is highly recommended, data about feasibility and safety of direct scaffold implantation is so limited to give strong evidence for decision making.

Adding to the difficulty, the spectrum of ACS is not homogenous. The culprit lesion in STEMI versus NSTE-ACS has different morphologic patterns. Lesions in STEMI tends to be softer, more lipid-rich, with thinner cap with more thrombotic burden mainly red thrombus [7] making them an ideal substrate for BVS which is not the case for the NSTE-ACS.

To the best of our knowledge, our study is the first one to evaluate the immediate and one-year outcome of direct BVS implantation in a cohort of patient with NSTE-ACS based only on angiographic assessment without use of intracoronary imaging. We achieved procedural and clinical success in all patients. We didn't report any in-hospital adverse events. At 12 months follow up there was no significant difference between direct implantation group and pre dilatation group as regard composite endpoints or scaffold thrombosis.

Rzeszutko et al., [17] reported the in-hospital outcome of 50 ACS patients who received direct scaffold implantation. NSTEMI represent 62 % of their study population. They also didn't use OCT for sizing. They didn't report any in-hospital MI, scaffold thrombosis or TLR but long-term data were not reported.

Suarez de Lezo et al., [18] studied the outcome of direct scaffold implantation and reported a 5.9% MACE rate 12 months. They reported 0.6% death due scaffold thrombosis and 4% TLR however there was no significant difference between direct implantation group and pre dilatation group. Importantly they use intracoronary Imaging (IVUS or OCT) in nearly 86%of lesions which allow better sizing and ensure good scaffold apposition.

In the BVS-STEMI-STRATEGY-IT study, Alfonso Ielasi et al., [19] evaluate the 30-day outcome of BVS in STEMI patients using pre-specified strategy. The strategy involved using direct implantation only when there is TIMI 2-3 flow after wiring the culprit lesion and/or after thrombus aspiration and only when the residual stenosis is less than 30%. Otherwise, pre-dilatation was done. They reported DOCE 0.6% (0.4% death and 0.2% TLR) and scaffold thrombosis in 0.2%. The used intracoronary imaging before implantation in 26% of cases and at least after implantation in 52% of cases.

The most important finding of our study is the feasibility and good midterm outcome of direct BVS deployment in patients with NSTE-ACS. The value of an angiographic assessment of a scaffold invisible for fluoroscopy was supposed to be limited and does not provide information about the scaffold apposition. However, this was disproven by our results. However, it is not a randomised study, the study sample size is relatively small, and the results only allow for raising a new principle that needs larger randomised studies to prove.

In conclusion, with proper lesion selection, direct BVS implantation in all-comers NSTE-ACS patients is feasible and safe even without the aid of intracoronary imaging.

References

1. Stone GW, Rizvi A, Newman W, et al. Everolimus-Eluting versus Paclitaxel-Eluting Stents in Coronary Artery Disease. N Engl J Med. 2010; 362(18):1663-1674. https://doi.org/10.1056/NEJMoa0910496 PMid:20445180

2. Stefanini GG, Kalesan B, Serruys PW, et al. Long-term clinical outcomes of biodegradable polymer biolimus-eluting stents versus durable polymer sirolimus-eluting stents in patients with coronary artery disease (LEADERS): 4 year follow-up of a randomised non-inferiority trial. Lancet. 2011; 378(9807):1940-1948. https://doi.org/10.1016/S0140-6736(11)61672-3

3. Waksman R. Biodegradable stents: they do their job and disappear. J Invasive Cardiol. 2006; 18(2):70-74.

4. Tanaka A, Jabbour RJ, Latib A, et al. Bioresorbable vascular scaf¬folds: From patient selection to optimal scaffold implantation; tips and tricks to minimize device failure. Catheter Cardiovasc Interv. 2016; 88(S1):10-20. https://doi.org/10.1002/ccd.26812 PMid:27797460

5. Everaert B, Wykrzykowska JJ, Koolen J, et al. Recommenda¬tions for the use of bioresorbable vascular scaffolds in percu¬taneous coronary interventions: 2017 revision. Neth Heart J. 2017; 25(7-8):419-428. https://doi.org/10.1007/s12471-017-1014-z PMid:28643297 PMCid:PMC5513994

6. Ndrepepa G, Kastrati A. Mechanical strategies to enhance myocardial salvage during primary percutaneous coronary intervention in patients with STEMI. EuroIntervention. 2016; 12(3):319-328. https://doi.org/10.4244/EIJV12I3A52 PMid:27320426

7. Ino Y, Kubo T, Tanaka A, et al. Difference of culprit lesion morphologies between ST-segment elevation myocardial infarction and non-ST-segment elevation acute coronary syndrome: an optical coherence tomography study. JACC Cardiovasc Interv. 2011; 4(1):76-82. https://doi.org/10.1016/j.jcin.2010.09.022 PMid:21251632

8. Reczuch K, Milewski K, Wąsek W, et al. [Bioresorbable scaffolds in the treatment of coronary artery disease. Expert consensus statement of the Association of Cardiovascular Interventions of the Polish Cardiac Society (ACVI PCS)]. Kardiol Pol. 2017; 75(8):817-835. https://doi.org/10.5603/KP.2017.0160 PMid:28819961

9. Cutlip DE, Windecker S, Mehran R, et al. Clinical end points in coronary stent trials: A case for standardized definitions. Circulation. 2007; 115(17):2344-2351. https://doi.org/10.1161/CIRCULATIONAHA.106.685313 PMid:17470709

10. Thygesen K, Alpert JS, Jaffe AS, et al. Third universal definition of myocardial infarction. Eur Heart J. 2012; 33(20):2551-2567. https://doi.org/10.1093/eurheartj/ehs184 PMid:22922414

11. Chan YH. Biostatistics 102: quantitative data-parametric & non-parametric tests. blood pressure. 2003; 140(24.08):79-00.

12. Chan YH. Biostatistics 103: Qualitative Data -Tests of Independence. Singapore Med J. 2003; 44(10):498-503.

13. Möckel M, Vollert J, Lansky AJ, et al. Comparison of direct stenting with conventional stent implantation in acute myocardial infarction. Am J Cardiol. 2011; 108(12):1697-1703.

https://doi.org/10.1016/j.amjcard.2011.07.040 PMid:21906709

14. McCormick LM, Brown AJ, Ring LS, et al. Direct stenting is an independent predictor of improved survival in patients un¬dergoing primary percutaneous coronary intervention for ST elevation myocardial infarction. Eur Heart J Acute Cardiovasc Care. 2014; 3(4):340-346. https://doi.org/10.1177/2048872614530864 PMid:24719243

15. Li C, Zhang B, Li M, et al. Comparing direct stenting with conventional stenting in patients with acute coronary syn¬dromes: a meta-analysis of 12 clinical trials. Angiology. 2016; 67(4):317-325. https://doi.org/10.1177/0003319715585662 PMid:25964649

16. Dziewierz A, Siudak Z, Rakowski T, et al. Impact of direct stenting on outcome of patients with ST-elevation myocardial infarction transferred for primary percutaneous coronary in¬tervention (from the EUROTRANSFER registry). Catheter Cardiovasc Interv. 2014; 84(6):925-931. https://doi.org/10.1002/ccd.25266 PMid:24155092

17. Rzeszutko Ł, Węgiel M, Kleczyński P, et al. Direct Absorb bioresorbable scaffold implantation in acute coronary syndrome. Kardiol Pol. 2018; 76(10):1434-1440. https://doi.org/10.5603/KP.a2018.0147 PMid:30067276

18. Suárez de Lezo J, Martín P, Mazuelos F, et al. Direct bioresorb¬able vascular scaffold implantation: Feasibility and midterm results. Catheter Cardiovasc Interv. 2016; 87(5):E173-E182. https://doi.org/10.1002/ccd.26133 PMid:26268440

19. Ielasi A, Campo G, Rapetto C, et al. A prospective evaluation of a pre-specified Absorb BRS implantation strategy in ST-segment elevation myocardial infarction: the BRS STEMI STRATEGY-IT study. JACC Cardiovasc Interv. 2017; 10:1855-1864. https://doi.org/10.1016/j.jcin.2017.07.023 PMid:28935077

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Clinical Science

Pentraxin 3: A Potential Novel Predictor for Neonatal Pulmonary Hypertension

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Abstract

BACKGROUND: Persistent pulmonary hypertension of the newborn (PPHN) is a serious neonatal problem which has a high mortality rate even with advanced modes of mechanical ventilation. Pentraxin 3 is one of the long pentraxins, which plays an essential role in regulation of cell proliferation and angiogenesis.

AIM: This study aims to assess serum pentraxin 3 levels in neonates with pulmonary arterial hypertension and compare them in those who have other congenital heart diseases and healthy neonates. Also, we intended to evaluate serum levels of CRP as a mediator of inflammation in the studied groups.

METHODS: The study is a case-control study. Cases were recruited from El Galaa Teaching Hospital, classified into three groups; each group had thirty cases. The first one: cases with pulmonary hypertension (PHT), the second one: cases with congenital heart diseases (CHD) without pulmonary hypertension and the third group included healthy neonates. All participants were subjected to full history taking and full clinical examination. Diagnosis of congenital heart disease and pulmonary hypertension was made according to echocardiographic findings by pediatric cardiologist using echocardiography machine. Laboratory investigations included measurement of serum pentraxin 3, Routine CBC, CRP.

RESULTS: This study found that the mean serum pentraxin 3 in PHT neonates was significantly higher than that of the control and CHD neonates ($p \leq 0.001$, $p = 0.02$ respectively). Also, the mean Pentraxin3 of the CHD neonates was significantly higher than that of the control ($p = 0.06$). Also, the mean CRP of the PHT neonates was significantly higher than that of the control ($p = 0.01$). Regression analysis showed that Pentraxin3 was the main predictor of PAP ($P = 0.01$).

CONCLUSION: Serum pentraxin 3 is significantly elevated in neonates with pulmonary hypertension, so measurement of pentraxin 3 levels in neonates may be valuable as a predictor for pulmonary hypertension in neonates.

Introduction

Persistent pulmonary hypertension of the newborn (PPHN) is considered a serious condition that appears in the neonatal period, which is always complicated by high morbidity and mortality. It is due to that the neonate fails to make a postnatal change from a high resistance fetal pulmonary circulatory state to a low resistance pulmonary circulation [1]. This low pulmonary blood flow secondary to increased pulmonary vascular resistance counteract potent gas exchange in the lungs prompting severe respiratory distress and decreased blood oxygen level in the neonate [2]. Data suggested that PPHN happens in 1-2 infants / 1000 live births [3]. A recent study was performed in Egypt found that PPHN was found in 5% of the studied population [4]. The neonate has PPH is usually term or late-preterm and usually is not associated with congenital anomalies. Affected neonate complains shortly after birth with severe shortness of breath that needs mechanical ventilation [5]. In spite of different management procedures such as nitric oxide, aggressive mechanical ventilation and

extracorporeal membrane oxygenation (ECMO), the mortality rate for this disease is about 10-20% of affected neonates [6]. This aggressive treatment and occurrence of hypoxemia expose these neonates to have long-term sequelae including chronic lung disease, seizures, and neurological developmental problems [7].

Pentraxin 3 is a subclass of long pentraxins which is synthesised by fibroblasts, smooth muscle cells and endothelium. Also, innate immune cells during inflammation secrete pentraxin 3. It plays a vital role in cell proliferation regulation and angiogenesis [8]. Serum levels of CRP are mediators of inflammation [9]. CRP is considered one of the short pentraxin which is produced in the liver in case of systemic inflammation [10].

Using echocardiography has further expanded as an adjunct to physical examination to improve diagnostic accuracy and risk of cardiac diseases [11].

Fractional shortening (FS) is used to asses left ventricular dysfunction. It simply measures the degree of shortening of the diameter of left ventricle between end-diastole and end-systole. It is one of the most important measures in functional echocardiography [12].

The aim of this study was to evaluate serum pentraxin 3 levels in neonates with pulmonary arterial hypertension and compare them with those who have other congenital heart diseases and healthy neonates. Also, we intended to evaluate serum levels of high sensitive-CRP (hs-CRP), as a mediator of inflammation in the three studied groups.

Material and Methods

This study is a case-control study. Studied cases were recruited from El Galaa Teaching Hospital. It was approved by the local ethical committee of the National Research Center, and parental written informed consent was obtained from all study participants. Participants were classified into three groups; each group had thirty cases. The first one: cases with pulmonary hypertension, the second one: cases with congenital heart diseases with normal pulmonary pressure and the third group included healthy neonates. They were age, sex and gestation matched with neonates of other three groups. All participants were subjected to full history taking and full clinical examination. Demographic and clinical data such as age, sex, birth weight, and gestational age were documented in all participants.

Diagnosis of congenital heart disease, pulmonary hypertension and other cardiac measurements including fractional shortening (FS) were made according to echocardiographic findings performed by pediatric cardiologist using echocardiography machine (Sonosite®, USA, probe 5–8 Hz) [13]. Neonates meeting any of the following criteria were excluded from the study: diagnosis of proven sepsis by positive blood culture, disseminated intravascular coagulation, severe hypoxic respiratory failure, low Apgar score, non-congenital heart diseases (e.g., endocarditis) or maternal history of chorioamnionitis.

Venous blood sample (2 ccs) was taken from each participant and serum was separated and stored at -30 until collection of all the samples then laboratory investigations were done. Laboratory investigations included measurement of serum pentraxin 3, Routine CBC. Serum Pentraxin 3 (PTX3) level was assessed by enzyme-linked immunosorbent assay (ELISA) following instructions of the kits purchased from Sino Gene Clon Biotech Co., Ltd. Catalog No: SG-10465. Serum hs-CRP levels were determined with an enzyme-linked immunosorbent assay (ELIZA) technique using commercial kits (BioCheck, Inc 323 Vintage Park Drive Foster City, CA 94404) and the sensitivity of detection level was 0.01 mg/dl.

Statistical analysis

Data entry was carried out on excel sheet and analysis was done using SPSS software program version 22 (SSPS Inc., Pennsylvania, USA). Mean± SD was used to present quantitative data. T-test was done for comparison between two means. Pearson's correlation analysis was performed to estimate the association between variables. Linear regression analysis was performed to identify the main predictors of PAP. P-value was considered statistically significant when P was < 0.05.

Results

Subjects in this study were classified into three groups; each group had thirty subjects.

Table 1 shows the demographic and laboratory data of the studied groups. Comparison between PHT, CHD without pulmonary hypertension and control groups as regards different variables were shown in Table 1. No significant difference was found between the studied groups as regards age, GA and FS. As regards laboratory investigations, it was found that the mean CRP of the PHT group was significantly higher than that of the control ($p = 0.01$). Also, serum pentraxin 3 in PHT group was significantly higher compared to the control and CHD groups ($p \leq 0.001$, $p = 0.02$ respectively). The mean Pentraxin3 of the CHD group was significantly higher in comparison to

controls (p = 0.06).

Table 1: Demographic and Echocardiographic data in the studied groups

Variable	PHT (Mean ± SD)	CHD (Mean ± SD)	Control (Mean ± SD)	P1	P2	P3
Age (days)	3.95 ± 1.59	3.97 ± 2.36	4.37 ± 0.93	0.19	0.97	0.39
Weight (Kg)	2.64 ± .0.71	2.97 ± 0.44	2.85 ± 0.53	0.171	0.03*	0.34
GA (weeks)	37.03 ± 1.42	37.30 ± 1.37	37.53 ± 1.72	0112	0.42	0.46
PAP (mm Hg)	62.15 ± 14.48	28.7 ± 7.3			≤ 0.001	
FS (%)	40.38 ± 3.18	41.10 ± 2.06			0.28	
CRP (mg/dl)	2.88 ± 0.91	2.53 ± 0.90	2.40 ± 0.50	0.01*	0.12	0.48
Pentraxin 3 (µg/l)	7.60 ± 2.17	6.38 ± 2.20	5.53 ± 0.84	≤ 0.001*	0.02*	0.06*

P1: PHT Vs Control P2: PHT Vs CHD P3: CHD Vs Control; PHT: neonates with pulmonary hypertension PAP: Pulmonary artery pressure; CHD: Neonates with congenital heart disease without pulmonary hypertension; GA: Gestational age FS: Fractional shortening; *P < 0.05, the relationship is significant.

Multiple correlations were calculated between serum pentraxin 3 and all other variables. They showed that PAP was positively correlated with serum pentraxin 3 (as shown in Figure 1) and FS (p = 0.009, p = 0.011 respectively).

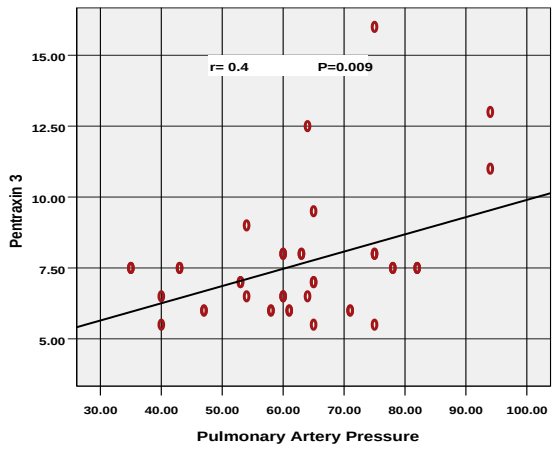

Figure 1: Correlation between PAP and serum pentraxin 3

To show the influence of pentraxin 3 and CRP on PAP as a dependent factor, we did linear regression analysis. This analysis showed that Pentraxin3 was the main predictor of PAP (P = 0.01), as shown in Table 2.

Table 2: Predictive factors for increased PAP in the PHT group as estimated by linear regression

Model		Unstandardized Coefficients		Standardised Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	39.727	11.163		3.559	.001
	Pentraxin3	2.742	1.011	.410	2.712	.010
	CRP	.550	2.405	.035	.229	.820

*P < 0.05, the relationship is significant.

Discussion

Pulmonary hypertension is one of the serious conditions in neonates which may be idiopathic, associated with congenital heart disease or lung disease and postoperative. In this study, it was found that the mean serum pentraxin 3 in neonates with PHT was significantly higher than that of the control and neonates with other CHD with no PHT. Also, the mean Pentraxin 3 of the later group was significantly higher than that of the control. This was in agreement with several studies which found that the mean serum pentraxin 3 in neonates with PHT was significantly higher than that of the control and neonates with other CHD [14], [15], [16]. Also, we found that the mean hs-CRP of the PHT neonates was significantly higher than that of the control.

Similarly, other studies found that hs-CRP was significantly elevated in PHT patients. They added that inflammation is a contributing factor to the progression of pulmonary hypertension [15], [16], hs-CRP is one of the short pentraxins and is strongly released secondary to inflammation. To clarify our results, multiple correlations were calculated between serum pentraxin3 and all other variables. It showed that PAP was positively correlated with serum pentraxin 3. This may be explained by that pentraxin 3 has a role in angiogenesis and vascular diseases. It is secreted at sites of inflammation not only by endothelial cells but also by macrophages and monocytes infiltrating sites of inflammation that happens in pulmonary hypertension [17]. No correlation was found between serum pentraxin 3 and hs-CRP as found in previous study [15]. To show the influence of pentraxin 3 and CRP on PAP as a dependent factor, we did linear regression analysis. This analysis showed that Pentraxin3 was the main sensitive biomarker of PAP than hs-CRP. This is similar to findings of Tamura et al., who added that hs-CRP is increased to a significant degree in the PAH group. This study also found that PAP was positively correlated with FS [15].

In conclusion, serum pentraxin 3 is an important sensitive biomarker of pulmonary artery pressure. It is elevated in pulmonary artery hypertension, so measuring serum pentraxin3 might be useful in prediction and progression of pulmonary artery hypertension in neonates.

References

1. Steinhorn RH. Neonatal pulmonary hypertension. Pediatr Crit Care Med. 2010; 11(2):79-84. https://doi.org/10.1097/PCC.0b013e3181c76cdc PMid:20216169 PMCid:PMC2843001

2. Ostrea EM, Villanueva-Uy ET, Natarajan G, Uy HG. Persistent pulmonary hypertension of the newborn. Pediatric Drugs. 2006; 8(3):179-88. https://doi.org/10.2165/00148581-200608030-00004 PMid:16774297

3. Abman SH. Recent advances in the pathogenesis and treatment of persistent pulmonary hypertension of the newborn. Neonatology. 2007; 91:283-90. https://doi.org/10.1159/000101343 PMid:17575471

4. Mohsen AH, Amin AS. Risk factors and outcomes of persistent pulmonary hypertension of the newborn in neonatal intensive care unit of Al-Minya University Hospital in Egypt. Journal of clinical neonatology. 2013; 2(2):78-82. https://doi.org/10.4103/2249-4847.116406 PMid:24049749 PMCid:PMC3775141

5. Suesaowalak M, Cleary JP, Chang AC. Advances in diagnosis and treatment of pulmonary arterial hypertension in neonates and children with congenital heart disease. World Journal of Pediatrics. 2010; 6(1):13-31. https://doi.org/10.1007/s12519-010-0002-9 PMid:20143207

6. Greenough A, Khetriwal B. Pulmonary hypertension in the newborn. Paediatric respiratory reviews. 2005; 6(2):111-6. https://doi.org/10.1016/j.prrv.2005.03.005 PMid:15911456

7. Latini G, Del Vecchio A, De Felice C, Verrotti A, Bossone E. Persistent pulmonary hypertension of the newborn: therapeutical approach. Mini reviews in medicinal chemistry. 2008; 8(14):1507-13. https://doi.org/10.2174/138955708786786507 PMid:19075808

8. Mantovani A, Garlanda C, Doni A, Bottazzi B. Pentraxins in innate immunity: from C-reactive protein to the long pentraxin PTX3. Journal of clinical immunology. 2008; 28(1):1-3. https://doi.org/10.1007/s10875-007-9126-7 PMid:17828584

9. El-Wakeel MA, El-Kassas GM, Fathy GA, El-Wakkad AS, Sebaii HM, El-Zayat SM. Diagnostic and prognostic values of high sensitive c-reactive protein, tumor necrosis factor and interleukin-1β in neonatal sepsis. Aust J Basic Appl Sci. 2012; 6:224-8.

10. Karakurt C, Çelik FS, Başpınar O, Şahin AD, Taşkapan Ç, Yoloğlu S. Serum pentraxin 3 and hs-CRP levels in children with severe pulmonary hypertension. Balkan medical journal. 2014; 31(3):219-23. https://doi.org/10.5152/balkanmedj.2014.13307 PMid:25625020 PMCid:PMC4299966

11. Omar AM, Bansal M, Sengupta PP. Advances in echocardiographic imaging in heart failure with reduced and preserved ejection fraction. Circulation research. 2016; 119(2):357-74. https://doi.org/10.1161/CIRCRESAHA.116.309128 PMid:27390337

12. Srinivasan A, Kim J, Khalique O, Geevarghese A, Rusli M, Shah T, Di Franco A, Alakbarli J, Goldburg S, Rozenstrauch M, Devereux RB. Echocardiographic linear fractional shortening for quantification of right ventricular systolic function-A cardiac magnetic resonance validation study. Echocardiography.; 34(3):348-58. https://doi.org/10.1111/echo.13438 PMid:28247463 PMCid:PMC5352481

13. Mourani PM, Sontag MK, Younoszai A, Ivy DD, Abman SH. Clinical utility of echocardiography for the diagnosis and management of pulmonary vascular disease in young children with chronic lung disease. Pediatrics. 2008; 121(2):317-25. https://doi.org/10.1542/peds.2007-1583 PMid:18245423 PMCid:PMC3121163

14. Farhadi R, Rafiei A, Hamdamian S, Zamani H, Yazdani J. Pentraxin 3 in neonates with and without diagnosis of pulmonary hypertension. Clinical biochemistry. 2017; 50(4-5):223-7. https://doi.org/10.1016/j.clinbiochem.2016.11.009 PMid:27838407

15. Tamura Y, Ono T, Kuwana M, Inoue K, Takei M, Yamamoto T, Kawakami T, Fujita J, Kataoka M, Kimura K, Sano M. Human pentraxin 3 (PTX3) as a novel biomarker for the diagnosis of pulmonary arterial hypertension. PLoS One. 2012; 7(9):e45834. https://doi.org/10.1371/journal.pone.0045834 PMid:23029266 PMCid:PMC3448700

16. Karakurt C, Çelik FS, Başpınar O, Şahin AD, Taşkapan Ç, Yoloğlu S. Serum pentraxin 3 and hs-CRP levels in children with severe pulmonary hypertension. Balkan medical journal. 2014; 31(3):219-23. https://doi.org/10.5152/balkanmedj.2014.13307 PMid:25625020 PMCid:PMC4299966

17. Vergadi E, Chang MS, Lee C, Liang OD, Liu X, Fernandez-Gonzalez A, Mitsialis SA, Kourembanas S. Early macrophage recruitment and alternative activation are critical for the later development of hypoxia-induced pulmonary hypertension. Circulation. 2011; 123(18):1986-95. https://doi.org/10.1161/CIRCULATIONAHA.110.978627 PMid:21518986 PMCid:PMC3125055

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Predictive Value of Hematologic Indices in the Diagnosis of Acute Coronary Syndrome

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Abstract

BACKGROUND: Distinguishing between Acute Coronary Syndrom (ACS) and SCAD (Stable Coronary Artery Disease) requires advanced laboratory instrument and electrocardiogram. However, their availabilities in primary care settings in developing countries are limited. Hematologic changes usually occur in the ACS patient and might be valuable to distinguish ACS from SCAD.

AIM: This study compares the hematologic indices between ACS and SCAD patients and analyses its predictive value for ACS.

MATERIAL AND METHODS: A total of 191 patients (79 ACS and 112 SCAD) were enrolled in this study based on the inclusion criteria. Patient's characteristic, hematologic indices on admission, and the final diagnosis were obtained from medical records. Statistical analyses were done using SPSS 23.0.

RESULTS: In this research MCHC value (33.40 vs. 32.80 g/dL; $p < 0.05$); WBC (11.16 vs. 7.40 x109/L; $p < 0.001$); NLR (6.29 vs. 2.18; $p < 0.001$); and PLR (173.88 vs 122.46; $p < 0.001$) were significantly higher in ACS compared to SCAD patients. While MPV (6.40 vs. 10.00 fL; $p < 0.001$) was significantly lower in ACS patients. ROC curve analysis showed MPV had the highest AUC (95%) for ACS diagnosis with an optimum cut-off point at ≤ 8.35 fL (sensitivity 93.6% and specificity 97.3%).

CONCLUSION: There was a significant difference between hematologic indices between ACS and SCAD patients. MPV is the best indices to distinguish ACS.

Introduction

Coronary Artery Disease (CAD) is the leading cause of death worldwide, including Indonesia. WHO reported that CAD caused 138.400 deaths of Indonesian in 2012 [1]. Most of CAD is caused by coronary artery narrowing from atherosclerosis [2]. The characteristics of atherosclerosis determine the clinical manifestation of CAD. Vulnerable plaque or unstable plaque will result in atherothrombosis event which is the hallmark of Acute Coronary Syndrome (ACS), while stable plaque consists of poor-lipid core and thick fibrous cap will be manifested as Stable Coronary Artery Disease (SCAD) [2], [3], [4].

Rapid coronary revascularisation is beneficial for ACS patients to reduce adverse events or death [5]. Therefore, early diagnosis of ACS is critical since mortality rates in the ACS patients are up to seven times higher than SCAD [6]. However, due to the limited availability of the electrocardiogram (ECG) and cardiac markers in the primary care setting, diagnosis of the ACS may become a big challenge for primary care physician in developing countries [7]. The previous study even showed that the availability of ECG in the rural primary care setting was only 63.3% [8]. Hence, easy and accessible screening approach for diagnosis of ACS in primary care settings is urgently needed.

Pathogenesis of atherosclerosis is related to the inflammation and hematologic responses. Various inflammatory substance and hematologic cells are involved in the pathogenesis of atherosclerotic lesion [9], [10], [11]. Leukocyte and platelets play major roles in the foam cell generation, cytokines secretion,

including Reactive Oxygen Species (ROS), and cardiomyocytes death, which contribute to the atherosclerosis progression [12]. The lesion in ACS exhibits acute condition and activates neutrophil as pro-inflammatory cells [4], [13]. ACS also usually followed by inflammation regulation by anti-inflammatory cells such as lymphocyte cells [14], [15]. Platelets also play a role in the ACS by inducing higher inflammatory activity and thrombogenicity [3], [4], [16]. Contrary, the lesion in SCAD exhibits chronic and lower grade of inflammation compared to ACS. Previous studies showed that white blood cell count and inflammatory markers are significantly higher in the ACS group compared to SCAD [9], [10], [11]. However, the comparison of other hematologic indices between ACS and SCAD is yet to be investigated.

Thus, this study compares hematologic indices between ACS and SCAD patients and analyse its predictive value to distinguish ACS.

Material and Methods

Study design, Sampling, and Participants

This retrospective cross-sectional study was conducted in Dr Soetomo General Hospital, Surabaya, Indonesia. Total sampling was done from all medical records of the patient diagnosed with ACS or SCAD from January to December 2017. Patients with kidney and liver abnormalities, active infection, cancer, haematological diseases, corticosteroid therapy, and chemotherapy are excluded. Dr Soetomo General Hospital Surabaya Ethical Committee in Health Research has approved this study (approval number 0485/KEPK/VIII/2018). Privacy and confidentiality of the information were guaranteed, as data did not include patient personal identities

Data Collection

Age, Sex, CAD type (ACS or SCAD), erythrocyte indices (MCHC, Hgb, Hct), leukocytes indices (WBC, Neutrophil Percentage, Lymphocyte Percentage) and platelet indices (MPV, PLT) were obtained from medical records. Diagnosis of ACS is defined by ICD10 diagnosis code I20.0 as Unstable Angina Pectoris (UAP), I21.0 and I21.1 as ST-Elevation Myocardial Infarction (STEMI), and I21.4 as Non-ST-Elevation Myocardial Infarction (NSTEMI). Diagnosis of SCAD is defined by ICD10 diagnosis code I25.0 with no history of ACS or myocardial infarction. Neutrophil to Lymphocyte Ratio (NLR) was calculated by dividing Neutrophil Percentage to Lymphocyte Percentage, while Platelet to Lymphocyte ratio (PLR) was calculated by dividing PLT to the multiplication of Lymphocyte Percentage with WBC.

Statistical Analysis

Statistical analyses were performed using SPSS Statistics 23.0. Continuous variables, presented as mean±SD, was compared using Independent T-test or Mann Whitney test based on the normality test. Specificity and sensitivity were obtained from the ROC curve and cut-off point analysis.

Results

Baseline Characteristics

Total of 191 medical records consisting of 79 ACS patient (9 UAP, 10 NSTEMI, and 60 STEMI) and 112 SCAD met the inclusion criteria and included in this study. In both groups, most of the participants were male and aged below sixty. There was no significant difference between the two groups (Table 1).

Table 1: Baseline characteristics of ACS and SCAD patients

Variable	ACS (n = 79)	SCAD (n = 112)	*p*-value
Sex, n (%)			
Male	64 (81.0%)	94 (83.9%)	0.741
Female	15 (19.0%)	18 (16.1%)	
Age (years)	56.82 ± 9.532	57.78 ± 9.300	0.490

Comparison of Erythrocyte Indices

This study focuses on comparing MCHC values between two groups, Hgb and Hct values are used to analyse the component of MCHC value since MCHC is the ratio between Hgb and Hct. MCHC values were significantly higher in ACS than SCAD (p = 0.019), while Hgb and Hct were not significantly different (Table 2).

Comparison of Leukocyte Indices

This study compares the WBC values between two groups: the percentage of Neutrophil and Lymphocyte values are used to calculate NLR and PLR value. WBC and percentage of Neutrophil were significantly higher, while the percentage of Lymphocyte was significantly lower in ACS than SCAD with all p-value less than 0.001, respectively (Table 2).

Comparison of Platelet Indices

This study compares the MPV values between two groups; PLT values are used to calculate PLR value. MPV was significantly lower in ACS than SCAD with p-value less than 0.001, while PLT was not significantly different (Table 2).

Comparison of Other Indices

Both NLR and PLR values were significantly higher in ACS than SCAD with the p-value of less than 0.001 (Table 2).

Table 2: Comparison of hematological indices between ACS and SCAD patients

Indices	ACS (n = 79)	SCAD (n = 112)	*p*-value
Erythrocyte			
Hgb (g/dL)[1]	13.94 ± 1.51	13.72 ± 1.41	0.303
Hct (%)[1]	41.79 ± 4.91	41.69 ± 4,04	0.877
MCHC (g/dL)	33.44 ± 1.85	32.92 ± 1.09	0.019*
Leukocyte			
WBC (x 10^9 /L)	11.72 ± 3.41	8.07 ± 5.31	< 0.001*
Neu% (%)	77.27 ± 11.03	59.94 ± 8.11	< 0.001*
Lymp% (%)	14.92 ± 8.51	27.58 ± 7.06	< 0.001*
Platelet			
PLT (x 10^9 /L)[1]	267.95 ± 75.57	257.91 ± 59.70	0.307
MPV (fL)	6.64 ± 1.29	10.04 ± 0.94	< 0.001*
Other			
NLR	7.45 ± 5.31	2.48 ± 1.58	< 0.001*
PLR	203.84 ± 111.72	136.38 ± 56.200	< 0.001*

[1]Data were normally distributed; *P < 0.05 was considered statistically significant; Hgb = Hemoglobin; Hct = Hematocrit; MCHC = Mean Corpuscular Hemoglobin Concentration; WBC = White Blood Cells; Neu% = Percentage of Neutrophil; Lymp% = Percentage of Lymphocyte; PLT = Platelets; MPV = Mean Platelet Volume; NLR = Neutrophil to Lymphocyte Ratio; PLR = Platelet to Lymphocyte Ratio.

Cut-off Point

ROC Curve of MCHC, WBC, NLR, and PLR is set to indicate ACS if its value is increasing (Figure 1A), while ROC Curve of MPV is set to indicate ACS if its value is decreasing (Figure 1B). MPV has highest AUC (95.0%) followed by WBC and NLR (88.4%), PLR (71.7%), and MCHC (60.0%) respectively (Table 3).

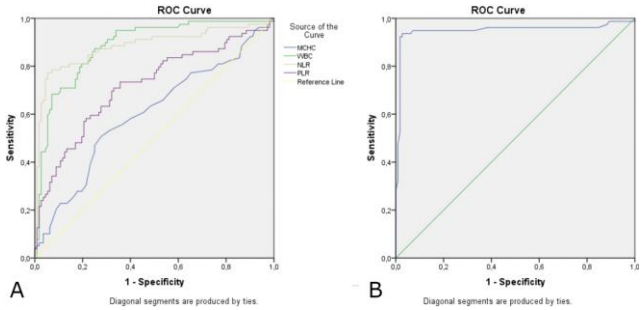

Figure 1: Receiving Operating Characteristics Curve of A) MCHC, WBC, NLR, and PLR; B) MPV

The cut-off point for MPV to distinguish ACS was 8.35 fL with very high sensitivity (93.6%) and specificity (97.3%). This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation as well as the experimental conclusions that can be drawn.

Table 3: ROC analysis and cut-off points for each index

Indices	AUC (%)	95% CI		Cut-Off	Sensitivity (%)	Specificity (%)
		Lower Bound	Upper Bound			
MCHC	60.0	0.517	0.683	33.050	58.2	59.8
WBC	88.4	0.835	0.933	9.170	81.0	80.4
MPV	95.0	0.907	0.992	8.350	93.6	97.3
NLR	88.4	0.828	0.939	3.187	81.0	85.7
PLR	71.7	0.641	0.793	147.087	69.6	67.9

Discussion

To our knowledge, this is the first study comparing various haematological indices simultaneously between ACS and SCAD patient, especially in Indonesia. Baseline characteristics for both ACS and SCAD are similar, which dominated by male and majority aged below sixty. This result is relatively similar to the previous study in Makassar, Indonesia [17]. Southeast Asian countries indeed have younger morbidity and mortality due to non-communicable disease, primarily cardiovascular disease compared to another region such as European. This difference may be due to a rapid epidemiological transition in Southeast Asian countries [17], [18].

Based on this study, MCHC value is significantly higher in the ACS group. This result is similar to previous studies, which showed MCHC value is significantly higher in CAD patients compared to healthy control [19], [20]. However, a study showed MCHC is lower in acute myocardial infarction compared to SCAD patients, yet it is not statistically significant (32.09 ± 1.34 vs 32.70 ± 1.45, p = 0.071) [20]. The previous theory stated there is a complex interaction between inflammation, iron metabolism, and anaemia, which affect MCHC value. During inflammation, the body will decrease iron serum levels by duodenal absorption and macrophage regulation [21]. Low iron serum levels will lead to iron-deficiency anaemia and decrease MCHC value [22]. Despite a few studies explaining these results; we hypothesise that high grade of inflammation in ACS leads to higher oxidative stress resulting in hemolysis and increasing MCHC value. Oxidative stress impairs erythrocyte metabolism and causes hemolysis [23]. Hemolysis will lead to increased haemoglobin production, followed by increased MCHC value as the ratio between haemoglobin and hematocrit. Other than a high grade of inflammation, risk factor such as smoking also contributes to oxidative stress [23]. Study in Indonesia has shown that ACS patients have a higher number of smoker compared to SCAD patient [17].

The result of WBC is similar to the previous study, which showed WBC of ACS patients is significantly higher compared to SCAD patients [10], [11]. Based on the previous study, WBC of ACS patients ranging from 7.07 ± 2.02 to 9.40 ± 3.30 x 10^9/L, while SCAD patients are ranging from 6.63 ± 1.57 to 6.60 ± 1.40 x 10^9/L [10], [11]. In this study, WBC for ACS is higher than the previous study (11.72 ± 3.41 x 10^9/L) since the majority of participants in this study were STEMI patient (75.95%), while the previous study was UAP [10]. A study comparing WBC of STEMI and NSTEMI group showed higher WBC in the STEMI group (11.850 vs 8.460 x 10^9/L, p = 0.01). Elevation of WBC is related to the complex and dynamic inflammation response in local and systemic level. Leukocyte has major role in

pathogenesis and progression of the atherosclerotic lesion. Local low-grade inflammation during early lesion, endothelial dysfunction, and foam cell production is related to leukocytes activities [3]. Leukocytes activities are also responsible for plaque stability. During various atherosclerosis phases, there is continuous activation and infiltration of neutrophils resulting in plaque instability via myeloperoxidase (MPO) and metalloproteinase (MMP) release [13]. Myocardial damage from the atherosclerosis occlusion can also increase the neutrophil and macrophage numbers via cytokines, chemokine, and other substance stimulation [24].

Previous studies showed that ACS patients have higher MPV compared to SCAD patients [25], [26], [27]. Higher MPV is correlated to various cardiovascular risks and higher thrombogenicity due to platelet metabolic and enzymatic activity [28], [29]. Interestingly, our study showed a different result, which showed that ACS patients had significantly lower MPV compared to SCAD patients. We hypothesise there was dynamic and complex platelet regulation during ACS, including production and consumption of platelet. During inflammation, larger platelet was produced. However, atherothrombotic lesion exhibits high consumption of large and hyperactive platelet [28].

Furthermore, high-grade inflammation diseases such as rheumatoid arthritis and inflammatory bowel disease also showed lower MPV due to local active and large platelet consumption [28]. This theory is supported by the fact that activated platelet is six times more potent to adhere to polymorphonuclear cells and monocytes compared to inactive platelet [30]. Other theories suggest that during ACS, there is acute and general activation of platelet without followed by increased MPV [31].

In this research, both NLR and PLR are higher in ACS compared to SCAD. Similarly, the previous study showed NLR is elevated in both ACS and SCAD compared to healthy controls [32], [33], [34]. Higher NLR in ACS is related to acute and higher-grade inflammation response which neutrophils act as acute phase pro-inflammatory agents and lymphocytes as anti-inflammatory agents. Low lymphocyte level is likely due to the complex interaction between cytokines, neutrophils, and lymphocytes. ACS has the highest levels of circulating IFN-γ, followed by SCAD and healthy control [35]. Neutrophils activated IFN-γ suppresses lymphocyte proliferation through Programmed Death Ligand 1 expression [36].

The previous study also showed PLR is elevated in both ACS and SCAD compared to healthy controls [32], [37], [38]. This study shows that elevated PLR is mainly due to lower lymphocyte count. Low lymphocyte count in ACS condition may be due to cortisol release or lymphocyte migration from blood circulation [37], [38]. Platelet count in ACS and SCAD showed inconsistent results, several studies showed platelet count is higher in ACS group compared to SCAD and healthy control [11], [39], while others showed lower platelet count [32], [40]. A study also showed platelet count in myocardial infarction patient is higher compared to healthy control but lower in unstable angina patient [41]. We hypothesise this inconsistency is due to the complex relation between thrombopoietin and regulation of platelet in inflammation settings. Thrombopoietin, a platelet production regulatory hormone, is elevated in unstable angina patient compared to SCAD and healthy control [42]. This elevation is due to platelet consumption during the acute myocardial attack to stimulate megakaryocytes proliferation [43]. Other theory suggested that interaction between thrombopoietin and its receptor on the platelet surface will decrease thrombopoietin, resulting in the low production of the platelet. Platelet with high MPV will have many receptors which induces inhibitory feedback resulted in lower platelet count [44].

This study analysed the cut-off point for five indices. MPV cut-off point was 8.35 fL, and lower MPV suggests a diagnosis of ACS. This result is different from the previous study, which showed MPV cut-off point was 9.15 fL or higher with sensitivity 72% and specificity 40% [25]. In this research, the cut-off point of NLR was 3.187, which higher NLR is suggestive for ACS diagnosis. The previous study showed that NLR above 2.5 could diagnose ACS with the sensitivity of 63.6% and specificity 80.2% [32]. A meta-analysis also showed that NLR cut-off point from 1.95 to 3.97 could predict severe atherosclerotic lesion [45]. In this research, WBC of more than 9.170 is suggestive for ACS diagnosis. Previously, WBC been reported to have a cut-off point of 6.91; 7.37; and 8.89 x 10^3/μL with each sensitivity and specificity are 86% and 37%; 45% and 54%; 54% and 71% respectively [46]. Overall, our study showed that MPV, NLR, and WBC is not inferior to other inflammation markers such as IL-6 to diagnose ACS. Previously, a study in Indonesia showed IL-6 with cut-off point 4.43 pg/mL can distinguish ACS and SCAD with sensitivity and specificity are 80.95% and 77.42%, respectively [9].

Benefits in Further Clinical Practice: Descriptions of chest pain from CAD patients are often subjective, dependent on communication skills, and different from Diamond and Forrester angina classification [47]. Moreover, the general practitioner ability to diagnose ACS and SCAD based on sign and symptom is considered low [48]. The complete blood count is a simple and accessible examination in the primary care setting. General practitioner with limited resources could consider MPV, NLR, and WBC to distinguish chest pain originated from ACS or SCAD.

Strength and Limitations of the Study: To our knowledge, this is the first study comparing various haematological indices simultaneously between ACS and SCAD patient, especially in Indonesia. Moreover, this study also firstly showed the difference in MCHC,

NLR, and PLR value in ACS and SCAD group. However, this study may yet to be generalised since it only involved single-centre as the source of data. This study also used consecutive samplings from all patients who were admitted for ACS or SCAD diagnosis. Hence, selection bias might occur. In the future, it is suggested to involve more hospital and stratify the sample based on several risk factors such as race and social status to ensure the validity of the hematologic indices among various demographic characteristics.

In conclusion, there was a significant difference in hematologic indices between ACS and SCAD patients. ACS had higher MCHC, WBC, NLR, PLR, and lower MPV compared to SCAD group. MPV had highest AUC (95.0%) with optimum cut-off point was 8.35 fL (sensitivity 93.6% and specificity 97.3%).

Author Contributions

Conceptualisation, Y.H.O. and K.L.; methodology, Y.H.O., K.L., L.H., and B.P.; software, K.L. and M.J.A.; formal analysis, K.L., B.P., M.J.A.; investigation, K.L.; data curation, K.L., L.H.; writing—original draft preparation, K.L., B.P., L.H.; writing—review and editing, L.H., Y.H.O and M.J.A.

References

1. WHO. Indonesia: WHO Statistical Profile [Internet], 2015. [cited 2019 Jun 13]. Available from: https://www.who.int/gho/countries/idn.pdf?ua=1

2. Libby P, Theroux P. Pathophysiology of coronary artery disease. Circulation. 2005; 111(25):3481-8. https://doi.org/10.1161/CIRCULATIONAHA.105.537878 PMid:15983262

3. Cimmino G, Loffredo FS, Morello A, D'Elia S, De Palma R, Cirillo P, et al. Immune-Inflammatory Activation in Acute Coronary Syndromes: A Look into the Heart of Unstable Coronary Plaque. Curr Cardiol Rev. 2016; 13(2):110-7. https://doi.org/10.2174/1573403X12666161014093812 PMid:27758696 PMCid:PMC5452145

4. Acet H, Ertaş F, Akıl MA, Özyurtlu F, Polat N, Bilik MZ, et al. Relationship Between Hematologic Indices and Global Registry of Acute Coronary Events Risk Score in Patients With ST-Segment Elevation Myocardial Infarction. Clin Appl Thromb. 2016; 22(1):60-8. https://doi.org/10.1177/1076029614533145 PMid:24816530

5. Cassar A, Holmes DR, Rihal CS, Gersh BJ. Chronic coronary artery disease: Diagnosis and management. Mayo Clin Proc. 2009; 84(12):1130-46. https://doi.org/10.4065/mcp.2009.0391 PMid:19955250 PMCid:PMC2787400

6. Agewall S. Acute and stable coronary heart disease: Different risk factors. Eur Heart J. 2008; 29(16):1927-9. https://doi.org/10.1093/eurheartj/ehn321 PMid:18621774

7. Bruins Slota MHE, Ruttena FH, van der Heijdena GJMG, Geersinga GJ, Glatzb JFC, Hoesa AW. Diagnosing acute coronary syndrome in primary care: Comparison of the physicians' risk estimation and a clinical decision rule. Fam Pract. 2011; 28(3):323-8. https://doi.org/10.1093/fampra/cmq116 PMid:21239470

8. Oikonomidou E, Anastasiou F, Dervas D, Patri F, Karaklidis D, Moustakas P, Andreadou N, Mantzanas E, Merkouris B. Rural primary care in Greece: working under limited resources. International Journal for Quality in Health Care. 2010; 22(4):333-7. https://doi.org/10.1093/intqhc/mzq032 PMid:20581119

9. Alwi I, Santoso T, Suyono S, Sutrisna B, Kresno SB. The cut-off point of interleukin-6 level in acute coronary syndrome. Acta Med Indones. 2007; 39(4):174-8.

10. Yip HK, Wu CJ, Hang CL, Chang HW, Yang CH, Hsieh YK, et al. Levels and values of inflammatory markers in patients with angina pectoris. Int Heart J. 2005; 46(4):571-81. https://doi.org/10.1536/ihj.46.571 PMid:16157948

11. Hung MJ, Cherng WJ, Cheng CW, Li LF. Comparison of Serum Levels of Inflammatory Markers in Patients With Coronary Vasospasm Without Significant Fixed Coronary Artery Disease Versus Patients With Stable Angina Pectoris and Acute Coronary Syndromes With Significant Fixed Coronary Artery Diseas. Am J Cardiol. 2006; 97(10):1429-34. https://doi.org/10.1016/j.amjcard.2005.12.035 PMid:16679078

12. Bobryshev YV, Ivanova EA, Chistiakov DA, Nikiforov NG, Orekhov AN. Macrophages and Their Role in Atherosclerosis: Pathophysiology and Transcriptome Analysis. Biomed Res Int. 2016; 2016. https://doi.org/10.1155/2016/9582430 PMid:27493969 PMCid:PMC4967433

13. Soehnlein O. Multiple roles for neutrophils in atherosclerosis. Circ Res. 2012; 110(6):875-88. https://doi.org/10.1161/CIRCRESAHA.111.257535 PMid:22427325

14. Hedrick CC. Lymphocytes in Atherosclerosis. Arterioscler Thromb Vasc Biol. 2015; 35(2):253-7. https://doi.org/10.1161/ATVBAHA.114.305144 PMid:25609772 PMCid:PMC4327776

15. Chen C, Cong BL, Wang M, Abdullah M, Wang XL, Zhang YH, et al. Neutrophil to lymphocyte ratio as a predictor of myocardial damage and cardiac dysfunction in acute coronary syndrome patients. Integr Med Res. 2018; 7(2):192-9. https://doi.org/10.1016/j.imr.2018.02.006 PMid:29984180 PMCid:PMC6026362

16. Budzianowski J, Pieszko K, Burchardt P, Rzeźniczak J, Hiczkiewicz J. The Role of Hematological Indices in Patients with Acute Coronary Syndrome. Dis Markers. 2017; 2017. https://doi.org/10.1155/2017/3041565 PMid:29109595 PMCid:PMC5646322

17. Qanitha A, Uiterwaal CSPM, Henriques JPS, Alkatiri AH, Mappangara I, Mappahya AA, et al. Characteristics and the average 30-day and 6-month clinical outcomes of patients hospitalised with coronary artery disease in a poor South-East Asian setting : the first cohort from Makassar Cardiac Center , Indonesia. BMJ Open. 2018; (8):1-11. https://doi.org/10.1136/bmjopen-2018-021996 PMid:29950477 PMCid:PMC6020938

18. WHO. Noncommunicable Diseases in the South-East Asia Region, 2011.

19. Nagula P, Karumuri S, Otikunta AN, Yerrabandi SRV. Correlation of red blood cell distribution width with the severity of coronary artery disease-A single center study. Indian Heart J. 2017; 69(6):757-61. https://doi.org/10.1016/j.ihj.2017.04.007 PMid:29174254 PMCid:PMC5717282

20. Khode V, Ruikar K, Nallulwar S, Sindhur J, Kanabur D. Association of red cell distribution width, haematocrit and other RBC indices with coronay artery disease: A case control study. Niger J Cardiol. 2014; 11(2):88. https://doi.org/10.4103/0189-7969.142088

21. Koorts AM, Levay PF, Becker PJ, Viljoen M. Pro- and Anti-Inflammatory Cytokines during Immune Stimulation: Modulation of Iron Status and Red Blood Cell Profile. 2011; 2011. https://doi.org/10.1155/2011/716301 PMid:21547258 PMCid:PMC3086355

22. Huang Y, Hu Z. Lower mean corpuscular hemoglobin concentration is associated with poorer outcomes in intensive care unit admitted patients with acute myocardial infarction. 2016; 4(5):1-8. https://doi.org/10.21037/atm.2016.03.42 PMid:27294086 PMCid:PMC4885905

23. Metta S, Uppala S, Basalingappa D, Gunti S, Badeti S. Impact of smoking on erythrocyte indices and oxidative stress in acute myocardial infarction. J Dr NTR Univ Heal Sci. 2015; 4(3):159. https://doi.org/10.4103/2277-8632.165400

24. Fang L, Moore XL, Dart AM, Wang LM. Systemic inflammatory response following acute myocardial infarction. J Geriatr Cardiol. 2015; 12(3):305-12.

25. Dehghani MR, Taghipour-Sani L, Rezaei Y, Rostami R. Diagnostic importance of admission platelet volume indices in patients with acute chest pain suggesting acute coronary syndrome. Indian Heart J. 2014; 66(6):622-8. https://doi.org/10.1016/j.ihj.2014.10.415 PMid:25634396 PMCid:PMC4310955

26. Khandekar MM, Khurana AS, Deshmukh SD, Kakrani AL, Katdare AD, Inamdar AK. Platelet volume indices in patients with coronary artery disease and acute myocardial infarction: An Indian scenario. J Clin Pathol. 2006; 59(2):146-9. https://doi.org/10.1136/jcp.2004.025387 PMid:16443728 PMCid:PMC1860313

27. Abef NK-çamur, B RD, Def CK. Could mean platelet volume be a predictive marker for acute myocardial infarction ? Med Sci Monit. 2005; 11(8):387-93.

28. Gasparyan AY, Ayvazyan L, Mikhailidis DP, Kitas GD. Mean platelet volume: a link between thrombosis and inflammation? Curr Pharm Des. 2011; 17(1):47-58. https://doi.org/10.2174/138161211795049804 PMid:21247392

29. Chu S, Becker R, Berger P, Bhatt D, Eikelboom J, Konkle B, et al. Mean platelet volume as a predictor of cardiovascular risk: a systematic review and meta-analysis. J Thromb Haemost. 2010; 8(1):148-56. https://doi.org/10.1111/j.1538-7836.2009.03584.x PMid:19691485 PMCid:PMC3755496

30. Rinder HM, Bonan JL, Rinder CS, Ault KA, Smith BR. Activated and Unactivated Platelet Adhesion to Monocytes and Neutrophils. Blood. 1991; 78(7):1760-9.

31. Mathur A, Robinson MSC, Cotton J, Martin JF, Erusalimsky JD. Platelet reactivity in acute coronary syndromes: Evidence for differences in platelet behaviour between unstable angina and myocardial infarction. Thromb Haemost. 2001; 85(6):989-94. https://doi.org/10.1055/s-0037-1615952 PMid:11434707

32. Ertürk M, Turhan Caglar FN, Bıyık İ. Correlations between hematological indicators and other known markers in acute coronary syndromes. E J Cardiovasc Med. 2018; 5(4):67-74. https://doi.org/10.15511/ejcm.17.00467

33. Bhuiyan M, Sultana S, Hasan A, Barua D, Ahmed M, Aa C. Neutrophil Lymphocyte Ratio (NLR) is an Indicator of Coronary Artery Disease (Cad) in Type 2 Diabetes Mellitus Patients. 2018; 76:1-6.

34. Tabrez S, Alama MN, Jabir NR, Firoz CK. Neutrophils to Lymphocyte Ratio as a Biomarker of Coronary Artery Disease. Acta Medica Mediterr. 2016; 32:1637-42.

35. Min X, Lu M, Tu S, Wang X, Zhou C, Wang S, et al. Serum Cytokine Profile in Relation to the Severity of Coronary Artery Disease. Biomed Res Int. 2017; 2017. https://doi.org/10.1155/2017/4013685 PMid:28349060 PMCid:PMC5352875

36. de Kleijn S, Langereis JD, Leentjens J, Kox M, Netea MG, Koenderman L, et al. IFN-γ-Stimulated Neutrophils Suppress Lymphocyte Proliferation through Expression of PD-L1. PLoS One. 2013; 8(8). https://doi.org/10.1371/journal.pone.0072249 PMid:24015224 PMCid:PMC3756078

37. Harun H, Bahrun U, Darmawaty E. Platelet-Lymphocyte Ratio (PLR) Markers in Acute Coronary Syndrome. Indones J Clin Pathol Med Lab. 2016; 23(1):7-11. https://doi.org/10.24293/ijcpml.v23i1.1176

38. Akboga MK, Canpolat U, Yayla C, Ozcan F, Ozeke O, Topaloglu S, et al. Association of Platelet to Lymphocyte Ratio with Inflammation and Severity of Coronary Atherosclerosis in Patients with Stable Coronary Artery Disease. Angiology. 2016; 67(1):89-95. https://doi.org/10.1177/0003319715583186 PMid:25922197

39. Turk U, Tengiz I, Ozpelit E, Celebiler A, Pekel N, Ozyurtlu F, et al. The relationship between platelet indices and clinical features of coronary artery disease. Kardiol Pol. 2013; 71(11):1129-34. https://doi.org/10.5603/KP.2013.0293 PMid:24297710

40. Ranjani G, Manikandan S, Rohini I, Kalaiselvi S. A study on platelet volume indices in acute coronary syndrome. Int Arch Integr Med IAIM. 2016; 3(38):146-52.

41. Assiri AS, Jamil AM, Mahfouz AA, Mahmoud ZS, Ghallab M. Diagnostic importance of platelet parameters in patients with acute coronary syndrome admitted to a tertiary care hospital in southwest region, Saudi Arabia. J Saudi Hear Assoc. 2012; 24(1):17-21. https://doi.org/10.1016/j.jsha.2011.08.004 PMid:23960663 PMCid:PMC3727553

42. Lupia E, Bosco O, Bergerone S, Dondi AE, Goffi A, Oliaro E, et al. Thrombopoietin Contributes to Enhanced Platelet Activation in Patients With Unstable Angina. J Am Coll Cardiol. 2006; 48(11):2195-203. https://doi.org/10.1016/j.jacc.2006.04.106 PMid:17161245

43. Amraotkar AR, Song DD, Otero D, Trainor PJ, Ismail I, Kothari V, et al. Platelet Count and Mean Platelet Volume at the Time of and after Acute Myocardial Infarction. Clin Appl Thromb. 2017; 23(8):1052-9. https://doi.org/10.1177/1076029616683804 PMid:28294633 PMCid:PMC5572529

44. Loo B van der, Martin JF. A Role for Changes in Platelet Production in the Cause of Acute Coronary Syndromes. Arterioscler Thromb Vasc Biol. 1999; 19:672-9. https://doi.org/10.1161/01.ATV.19.3.672 PMid:10073972

45. Li X, Ji Y, Kang J, Fang N. Association between blood neutrophil-to- lymphocyte ratio and severity of coronary artery disease. Medicine (Baltimore). 2018; 97(39):1-9. https://doi.org/10.1097/MD.0000000000012432 PMid:30278521 PMCid:PMC6181556

46. Meissner J, Irfan A, Twerenbold R, Mueller S, Reiter M, Haaf P, et al. Use of neutrophil count in early diagnosis and risk stratification of AMI. Am J Med. 2011; 124(6):534-42. https://doi.org/10.1016/j.amjmed.2010.10.023 PMid:21507368

47. Jones MM, Somerville C, Feder G, Foster G. Patients ' descriptions of angina symptoms : a qualitative study of primary care patients. 2010; (October 2009):735-41. https://doi.org/10.3399/bjgp10X532378 PMid:20883622 PMCid:PMC2944932

48. Bösner S, Hani A, Keller H, Sönnichsen AC, Karatolios K, Schaefer JR, et al. Accuracy of General Practitioners ' Assessment of Chest Pain Patients for Coronary Heart Disease in Primary Care : Cross-sectional Study with Follow-up. 2010; 243-9. https://doi.org/10.3325//cmj.2010.51.243 PMid:20564768 PMCid:PMC2897083

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Clinical Science

The Relation between Serum Hepcidin, Ferritin, Hepcidin: Ferritin Ratio, Hydroxyurea and Splenectomy in Children with β-Thalassemia

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Abstract

BACKGROUND: Hepcidin, a small peptide hormone, is established as the main regulator of iron homeostasis.

AIM: To estimate serum hepcidin, ferritin, and hepcidin: ferritin ratio in β-thalassemia patients and to determine the effect of splenectomy and hydroxyurea on serum hepcidin.

METHODS: A study was conducted on 30 thalassemia major (βTM), 29 thalassemia intermedia (βTI) and 29 healthy children's controls. Data were collected by patient interviewing where detailed history-taking and thorough clinical examinations were carried out. Serum ferritin and hepcidin were measured by ELISA assay (Bioneovan Co. Ltd Beijing, China).

RESULTS: Beta-thalassemia patients had higher serum ferritin, serum hepcidin and lower Hb and hepcidin: ferritin ratio compared to the controls ($p < 0.001$, 0.010, 0.001, 0.001) respectively. B-TM patients had higher mean serum hepcidin and serum ferritin compared to β-TI, with statistically significant difference ($P = 0.042$, $P < 0.001$, respectively). Twenty-one patients out of 29 βTI was on hydroxyurea therapy; these patients had significantly lower levels of serum ferritin ($P < 0.004$) and significantly higher levels of Hb ($P < 0.004$). Serum ferritin was statistically significantly higher in splenectomized patients $P < 0.009$. Serum hepcidin level was insignificantly higher in splenectomized patients than non-splenectomized patients (21.6 ± 14.75, 17.76 ± 10.01 ng/mL). Hepcidin showed a significantly positive correlation with hepcidin: ferritin ratio in all studied groups.

CONCLUSION: Serum hepcidin was elevated in β-thalassemia children with more evident elevation in βTM patients. Splenectomy played no major role in hepcidin regulation. Knowing that hepcidin in serum has a dynamic and multi-factorial regulation, individual evaluation of serum hepcidin and follow up, e.g. every 6 months could be valuable, and future therapeutic hepcidin agonists could be helpful in management of iron burden in such patient.

Introduction

Thalassemia remains a major haematological health problem in Egypt. B- thalassemia is autosomal recessive anaemia resulting from impaired biosynthesis of the β-globin chain [1]. This disorder of β-chain synthesis leads to ineffective erythropoiesis. Erythroid progenitor cells undergo intramedullary apoptosis and do not develop into mature erythrocytes.The basic mechanism of iron overload in children with β-thalassemia intermedia (TI) is the acceleration of the production of RBCs due to ineffective erythropoiesis [2], [3]. In children with β-thalassemia major (βTM) an adequate blood transfusion program decreases the complications of anaemia and compensatory bone marrow expansion, allows normal development throughout childhood, and extends survival [4]. Iron overload due to both transfused iron and increased iron absorption is common in βTM.

Significant progress was developed in clarifying the iron metabolism pathway. Hepcidin a small peptide hormone is established as main regulator of iron homeostasis. It inhibits iron entry into the plasma from the main sources of iron: dietary absorption, the release of stored iron from hepatocytes and the release of recycled iron from macrophages. However, in transfused patients higher hepcidin levels resulted in macrophage iron loading, whereas in nontransfused thalassemia, iron was deposited in hepatocytes [5]. Hepcidin is synthesized

as prohepcidin that is converted to its bioactive form by hepatocytes, secreted into the bloodstream, and excreted by the kidneys [5].

Furthermore, hepcidin is also synthesised in the spleen of normal mice and induced by lipopolysaccharide [6]. Camberlein et al. reported that hepcidin has led to restriction of iron export from the spleen, in a mouse model of secondary iron overload [7]. However, many authors reported that hepcidin is also produced from various tissues and organs, including adipose tissue, macrophages, monocytes and kidney, but at a lower level than that produced by the liver and the role of hepcidin in these cell types is unclear [8], [9], [10], [11]. Thalassemia is a good model to show the dynamic regulation of hepcidin in the face of competing influences of coexistent anaemia, expanded erythropoiesis and iron loading. Hepcidin level is variable in children with β-thalassemia [12], [13]. Cases with marked anaemia and high erythropoietic activity had hepcidin deficiency [14], but in case of iron overload, hepcidin expression will be increased [15]. In β-thalassemia, usually we depend on serum ferritin measurements to diagnose iron overload [16]. Knowing that, erythropoiesis measurably fluctuates over the inter-transfusion interval, thus we must standardized the timing of measurement of indices for the research purposes (e.g. immediately pre-transfusion) [12].

Spleen is the most commonly affected organ in children with thalassemia. Splenomegaly is the result of extramedullary hematopoiesis, excessive destruction of abnormal RBCs, and transfusion overload. Splenomegaly further increases transfusion requirement. Splenic macrophages remove damaged RBCs passing through the red pulp of the spleen. Splenectomy is one possible therapeutic approach to the management of severely affected patients. Splenectomy is indicated when hypersplenism increases blood transfusion requirement and prevents adequate control of body iron with chelating therapy [17].

This study was conducted to estimate serum hepcidin, ferritin and hepcidin: ferritin ratio in β-thalassemia patients treated with hydroxyurea and different chelating therapy. Also, we aimed to determine the effect of splenectomy on serum hepcidin.

Patients and Methods

The current case-control study was conducted in Pediatrics Clinic in the Centre of Excellence in National Research Centre and El Helal Giza Children Hospital haematology Clinic in 2018.The study included 59 patients, 30 TM and 29 TI. Patients consisted of 35 (59.3%) boys and 24(40.7%) girls. Their age ranged from 3 – 17 y (mean age was 9.1 ± 3.7 y) and were diagnosed as β-TM and β-TI based on conventional clinical and hematologic criteria [18]. The exclusion criteria were ethose with concurrent infection, chronic inflammatory disorders or liver dysfunction. Also, 29 healthy subjects with matching age and sex were included as a control group. All patients and healthy subjects gave informed consent form to participate in the present study, approved by National Research Centre Ethics Committee. Data were collected by reviewing medical records as well as patient interviewing where detailed history-taking and thorough clinical examinations were carried out. Blood was collected before blood transfusion.

Sample Collection

Four ml of venous blood were withdrawn under aseptic conditions; blood was centrifuged to get serum to measure liver function and kidney function tests. Serum samples then were stored in -80°C for assessment of serum ferritin and hepcidin.

Laboratory Investigation

Liver function and kidney function tests were done by using an autoanalyser Olympus AU400 (Olympus America Inc, Center Valley, Pa, USA). Serum ferritin and hepcidin were measured by ELISA assay (Bioneovan Co. Ltd Beijing, China)

Statistical analysis

The standard computer program Statistical Package for the Social Sciences (SPSS) for Windows, release 17.0 (SPSS Inc., USA) was used for data entry and analysis. All numeric variables were expressed as mean ± standard deviation (SD). The intergroup comparisons were performed by using an independent-sample t-test and a one-way analysis of variance and Chi-Square tests for categorical variables. Pearson's and Spearman's correlation tests (r=correlation coefficient) were used for correlating normal and nonparametric variables, respectively. Linear multiple regression was run to predict Hepcidin. Receiver operating curve (ROC) analysis was done to test the validity of hepcidin vs hepcidin to ferritin ratio in detecting iron overload. For all tests, a P-value of less than 0.05 was considered significant.

Results

The mean age of the children with TM, TI and control were 10.3 ± 4.2 years (from 4 to 17 years), 9.1 ± 3.7 years (from 3 to 16 years) and 10.4 ± 4.2 years (from 2 to 18 years old) respectively, and there was

no statistically significant difference between the three groups (P = 0.43) by ANOVA. Considering gender distributions, 21 (43.8%) and 22 (55%).

Of the subjects in the TM and TI groups were female, respectively; Considering gender distributions, patients consisted of 35 (59.3%) boys and 24 (40.7%) girls, there was no statistically significant difference (P = 0.39). Also, there were no statistically significant differences in serum hepcidin levels between males and females in patients with TM and TI (P = 0.30 and P = 0.56 respectively). Table 1 shows the comparison of Clinical and Laboratory Data of β- thalassemia Patients with controls. B-thalassemia patients had higher serum ferritin, serum hepcidin, lower Hb and hepcidin: ferritin ratio compared to the control group (p < 0.001, 0.010, 0.001, 0.001, respectively).

Table 1: Comparison of Clinical and Laboratory Data of β-thalassemia Patients With controls

		N	Mean	Std. Deviation	Sig. (2-tailed)
Age (Years)	β-thalassemia Patients	59	9.18	3.70	0.193
	Controls	29	10.34	4.24	
Hb (g/dL)	β-thalassemia Patients	59	7.24	1.03	0.001
	Controls	29	11.68	1.08	
Ferritin (ng/mL)	β-thalassemia Patients	59	1581.6	787.64	0.001
	Controls	29	141.9	55.33	
Hepcidin (ng/mL)	β-thalassemia Patients	59	19.73	12.98	0.010
	Controls	29	13.01	6.46	
Hepcidin:ferritin Ratio	β-thalassemia Patients	59	0.023	0.043	0.001
	Controls	29	0.094	0.0365	

When the two groups of thalassemia (TM, TI) were considered separately, age and hepcidin: ferritin ratio showed no statistically significant difference between the two groups. On the other hand, β-TM patients had higher mean serum hepcidin, serum ferritin, lower Hb and lower number of weeks between transfusions compared to β-TI, with statistically significant difference (P.042, P < 0.001 respectively) (Table 2).

Table 2: Comparison of Clinical and Laboratory Data of Patients With β-Thalassemia Major and Intermedia

		N	Mean	Std. Deviation	Sig. (2-tailed)
Age (Years)	Btm	30	9.23	3.80	0.922
	Bti	29	9.13	3.67	
BMI (Kg/m^2)	Btm	30	16.93	3.58	0.791
	Bti	29	17.16	3.02	
No of weeks between blood transfusion	Btm	30	3.80	0.407	0.001
	Bti	29	8.86	5.79	
Hb (g/L)	Btm	30	6.59	0.94	0.001
	Bti	29	7.91	0.59	
Ferritin (ng/ml)	Btm	30	2099.10	574.50	0.001
	Bti	29	1046.37	599.67	
Hepcidin (ng/ml)	Btm	30	21.74	13.11	0.042
	Bti	29	16.04	9.31	
Hepcidin:Ferritin Ratio	Btm	30	0.026	0.057	0.598
	Bti	29	0.020	0.01	

Table 3 shows iron chelation therapy (deferoxamine = DFO; deferiprone = DFP and deferasirox = DFX) in patients with β-thalassemia major and intermedia. There was a statistically significant difference between the two groups regarding the type of chelating agents and the number of patients (P < 0.001). Out of 59 patients 17 patients with β-thalassemia intermedia did take iron chelation therapy. None chelated group had significantly lower levels of serum ferritin (787.00 ± 272.04 and 1903.31 ± 693.48 P < 0.001). Also, they had a significantly higher mean Hb level (8.16 ± 0.499 and 6.87 ± 0.95 P < 0.001).

Table 3: Types of Chelating Agents in Patients With β-Thalassemia Major and Intermedia

		Type of chelation					Total
		0	DFO	DFO, DFP	DFP	DFX	
β-TM	Count	0	2	1	9	18	30
	% within Thalassemia Major Thalassemia Intermedia	.0%	6.7%	3.3%	30.0%	60.0%	100.0%
β-TI	% within Type of chelation	.0%	100.0%	100.0%	60.0%	75.0%	50.8%
	Count	17	0	0	6	6	29
	% within Thalassemia Major Thalassemia Intermedia	58.6%	0%	0%	20.7%	20.7%	100.0%
	% within Type of chelation	100.0%	0%	0%	40.0%	25.0%	49.2%
Total	Count	17	2	1	15	24	59
	% within Thalassemia Major Thalassemia Intermedia	28.8%	3.4%	1.7%	25.4%	40.7%	100.0%
	% within Type of chelation	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

Chi-Square Tests P > 0.001; DFO = deferoxamine DFP = deferiprone; DFX = deferasirox.

Twenty-one patients out of 29 βTI were on hydroxyurea therapy, these patients had significantly lower levels of serum ferritin (P < 0.004) and significantly higher levels of Hb (P < 0.004) than other patients. The insignificant difference was found in serum hepcidin and hepcidin:ferritin ratio between the two groups.

Transfusion dependent TI patients had significantly higher serum ferritin (P < 0.001) and significantly lower Hb (P < 0.007). Serum hepcidin and hepcidin: ferritin ratio showed no statistically significant difference between the dependent and non-dependent groups.

The relationship between iron overload and laboratory data among thalassemia patients are shown in Table 4. There was a statistically significant difference in number of weeks between blood transfusion, Hb g/L, ferritin ng/mL, hepcidin ng/mL and hepcidin: ferritin ratio (P < 0.005, 0.001, 0.001, 0.007 and 0.001) respectively.

Table 4: Comparison of Clinical and Laboratory Data of Patients with Iron Overload and Without

	Iron overload (SF ≥ 1000 ng/ml)	N	Mean	Std. Deviation	Sig. (2-tailed)
Age (Years)	No iron over load	49	9.69	3.92	0.737
	Iron overload	39	9.41	3.92	
BMI (Kg/m^2)	No iron over load	20	16.78	3.31	0.671
	Iron overload	39	17.17	3.31	
No of weeks between blood transfusion	No iron overload	20	8.65	5.84	0.005
	Iron overload	39	5.08	3.64	
Hb (g/L)	No iron over load	49	10.17	2.04	0.001
	Iron overload	39	6.86	1.01	
Ferritin (ng/ml)	No iron over load	49	369.70	293.53	0.001
	Iron overload	39	2033.80	564.25	
Hepcidin (ng/ml)	No iron over load	49	14.55	8.68	0.007
	Iron overload	39	21.25	13.79	
Hepcidin:Ferritin Ratio	No iron over load	49	0.066	0.046	0.001
	Iron overload	39	0.023	0.051	

Considering splenectomy, 18 cases out of 59 (30.5%) had a splenectomy. Although serum hepcidin level was higher in splenectomized patients (21.6 ± 14.75, 17.76 ± 10.01 ng/mL), there was no statistically

significant difference in number of weeks between blood transfusion, Hb g/L, hepcidin ng/mL and hepcidin: ferritin ratio between splenectomized patients and non- splenectomized. Serum ferritin ng/mL was statistically significantly higher in splenectomized patients P < 0.009. Also, 15 out of 18 splenectomized patients had iron overload (serum ferritin ≥ 1000 ng/ml) as shown in Table 5.

Table 5: Results of splenectomized and non-splenectomized TM patients

	1 = splenomegaly 0 = splenectomized	N	Mean	Std. Deviation	Sig. (2-tailed)
Hepcidin	1	20	19.7505	11.09305	0.245
	0	10	25.7400	16.37024	
Hipcidin/ ferritin	1	20	0.019520	0.0254222	0.344
	0	10	0.041020	0.0947943	
Ferritin	1	20	2103.6250	676.38623	0.953
	0	10	2090.0600	312.33822	
Hb	1	20	6.6750	0.91931	0.514
	0	10	6.4300	1.03500	

Hepcidin showed a significantly positive correlation with Hepcidin: ferritin ratio in all studied groups. Also, it showed a significantly negative correlation with age in patients with B-thalassemia. Healthy children hepcidin showed a significantly positive correlation with ferritin (r = 0.645, P < 0.001), as shown in Table 6.

Table 6: Results of correlation between serum hepcidin levels and the evaluated parameters for all groups

Group	Age, Y		Ferritin, ng/mL		Hemoglobin/dL		Hepcidin/Ferritin	
	r	P	r	P	r	P	r	P
B-Thalassemia	-0.317*	0.014	-0.014	0.917	0.012	0.930	0.751**	0.001
B-TM	-0.314	0.091	-0.058	0.760	0.082	0.668	0.747**	0.001
B-TI	-0.342	0.070	-0.160	0.408	0.199	0.301	0.914**	0.001
Controls	0.300	0.114	0.645**	0.001	0.173	0.368	0.583**	0.001

* Correlation is significant at the 0.05 level (2-tailed); ** Correlation is significant at the 0.01 level (2-tailed).

Multiple linear regression was run to predict hepcidin from Predictors: (Constant), hydroxyurea therapy, hepcidin/ ferritin, transfusion, hepcidin, and ferritin in diseased children. Serum ferritin and hepcidin: ferritin ratio variables were a statistically significant predictor of serum hepcidin level (P < 0.001).

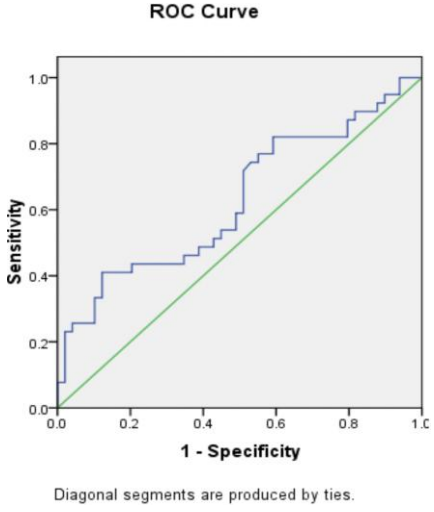

Figure 1: ROC curve of hepcidin

A receiver operating characteristic curve (ROC curve) was done to test the sensitivity and specificity of hepcidin and hepcidin/ferritin ratio, in predicting iron overload (SF ≥ 1000 ng/ml) at different cutoff values. The area under the curve of hepcidin was found as 0.628 (P = 0.040) while that of hepcidin/ferritin ratio was 0.180 (non-sig), indicating that overall predictability of hepcidin is significantly high when compared to hepcidin/ferritin ratio (Figure 1).

Discussion

Hepcidin level in serum has a dynamic and multifactorial regulation. The current study showed that β-thalassemia patients of both βTM and βTI had significantly elevated serum hepcidin level compared to healthy children. Our results were in agreement with those of previous studies [19], [20], [21]. On other hand, several authors reported undetectable or low serum hepcidin in β-TM subjects as compared to controls [22], [23], [24], [25], [26]. Hepcidin has a complex regulation in β-thalassemia patients. Its production is controlled by opposing effects from erythropoiesis, anaemia and iron overload. Hepcidin is up-regulated by increased body iron levels, infection and inflammation; it is down-regulated by many factors as anaemia, hypoxia, iron deficiency, ineffective erythropoiesis and by increased levels of erythropoietin [13], [27], [28], [29].It may be valuable to know that other factors might modify the hepcidin synthesis in β-thalassemia patients such as genetic or laboratory variables [27], [30]. Our data showed that patients with βTM had significantly higher serum hepcidin than βTI cases. The rate of iron loading is higher in βTM than in βTI due to regular transfusions also transfusions inhibit erythropoietic drive; both lead to an increase of hepcidin in β-TM patients [14], [19], [21], [23], [24].

Our data showed that serum hepcidin is not affected by gender in children with β-thalassemia this was in agreement with previous studies [21], [22], [23], [24].

The current study showed no significant difference in serum hepcidin between TI patients receiving hydroxyurea and those not receiving hydroxyurea, although they had significantly higher levels of Hb (P < .004). This means that hydroxyurea intake did not affect hepcidin production directly or through increasing total haemoglobin level. Or probably Hb was not high enough to suppress ineffective erythropoiesis and consequently, was not able to decrease serum hepcidin level. Our result was in agreement with Haghpanah et al., [21], [29].

Considering splenectomy, eighteen (30.5%) of our patients had been splenectomized. Although

serum hepcidin level was higher in splenectomized patients, the difference was of no statistical significance. Jones et al., reported that the inflammatory marker CRP and transferrin saturation both were higher in splenectomized patients. Both inflammation and transferrin bound iron is known to induce hepcidin [1]. Splenectomy in Hb E β-thalassemia patients was associated with lower serum hepcidin; the authors reported that the differences in hepcidin were not mediated by a phenotype-splenectomy interaction [31]. Similar to a previous study, we found no difference in Hb between splenectomized and non-splenectomized patients [1], [32]. There was no apparent difference in hepcidin: ferritin ratio between splenectomized patients and non- splenectomized patients.

The authors found that age has no relation with hepcidin level among healthy children; this was in agreement with previous report [21], [29]. But in children with βTM, negative correlation between age and hepcidin was reported. Based on the current study results, there was insignificant correlation between serum hepcidin and ferritin levels in patients with βTM and βTI, which supported the results of previous studies that regulation of serum hepcidin in thalassemia is more affected by erythropoietic activity than by iron, overload [29], [33].

The hepcidin/ferritin ratio in β-thalassemia children was significantly lower ($P < 0.0001$) than in controls suggesting that serum hepcidin level was not increased in proportion to the iron overload [13], [29]. Hepcidin showed a significantly positive correlation with hepcidin: ferritin ratio in all studied groups.

Using a receiver operating characteristic curve (ROC curve) in predicting iron overload, it was found that area under the curve (AUC) of hepcidin was 0.628; which indicates that the overall predictability of hepcidin is significant (p = 0.040). Accuracy is measured by the area under the ROC curve. An area of .60-.70 represents a poor test; this result was similar to a previous study [21]. The result means that serum hepcidin was not as good as serum ferritin in predicting severe iron overload.

There were some limitations in the study since we did not evaluate molecular erythropoietic activity. The liver iron concentration was not available for the present cases to be the gold standard of assessment of iron overload.

In conclusion, based on the current study results, serum hepcidin was elevated in β-thalassemia children with more evident elevation in βTM patients. Also serum hepcidin levels were significantly higher in the βTM than in βTI patients, which could be due to higher erythropoietic activity in TI. Splenectomy played no major role in hepcidin regulation. There was insignificant correlation between serum hepcidin level and serum ferritin level as a marker of iron overload in patients with βTM and βTI. Knowing that hepcidin in serum has a dynamic and multi-factorial regulation, individual evaluation and follow up, e.g. every 6 months could be valuable in diagnosing and managing iron burden in such patient. Future therapeutic hepcidin agonists could be helpful in management of iron burden in β thalassemia.

References

1. Ismail NA, Moustafa NO, Habib SA, El-Ghaffar Mohammad NA, EL Kafoury MR, Talaat AA. Impact of Splenectomy and Chelating Agents on Serum Cystatin C Levels in Egyptian Children with Beta-Thalassemia. Australian Journal of Basic and Applied Sciences. 2012; 6(2):85-89.

2. Taher AT, Musallam KM, Cappellini MD, Weatherall DJ. Optimal management of beta thalassaemia intermedia. Br J Haematol. 2011; 152:512-23. https://doi.org/10.1111/j.1365-2141.2010.08486.x PMid:21250971

3. Rivella, S. The role of ineffective erythropoiesis in non-transfusion-dependent thalassemia. Blood Rev. 2012; 26(Suppl. S1):S12-S15. https://doi.org/10.1016/S0268-960X(12)70005-X

4. Olivieri N, Brittenham G. Iron-chelating therapy and the treatment of thalassemia. Blood. 1997; 89:739-761.

5. Elizabeta Nemeth Hepcidin in β-thalassemia. Ann N Y. Acad Sci. 2010; 1202:31-35. https://doi.org/10.1111/j.1749-6632.2010.05585.x PMid:20712769 PMCid:PMC2924878

6. Liu XB, Nguyen NB, Marquess KD, Yang F, Haile DJ. Regulation of hepcidin and ferroportin expression by lipopolysaccharide in splenic macrophages. Blood Cells Mol Dis. 2005; 35(1):47-56. https://doi.org/10.1016/j.bcmd.2005.04.006 PMid:15932798

7. Camberlein E, Abgueguen E, Fatih N, Canonne-Hergaux F, Leroyer P, Turlin B, Ropert M, Brissot P, Loréal O. Hepcidin induction limits mobilisation of splenic iron in a mouse model of secondary iron overload. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 2010; 1802(3):339-46. https://doi.org/10.1016/j.bbadis.2009.12.007 PMid:20045050

8. Kulaksiz H, Theilig F, Bachmann S, Gehrke SG, Rost D, Janetzko A, et al. The iron-regulatory peptide hormone hepcidin: v expression and cellular localization in the mammalian kidney. J Endocrinol. 2005; 184:361-70. https://doi.org/10.1677/joe.1.05729 PMid:15684344

9. Bekri S, Gual P, Anty R, Luciani N, Dahman M, Ramesh B, et al. Increased adipose tissue expression of hepcidin in severe obesity is independent from diabetes and NASH. Gastroenterology. 2006; 131:788-96. https://doi.org/10.1053/j.gastro.2006.07.007 PMid:16952548

10. Sow FB, Florence WC, Satoskar AR, Schlesinger LS, Zwilling BS, Lafuse WP. Expression and localization of hepcidin in macrophages: a role in host defense against tuberculosis. J Leukoc Biol. 2007; 82:934-45. https://doi.org/10.1189/jlb.0407216 PMid:17609338

11, Theurl I, Theurl M, Seifert M, Mair S, Nairz M, Rumpold H, et al. Autocrine formation of hepcidin induces iron retention in human monocytes. Blood. 2008; 111:2392-9. https://doi.org/10.1182/blood-2007-05-090019 PMid:18073346

12. Pasricha SR, Frazer DM, Bowden DK, Anderson GJ. Transfusion suppresses erythropoiesis and increases hepcidin in adult patients with â-thalassemia major: A longitudinal study. Blood. 2013; 122:124-32. https://doi.org/10.1182/blood-2012-12-471441 PMid:23656728

13. Chauhan R, Sharma S, Chandra J. What regulates hepcidin in poly-transfused β-Thalassemia Major: Erythroid drive or store drive? Indian J PatholMicrobiol. 2014; 57:39-42. https://doi.org/10.4103/0377-4929.130891 PMid:24739829

14. Kattamis A, Papassotiriou I, Palaiologou D, et al. The effects of

erythropoetic activity and iron burden on hepcidin expression in patients with thalassemia major. Haematologica. 2006; 91:809-12.

15. Origa R, Galanello R, Ganz T, et al. Liver iron concentrations and urinary hepcidin in beta-thalassemia. Haematologica. 2007; 92:583-8. https://doi.org/10.3324/haematol.10842 PMid:17488680

16. Ho PJ, Tay L, Lindeman R, Catley L, Bowden DK. Australian guidelines for the assessment of iron overload and iron chelation in transfusion-dependent thalassaemia major, sickle cell disease and other congenital anaemias. Internal medicine journal. 2011; 41(7):516-24. https://doi.org/10.1111/j.1445-5994.2011.02527.x PMid:21615659

17. Iolascon A, Andolfo I, Barcellini W, Corcione F, Garçon L, De Franceschi L, Pignata C, Graziadei G, Pospisilova D, Rees DC, de Montalembert M. Recommendations regarding splenectomy in hereditary hemolytic anemias. haematologica. 2017; 102(8):1304-13. https://doi.org/10.3324/haematol.2016.161166 PMid:28550188 PMCid:PMC5541865

18. Galanello R, Origa R. Beta-thalassemia. Orphanet J Rare Dis. 2010; 5:11. https://doi.org/10.1186/1750-1172-5-11 PMid:20492708 PMCid:PMC2893117

19. Gardenghi S, Marongiu MF, Ramos P, Guy E, Breda L, Chadburn A, et al. Ineffective erythropoiesis in beta-thalassemia is characterized by increased iron absorption mediated by down regulation of hepcidin and up-regulation of ferroportin. Blood. 2007; 109(11):5027-35. https://doi.org/10.1182/blood-2006-09-048868 PMid:17299088 PMCid:PMC1885515

20. Kaddah NA. El Gindi HD, Mostafa NO, Abd El Aziz NMS, Kamhawy AHA. Role of hepcidin in the pathogenesis of iron overload in children with B-thalassemia. Int J Acad Res. 2011; 3:62-9.

21. Kaddah AM, Abdel-Salam A, Farhan MS, Reham R. Serum hepcidin as a diagnostic marker of severe iron overload in beta-thalassemia major. Indian J Pediatr. 2017; 84:745. https://doi.org/10.1007/s12098-017-2375-4 PMid:28600663

22. Kemna EHJM, Tjalsma H, Podust VN, Swinkels DW. Mass spectrometry-based hepcidin measurements in serum and urine: analytical aspects and clinical implications. Clin Chem. 2007; 53:620-8. https://doi.org/10.1373/clinchem.2006.079186 PMid:17272487

23. Kearney SL, Nemeeh E, Neufeld EJ, Thapa D, Ganz T, Weinstein DA. Urinary hepcidin in congenital chronic anemias. Pediatr Blood Cancer. 2007; 48:57-63. https://doi.org/10.1002/pbc.20616 PMid:16220548

24. El Beshlawy A, Alaraby I, Abdel Kader MSEM, Ahmed DH, Abdelrahman HE. Study of serum hepcidin in hereditary hemolytic anemias. Hemoglobin. 2012; 36:555-70. https://doi.org/10.3109/03630269.2012.721151 PMid:23088733

25. Papanikolaou G, Tzilianos M, Christakis JI, et al. Hepcidin in iron overload disorders. Blood. 2005; 105:4103-5. https://doi.org/10.1182/blood-2004-12-4844 PMid:15671438 PMCid:PMC1895089

26. Hendy OM, Allam M, Allam A, et al. Hepcidin levels and iron status in beta-thalassemia major patients with hepatitis C virus infection. Egypt J Immunol. 2010; 17:33-44.

27. Bansal D. Hepcidin and Thalassemia. Indian J Pediatr. 2017. https://doi.org/10.1007/s12098-017-2439-5 PMid:28840480

28. Nemeth E. Hepcidin and β-thalassemiamajor. Blood. 2013; 122:3-4. https://doi.org/10.1182/blood-2013-05-502617 PMid:23828883

29. Haghpanah S, Esmaeil zadeh M, Honar N, et al. Relationship between serum hepcidin and ferritin levels in patients with thalassemia major and intermedia in southern Iran. Iran Red Crescent Med J. 2015; 17:e28343. https://doi.org/10.5812/ircmj.17(5)2015.28343

30. Sharma V, Panigrahi I, Dutta P, Tyagi S, Choudhry VP, Saxena R. HFE mutation H63D predicts risk of iron overload in thalassemia intermedia irrespective of blood transfusions. Indian J Pathol Microbiol. 2007; 50:82-5.

31. Jones E, Pasricha SR, Allen A, Evans P, Fisher CA, Wray K, Premawardhena A, Bandara D, Perera A, Webster C, Sturges P. Hepcidin is suppressed by erythropoiesis in hemoglobin E β-thalassemia and β-thalassemia trait. Blood. 2015; 125(5):873-80. https://doi.org/10.1182/blood-2014-10-606491 PMid:25519750 PMCid:PMC4321326

32. Pratummo K, Jetsrisuparb A, Fucharoen S,Tripatara A. Hepcidin expression from monocyte of splenectomized and non-splenectomized patients with HbE-b-thalassemia. Hematology. 2014; 19(3):175-180. https://doi.org/10.1179/1607845413Y.0000000110 PMid:23905873

33. Jawad M, Aftab I, Saeed MT, Mumtaz G, Iram S, Mohsin S. Hepcidin levels inmulti transfused β thalassemia major patients. J Rawalpindi Med Col. 2016; 20:206-8.

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Clinical Science

Sodium Valproate versus Continuous Infusion of Haloperidol in Management of Agitated Critically Ill Patients

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Abstract

AIM: Describe the efficacy and safety of valproate and haloperidol infusion in controlling agitation in the intensive care unit (ICU).

MATERIAL AND METHODS: Prospective study on 100 critically ill patients with agitation in Kasralainy Hospital over the period from May 2016 to June 2017.patients were divided into two groups, each group included 50 patients, 1st group patients received Depakene orally, and 2nd group patients received haloperidol by i.v infusion for 72 h. Richmond agitation sedation score and doses of additional sedative drugs were noted and calculated daily in the first three days.

RESULTS: Our study showed that valproate was equal in efficacy in controlling agitation; decreasing the RAAS significantly after 48 h from initiation (2.52 ± 0.61 vs 0.28 ± 0.54 with $p < 0.001$) for Depakene and (2.6 ± 0.67 vs 0.34 ± 0.48 with $p < 0.001$) for haloperidol. There was also a decrease in the doses of additional sedative drugs used to control agitation (midazolam & propofol) after 48 h from drug initiation. Both drugs therapy was associated with decrease in heart rate (89 ± 20 vs 86.6 ± 13.6 with $p = 0.002$ for valproate and 99.8 ± 23.3 vs 91 ± 16.7 with $p < 0.001$ for haloperidol). They did not affect blood pressure. Haloperidol therapy was associated with significant QTc prolongation.

CONCLUSION: Valproate was equal in efficacy as haloperidol infusion in controlling agitation in ICU and decreasing the doses of additional sedative drugs used after 48 h from initiation.

Introduction

Agitation occurs in up to 70% of critically ill patients and is a significant source of distress for patients, families, and providers (Fraser GL et al., 2000). Sedatives are administered to 50% of intensive care unit (ICU) patients to alleviate agitation [1]. Choice of sedative is complex and largely driven by patient context. No sedative has consistently been shown to be superior to the rest, and alternative agents are greatly needed [2]. Most ICU patients, especially those requiring mechanical ventilation, are treated with opioids, propofol, and/or benzodiazepines [1], [3]. Use of these agents is limited by adverse effects (e.g., hemodynamic derangement) for safe administration [4]. New therapies for treating agitation are rarely introduced into practice, with dexmedetomidine being the most recent in 1999. Consequently, providers have increasingly repurposed older pharmacologic agents as ICU sedatives (e.g., clonidine, Phenobarbital, and valproate (Depakene) [5], [6].

Haloperidol is a butyrophenone that works by blocking D2 receptors, probably in the mesolimbic region [7]. Its side effects include extrapyramidal symptoms, and rarely, the neuroleptic malignant syndrome [8]. The patient should be monitored for precipitation of arrhythmias such as torsade de points [9] and haloperidol should be used with caution in patients with a QTc interval of over 450 msec. National guidelines for the management of agitation/delirium recommend short term haloperidol if non-pharmacological measures are not effective.

Depakene is an antiepileptic and mood stabiliser approved for the treatment of seizures, manic episodes associated with bipolar disorder, and migraine prophylaxis. Mechanistically, it blocks voltage-dependent sodium and calcium channels, increases γ-aminobutyric acid (GABA) synthesis, potentiates GABA activity at postsynaptic receptors, blocks GABA degradation, and attenuates the activity of glutamate upon N-methyl-D-Aspartate receptors

[10], [11].

Recently, valproate has been administered to critically ill patients to treat agitation and delirium, but there are few published reports to support this practice [12], [13], [14]. Valproate is an emerging treatment for ICU agitation because it allows patients to interact with their caregivers; can be administered outside of the ICU; has both an intravenous (IV) and enteral formulation; has a low drug acquisition cost; and has not been associated with respiratory depression, hemodynamic derangements, or delirium. In this study, we describe the use of Depakene & haloperidol for agitation in critically ill patients and examine their safety.

We aimed to evaluate the adding effect of a calcium sensitiser (levosimendan) compared to the conventional inotropic and vasoactive agent used in the patient with poor left ventricular function undergoing cardiac surgery on different measured hemodynamic variables and the effect on the outcome.

Material and Methods

Study design

Our study was a prospective one on Agitated critically ill patients in kasralainy hospital, critical care department over the period from May 2016 to June 2017. Patients included if they were above 18 years old and has severe agitation (score ≥ 2 on RAAS) patients were excluded if they have advanced cardiac diseases, hepatic diseases, advanced malignancy or if they were on antiepileptic. Our study was conducted on 100 patients divided in two groups; each group included 50 patients. Patients in group I received Depakene orally, and patients in group II received haloperidol by i.v. Infusion for 72 h.

Demographic data and patients' characteristics

Patient demographic data included age, sex, weight, history of psychiatric diagnosis, alcohol or substance abuse, the reason for ICU admission, need for mechanical ventilation. Clinical outcomes were descriptive and included ICU length of stay and ICU mortality.

Efficacy outcomes

Efficacy data were collected from day of drug (valproate or haloperidol) initiation and continuing for 72 h or until discontinuation, whichever came first. The 3-day interval was selected to allow a reasonable time to observe efficacy or lack thereof, the agitated patients were examined daily and Richmond Agitation-Sedation Scale (RASS) was assessed daily from drug initiation up to 3days. Also, the doses of additional sedative drugs were noted and documented daily during drug therapy.

Safety outcomes

Safety parameters were examined for the hospital duration of valproate and haloperidol therapy. Records were specifically reviewed for possible hepatotoxicity, hematologic toxicity for both drugs and QTc prolongation for haloperidol. The number of patients who had discontinued because of a suspected adverse event was also recorded. Hepatotoxicity was defined as a new alanine aminotransferase 3 times the upper limit of normal (120 U/L), alkaline phosphatase 2 times the ULN (234 U/L), total bilirubin 2 times the ULN (2 mg/dL), or a doubling of the baseline value if it was already abnormal following drug initiation. Suspected cases of hepatotoxicity were further assessed using the validated Roussel Uclaf Causality Assessment Method (RUCAM) [15]. Hematologic toxicity was defined as a new leukocyte count 4200 cells/mm3, absolute neutrophil count 2400 cells/mm3, or platelet count 140 000 cells/mm3 or platelet drop by 50% if platelets were already 140 000 cells/mm3 following valproate initiation.

Results

Demographic data and patient characteristics

One hundred patients were included in the study. The mean age of the studied population was 63.7 ± 15 years; the youngest was 22 years; they included 54 Males and 46 Females. Regarding Comorbidities; in our study 36 Smokers, 42 with pre-existing Cardiac patients (Ischemic heart disease and chronic heart failure) and 39 with pre-existing Renal patients (serum creatinine ≥ 1.4 mg/dl). End organ failure (45%), sepsis (40%) and Stroke (hemorrhagic and non-hemorrhagic) (15%) were the most common reasons for ICU admissions. The mean length of ICU stay was 7 days, 21 patients died in the ICU.

Table 1: Baseline characteristic data of the total population

Age (mean ± SD) years	63 ± 15
Gender-females n	46
Weight (mean ± SD) kg	76.5 ± 14
Smokers n (%)	36
Pre exciting Cardiac disease n (%)	42
Pre exciting Renal disease n (%)	39
ICU Mortality (%)	21%
ICU stay (mean) days	7
Causes of admission	
End Organ failure (%)	45%
Sepsis (%)	40%
Stroke (%)	15%

Efficacy outcomes

Regarding valproate group; the severity of agitation decreased after 48 h from starting valproate therapy; RASS score decrease from 2.52 ± 0.61 to 0.28 ± 0.54 before and after depakene initiation respectively with P value < 0.001. The doses of concomitant sedative drugs used to control agitation (midazolam & propofol) decreased after 48h from drug initiation (77.2 ± 82.4 mg/day vs. 0 mg/day) and (3.5 ± 2.0 gm/day vs. 0.35 ± 0.49 gm/day) with P value 0.004 and 0.135 respectively. Regarding haloperidol group; the severity of agitation decreased after 48 h from starting valproate therapy; RASS score decrease from 2.6 ± 0.67 Vs 0.34 ± 0.48 before and after depakene initiation respectively with P value < 0.001. The doses of concomitant sedative drugs used to control agitation (midazolam & propofol) decreased after 48 h from drug initiation (101 ± 141 mg/day vs. 16 ± 33 mg/day) and (1.6 ± 1.9 gm/day vs. 0.33 ± 0.7 gm/day) with P value < 0.001.

Hemodynamic response

The Depakene group showed a statistically significant difference regarding HR after 48 h from valproate therapy (89 ± 20 vs 86.6 ± 13.6) with P-value 0.002, there was no effect on systolic or diastolic blood pressure. Haloperidol group showed a statistically significant difference regarding HR after 48 h from haloperidol (99.8 ± 23.3 vs 91 ± 16.7) with P-value < 0.001, there was no effect on systolic or diastolic blood pressure.

Table 2: Efficacy outcomes and hemodynamic response for both groups

	Depakene group		Haloperidol group	
	Day 0	Day 2	Day 0	Day 2
RASS	2.52 ± 0.61	0.28 ± 0.54	2.6 ± 0.67	0.34 ± 0.48
Midazolam	0.77 ± 0.82	0	101 ± 141	16 ± 33
Propofol	3.5 ± 2.0	0.35 ± 0.49	1.6 ± 1.2	0.33 ± 0.7
Heart rate	89 ± 20	86 ± 13	99.8 ± 23	91 ± 16.7
Systolic BP	125 ± 18.8	121.8 ± 10	124.8 ± 17.7	122.6 ± 11.7
Diastolic BP	79.4 ± 8.6	78 ± 5.6	80.6 ± 6	80.4 ± 5

Safety outcomes

All patients had complete blood picture & liver function tests monitored during drug therapy, In valproate group; Hemoglobin level and Total leucocytic count showed significant decrease after 48 h from valproate therapy with p = 0.002 & 0.025 respectively. Liver enzymes (ALT & AST) showed a significant increase after 48 h from valproate therapy with p < 0.0001. One patient developed hepatotoxicity (elevation of ALT ≥ 120 IU/ml) but was unrelated to the drug as his RUCAM score was 1. Regarding haloperidol group; Hemoglobin level & Total leucocytic count showed significant decrease after 48 h from haloperidol therapy with p = 0.004 & < 0.001 respectively. Liver enzymes (ALT & AST) showed no significant differences after 48 h from haloperidol therapy. One patient developed hepatotoxicity (elevation of ALT ≥ 120 IU/ml) but was unrelated to the drug as his RUCAM score was 3. There was significant QTc prolongation after 48 h from haloperidol initiation with p < 0.001, but this was not associated with major cardiac events (VT or VF).

Table 3: Safety outcomes and hemodynamic response for both groups

	Depakene group		Haloperidol group	
Lab	Day 0	Day 2	Day 0	Day 2
Hemoglobin gm/dl	10.6 ± 1.9	10.2 ± 1.5	10.6 ± 1.9	10 ± 1.7
Total leucocytic count cells x 10^3	10.7 ± 9	9.2 ± 7	13 ± 8	10 ± 5
Platelets cells x 10^3	253 ± 103	245 ± 102	209 ± 88	212 ± 94
ALT IU/ml	44 ± 32	49 ± 100	49 ± 83	39 ± 36
AST IU/ml	39 ± 25	48 ± 126	47 ± 66	39 ± 33
Bilirubin IU/ml	0.62 ± 0.5	0.58 ± 0.47	1.32 ± 2.5	1.2 ± 2.3

Discussion

Regarding efficacy outcomes in Depakene & haloperidol groups of our study, there was a significant decrease in the severity of agitation after 48 h from drug initiation with Richmond agitation sedation scores p < 0.001. Also, there was a marked decrease in the doses of concomitant sedative drugs used to control agitation (Midazolam & propofol) after 48 h from drug initiation. Similarly, *Gagnon et al., (2017)* performed a retrospective cohort study evaluated critically ill adults treated with Depakene for agitation on Fifty-three patients and showed that the incidence of agitation significantly decreased following the initiation of Depakene from 96% to 61% on Depakene day 3 (P < 0.001). Also, in this study, Depakene therapy was associated with reduced doses of concomitant sedative drugs [16]. *Riker et al. (1994)* evaluated the Continuous infusion of haloperidol in critically ill agitated patients. This study was performed on eight patients required mechanical ventilation who had severe agitation which was refractory to intermittent bolus treatment with benzodiazepines, narcotics, and haloperidol. These patients received Continuous infusion of haloperidol to maintain adequate sedation. This study showed that there was a significant decrease in the Sedation-Agitation Scale after 48 h from haloperidol initiation (+2.4 vs +0.8) and (P = 0.06). Also, there was a marked reduction in the daily total of non-haloperidol sedatives after 48h of continuous infusion of haloperidol with (P-value = 0.15) [17].

Regarding Hemodynamics response after Depakene & haloperidol initiation, our study showed that there was significant decrease in patients heart rate after 48 h from drug initiation with p = 0.002 & < 0.001 respectively, blood pressure showed no significant changes before and after drug initiation. *Sinha et al., (2000)* reviewed hospital records of 13 patients with status epileptics and hypotension who received IV Depakene therapy. There were no significant changes in blood pressure, pulse, or use of vasopressors. The data suggest that Depakene

loading is well tolerated, even in patients with cardiovascular instability [18], also *Riker, et al., (1994)* evaluated the Continuous infusion of haloperidol in eight critically ill agitated patients. There were no hypotension episodes noted [17].

Regarding safety outcomes, our study showed that hepatotoxicity (ALT ≥ 120 IU/ml) had developed for one patient (2%) hepatotoxicity is unlikely to be related to the drug (Depakene or haloperidol) as RUCAM score was 1 and 3 respectively which make hepatotoxicity is unlikely related haloperidol [15]. Possible reasons for hepatotoxicity in these patients are the underlying disease.

QTc prolongation for all patients in the haloperidol group with one patient had significant QTc prolongation ≥ 500msec after 48 h from haloperidol therapy, but this QTc prolongation was not associated with major cardiac events. That agrees with *Tisdale et al.,* who assessed the effect of intravenous haloperidol on QT interval dispersion in critically ill patients who received intravenous haloperidol for delusional agitation. QTc intervals were measured, and QT interval dispersion was calculated.

Haloperidol prolonged QTc interval compared to pretreatment values in Torsades de Pointes patients by a greater magnitude than in patients who did not experience Torsades de Pointes. It was concluded that intravenous haloperidol prolongs QTc intervals in critically ill patients [19].

Also, there was a significant decrease in Hemoglobin levels and total leucocytic count after 48 h from haloperidol therapy. Possible reasons for that decrease of the total leucocytic count are that TLC was elevated on day 0 of drug initiation due to sepsis which decreased with starting broad-spectrum antibiotics & control of sepsis. Also, the possible reasons for decreased level of hemoglobin are dilutional effect of intravenous fluids, blood sampling for lab and bone marrow suppression from sepsis.

References

1. Wunsch H, Kahn JM, Kramer AA, Rubenfeld GD. Use of intravenous infusion sedation among mechanically ventilated patients in the United States. Crit Care Med. 2009; 37(12):3031-9. https://doi.org/10.1097/CCM.0b013e3181b02eff PMid:19633543

2. Roberts DJ, Haroon B, Hall RI. Sedation for critically ill or injured adults in the intensive care unit: a shifting paradigm. Drugs. 2012; 72(14):1881-916. https://doi.org/10.2165/11636220-000000000-00000 PMid:22950534

3. Weinert CR, Calvin AD. Epidemiology of sedation and sedation adequacy for mechanically ventilated patients in a medical and surgical intensive care unit. Crit Care Med. 2007; 35(2):393-401. https://doi.org/10.1097/01.CCM.0000254339.18639.1D PMid:17205015

4. Riker RR, Fraser GL. Adverse events associated with sedatives, analgesics, and other drugs that provide patient comfort in the intensive care unit. Pharmacotherapy. 2005; 25(5 Pt 2):8S-18S. https://doi.org/10.1592/phco.2005.25.5_Part_2.8S PMid:15899744

5. Gagnon DJ, Riker RR, Glisic EK, Kelner A, Perrey HM, Fraser GL. Transition from dexmedetomidine to enteral clonidine for ICU sedation: an observational pilot study. Pharmacotherapy. 2015; 35(3):251-9. https://doi.org/10.1002/phar.1559 PMid:25809176

6. Fraser GL, Riker RR. Phenobarbital provides effective sedation for a select cohort of adult ICU patients intolerant of standard treatment: a brief report. Hosp Pharm. 2006; 41:17-23. https://doi.org/10.1310/hpj4101-17

7. Seeman P. Atypical neuroleptics: role of multiple receptors, endogenous dopamine, and receptor linkage. Acta Psychiatr Scand Suppl. 1990; 358:14-20. https://doi.org/10.1111/j.1600-0447.1990.tb05280.x PMid:1978482

8. Fraser Gl et al. Haloperidol should be used sparingly. Crit Care Med. 2002; 30(11):2614. https://doi.org/10.1097/00003246-200211000-00052

9. Hassaballa HA, Balk RA. Torsade de pointes associated with the administration of intravenous haloperidol. Am J Ther. 2003; 10(1):58-60. https://doi.org/10.1097/00045391-200301000-00013

10. Perucca E. Pharmacological and therapeutic properties of valproate: a summary after 35 years of clinical experience. CNS Drugs. 2002; 16(10):695-714. https://doi.org/10.2165/00023210-200216100-00004 PMid:12269862

11. Rosenberg G. The mechanisms of action of valproate in neuropsychiatric disorders: can we see the forest for the trees? Cell Mol Life Sci. 2007; 64(16):2090-103. https://doi.org/10.1007/s00018-007-7079-x PMid:17514356

12. Bourgeois JA, Koike AK, Simmons JE, Telles S, Eggleston C. Adjunctive valproic acid for delirium and/or agitation on a consultation-liaison service: a report of six cases. J Neuropsychiatry Clin Neurosci. 2005; 17(2):232-8. https://doi.org/10.1176/jnp.17.2.232 PMid:15939979

13. Sher Y, Miller AC, Lolak S, Ament A, Maldonado JR. Adjunctive valproic acid in management-refractory hyperactive delirium: a case series and rationale. J Neuropsychiatry Clin Neurosci. 2015; 27(4):365-70. https://doi.org/10.1176/appi.neuropsych.14080190 PMid:25803136

14. Fitz K, Harding A. Safety and efficacy of valproic acid for treatment of delirium in critically ill patients [abstract]. Crit Care Med. 2011; 39(Suppl. 12):239.

15. Danan, et al. Causality assessment of adverse reactions to drugs-I. A novel method based on the conclusions of international consensus meetings: application to drug-induced liver injuries. Journal of clinical epidemiology. 1993; 46(11):1323-1330. https://doi.org/10.1016/0895-4356(93)90101-6

16. Gagnon DJ, Fontaine GV, Smith KE, Riker RR, Miller III, Lerwick PA, et al. Valproate for agitation in critically ill patients: a retrospective study. Journal of critical care. 2017; 37:119-125. https://doi.org/10.1016/j.jcrc.2016.09.006 PMid:27693975

17. Riker RR, Richard R, GILLES L. Fraser, and Paul M. Cox. Continuous infusion of haloperidol controls agitation in critically ill patients. Critical care medicine. 1994; 22(3):433-440. https://doi.org/10.1097/00003246-199403000-00013 PMid:8124994

18. Sinha, Shobhit, and Dean K. Naritoku. Intravenous valproate is well tolerated in unstable patients with status epilepticus. Neurology. 2000; 55(5):722-724. https://doi.org/10.1212/WNL.55.5.722 PMid:10980746

19. Tisdale JE, Rasty S, Padhi ID, Sharma ND, Rosman H. The effect of intravenous haloperidol on QT interval dispersion in critically ill patients: comparison with QT interval prolongation for assessment of risk of torsades de pointes. The Journal of Clinical Pharmacology. 2005; 41(12):1310-1318. https://doi.org/10.1177/009127001220128896 PMid:11762558

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The Association between Obesity and Severity of Dengue Hemorrhagic Fever in Children at Wangaya General Hospital

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Keywords: Obesity; Children; Dengue hemorrhagic fever; Severity

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Abstract

BACKGROUND: Dengue is a mosquito-borne disease caused by any one of four closely related Dengue virus (DENV 1-4). The clinical sign Dengue virus infection can vary from mild (mild febrile illness), Dengue Fever (DF), Dengue Hemorrhagic Fever (DHF) to Dengue Hemorrhagic Fever with shock (Dengue Shock Syndrome, DSS).

AIM: This study was designed to determine the relationship of obesity with the severity of Dengue Hemorrhagic Fever in children.

METHODS: It is a case-control study. The data of patients were retrospectively collected from the Department of Child Health at the Wangaya General Hospital between March 2019 to May 2019. It uses consecutive sampling. The total sample of 22 children with DHF with shock and 22 children with DHF without shock were investigated. Statistical analysis has been performed by SPSS Statistics 20.0 for Mac (IBM Corp., Armonk, New York, USA). DHF positive results were compared by the Chi-square test and binary logistic regression.

RESULTS: Prevalence of DHF with shock is fifty per cent's and DHF without shock is 50%. Prevalence of obesity is 40.9%. The result of binary logistic regression analysis of obesity in children and the severity of DHF was significantly correlated with P-value 0.004 and OR = 7.734.

CONCLUSION: Obesity is associated with the severity of Dengue Hemorrhagic fever in children.

Introduction

Dengue is a mosquito-borne disease caused by any one of four closely related *Dengue virus* (DENV 1-4). The clinical sign *Dengue virus* infection can vary from mild (mild febrile illness), Dengue Fever (DF), Dengue Hemorrhagic Fever (DHF) to Dengue Hemorrhagic Fever with shock (Dengue Shock Syndrome, DSS). DHF is an infection commonly found in tropical countries such as Indonesia.

This Dengue infection usually affects children < 15 years old with a high mortality rate [1]. In 2009, Indonesia had the most case of DHF in Southeast Asia. In 2015, the top 3 provinces with a high rate of DHF were Bali (208.7 per 100 thousand population), East Kalimantan (183.12 per 100 thousand population) and Southeast Kalimantan (120.08 per 100 thousand population) [2]. Several risk factors associated with the Dengue severity in children and one of them is obesity. In DHF, there is a plasma leakage which can lead to hypovolemic shock that causes DSS. In an obese patient, there is an increase in the production of interleukin and Tumor Necrosis Factors (TNF). One of the effects of TNF is increased capillary permeability; therefore, the increased capillary permeability was higher and eventually will lead to DSS because of massive plasma leakage [3]. From study of Devi et al., in 2015, there is a relationship between obesity and Dengue severity, and also Elmy in 2008 have found that obesity is the risk factor of DSS, but from the study of Sugiyanto et al. and study in Thailand there is no relationship between obesity and Dengue severity [3].

This study was designed to determine the

relationship of obesity with the severity of Dengue Hemorrhagic Fever in children.

Material and Methods

Study Design

This study is an observational study with a case-control design. The data were retrospectively collected from the Department of Child Health at the Wangaya General Hospital between March 2019 to May 2019. This study consists of 44 serum sample. This study uses consecutive sampling. Subjects were divided into two groups. First group, the control group consist of subjects with DHF without shock (grade I or grade II), positive tourniquet test, 2 – 7 days of fever, thrombocytopenia and positive signs of plasma leakage such as increased hematocrit, or having pleural effusion, or ascites. Second group, the case group included patient diagnosed with DHF with shock (grade III or grade IV), who meet the criteria of DHF grade I or grade II plus sign of shock, such as weak pulse, narrowing pulse pressure, poor tissue perfusion, clammy skin, and decreased urine output according to WHO criteria [4]. Obesity was assessed with BMI for age p > 85 according to CDC growth chart [5].

Inclusion and Exclusion Criteria

Inclusion criteria: All inpatient with DHF grade I–IV in the Department of Child Health at the Wangaya General Hospital.

Exclusion criteria: Patients with severe malnutrition and congenital heart disease.

Statistical Analysis

Data were presented in distribution tabulation, and data analysis was performed with a computer-assisted statistical package (SPSS 20 for Mac). Bivariate data was analysed with Chi-square and multivariate analysis with binary logistic regression.

Ethics Statement

The study was performed following the principles of the Declaration of Helsinki. Participation in the study was fully voluntary and anonymous, and was approved by the Ethics Committee for Medical Research of Wangaya General Hospital.

Results

We included 44 children in this study which consist of 22 (50%) children of DHF without shock and 22 (50%) children of DHF with shock. The main characteristics of the children of both groups are shown in Table 1.

Table 1: Main Characteristics of the Study

Characteristics	Frequency, n (%)
Gender	
Male	28 (63.6%)
Female	16 (36.4%)
Obese	
Yes	18 (40.9%)
No	26 (59.1%)
Shock	
Yes	22 (50%)
No	22 (50%)
Age, years	11.11 (4.271) Min: 3, Max: 17
Body Weight	41.76 (20.050) Min: 12, Max: 99

Statistical analysis was performed to identify the association between obesity and Dengue severity (in this case, DSS). Statistical analysis revealed that there is an association between obesity and DSS with a p-value of 0.004 (< 0.05) and OR of 7.734 meaning it is the risk factor of DSS with OR value > 1. The result of bivariate and multivariate analysis is shown in Table 2.

Table 2: Results of the statistical analysis

	Shock, n (%)	No Shock, n (%)	Unadjusted (bivariate analysis chi-square)			Adjusted (multivariate analysis, binary logistic regression)		
			p value	OR	CI 95%	p-value	OR	CI 95%
Obese	14 (63.6%)	4 (18.2%)						
Normal	8 (36.4%)	18 (81.8%)	0.005	7.875	1.964-31.574	0.004	7.734	1.910-31.321

Discussion

This study found out that obesity is associated with Dengue severity (it is the risk factor of DSS). This finding was supported by the theory that obesity may affect the severity of dengue infection due to the increased production of white adipose tissue which causes increased inflammation mediator production. These inflammation mediators were TNF α *(tumour necrosis factor* α) and several interleukins (IL) such as IL-1 β, IL-6, and IL-8. In obese children, there are increasing of TNF α dan IL-6. This inflammation mediator will increase the permeability of the capillaries. Subsequently, progressive plasma leakage leads to a higher risk of DSS [4]. The results of this study was also supported by previous studies such as Ridha in 2018 with p-value of 0.000 [6]; Elmy et al., in 2009 with p-value of 0.009 with OR of 4.927 [3]; and from Chuansumrit et al., that show children with > 50th percentile body weight for age were more likely to have grade III and grade IV DHF than those with lesser body weight with P-value = 0.039 [7]. Those studies above stated that obesity was associated with

severity of dengue and therefore becoming a DSS risk factor. But this study contradicted with the study from Maria et all in 2013 that result in obesity is not a risk factor or associated with the Dengue severity with P-value = 0.07 [1]. And also contradicted with the study from Tantri in 2017 with P-value = 0.309 meaning there is no association between obesity with Dengue severity [8].

From this study, we can see that obesity is associated with the severity of Dengue Hemorrhagic fever in children. Obese children have a higher risk of shock when experiencing dengue hemorrhagic fever. From this study, we can predict the prognosis of obese children when experience DHF; therefore, we can anticipate and prevent obese children from went into shock when diagnosing with DHF.

References

1. Permatasari DY, Ramaningrum G, Novitasari A. Association of nutritional status, age, and gender with severity of dengue hemorrhagic fever in children. Journal Kedokteran Muhammadiyah. 2015; 2(1):24-28.

2. Pusat Data dan Informasi Kementerian Kesehatan RI. Dengue Hemorrhagic Fever status. Indonesia: InfoDATIN; 2016.

3. Elmy S, Arhana BNP, Suandi IKG, Sidiartha IGL. Obesity as risk factor of dengue shock syndrome. Sari Pediatri. 2009; 11(14):238-243.

4. Widiyati MMT, Laksanawati IS, Prawitohartono EP. Obesity as a risk factor for dengue shock syndrome in children. Paeditrica Indonesia. 2013; 53(4):187-192. https://doi.org/10.14238/pi53.4.2013.187-92

5. Centers for disease control and prevention. Nutritional status Indicators. CDC. 2016 March (cited 2019 May). Available from: https://www.cdc.gov/nccdphp/dnpao/growthcharts/training/overview/page5_1.html

6. Hanifah R, Darmawan MTS, Febriani TB. Correlation of nutritional status with Dengue Shock Syndrome in children 0-14 years old in PKU Muhammadiyah Bantul Hospital from January-December, 2016-2017. Jogjakarta: Islam Indonesia Univerity, 2018.

7. Chuansumrit A, Phimolthares V, Tardtong P, Tapaneya-Olam C, Tapaneya-Olam W, Kowsathit P, et al. Transfusion requirements in patients with dengue hemorrhagic fever. Southeast Asian J Trop Med Public Healt. 2000; 31: 0-14.

8. Safti TM. Association between obesity and severity of Dengue Hemorrhagic Fever in children. Faculty of Medicine Muhammadiyah Surakarta Univeristy; 2017.

ID Design Press, Skopje, Republic of Macedonia
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Transvenous Lead Extraction of Cardiac Implantable Electronic Devices Indications, Complications and Outcome: An Egyptian Two Years' Experience

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Abstract

BACKGROUND: The growing needs to extract cardiovascular implantable electronic devices warrants the need to improve the outcome and prevent complications.

AIM: This study aims to analyse the findings and identify factors associated with complications of Percutaneous Transvenous Lead Extraction in the Critical Care Department, Cairo University.

METHODS: We studied 52 candidates for Percutaneous Transvenous Lead extraction of a Permanent Pace Maker (PPM) regarding extraction indications, comorbidities, device type, complications and outcome. Extraction was first attempted by simple manual traction using regular non-locking stylet and if failed, locking stylet, and evolution dilator sheath were used.

RESULTS: We extracted 110 leads with a mean lead age of 4.67 ± 3.6 years. The most common extraction indication was an infection (71.15%). Indications correlated significantly with comorbidities ($p = 0.024$), the most common being Diabetes Mellitus (40.38%). Simple traction was successful in 31 % of the leads, while 69% were extracted using locking stylet and evolution dilator sheath. The method of lead extraction correlated significantly with lead age ($P \leq 0.001$). Complications were significantly higher with extraction by evolution dilator sheaths than by simple traction ($P = 0.003$) and in older patients ($P = 0.008$). Complications also correlated significantly with extractions indications ($p = 0.012$), type of PPM ($P = 0.037$), number of extracted leads ($P = 0.041$), and lead age ($p= 0.011$).

CONCLUSION: Among the studied variables, extraction indications particularly infection, was the only preventable factor significantly associated with complications. While focusing on preventable factors, improving, implantation and extraction techniques should also be addressed.

Introduction

In recent years, the number of Cardiac Implantable Electronic Device (CIED) complications has increased as a result of the increasing implantations. Concomitantly a rise in lead malfunctions and recalls accompanying the complex CIED procedures in high-risk patients has resulted in increased Transvenous Lead Extractions (TLE) [1], [2], [3], [4], [5]. Lead extraction is by far a complex procedure as it carries some risks for the patient [6], [7].

Age, sex, body mass index, lead implant duration, number of leads, even small calcifications or fibrosis along with the lead, venous thrombosis, systemic infection, presence of large vegetations, pacemaker dependency, and physical characteristics of the lead, e.g. intrinsic fragility are all factors associated with increased procedural risk. Infections, lead malfunction, actual or potential lead failure, interference between multiple leads, lead-induced life-threatening arrhythmias, lead interference with malignancy treatment, chronic pain and subclavian vein/superior vena cava thrombosis are clinical situations most commonly requiring lead extraction to be completely alleviated [6], [7].

Methods

From March 2016 to February 2018, 52 candidates for percutaneous Transvenous Lead Extractions (TLE) of Cardiac Implantable Electronic Devices (CIED), were enrolled in a prospective study conducted in Critical Care Department in Kasr Al-Ainy Hospital, Cairo University, Egypt. We aimed to analyse the findings and identify factors associated and can be predictive of complications of Percutaneous Transvenous Lead Extraction.

Patients were enrolled in the current study according to the Heart Rhythm Society (HRS) indications (2009) for lead extraction classes I & IIa [7]. All patients had undergone full medical history taking, body mass index (BMI) assessment, recording of indication, type, and functional status of CIED and indication of lead extraction. All patients were also subjected to full clinical examination & investigations in the form of full laboratory tests including blood culture and sensitivity, wound swab and blood typing, 12-lead surface ECG, Chest x-ray, Trans-thoracic echocardiography (TTE). Transesophageal echocardiography (TEE) was performed in every patient with suspected CIED-related infection to assess vegetations' place and size if present (Figure 1).

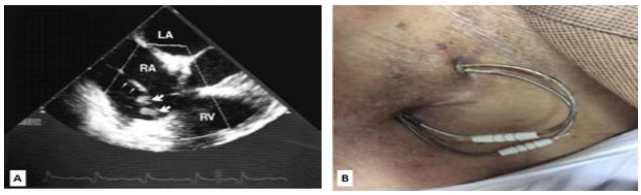

Figure 1: A patient with infective endocarditis secondary to pocket infection. A: TEE showing two large masses (bold arrows) attached to the pacemaker lead, B: A closed sinus of infected pocket and leads extrusion before extraction in our study

Lead extraction procedure

Following the induction of anaesthesia, proper skin preparation and sterile draping of the patient, a Temporary Pacing Lead was placed in the right ventricle via the right femoral vein. Invasive arterial blood pressure monitoring was connected via the right radial artery. Local anaesthesia and incision at the site of the pocket were done under deep sedation and analgesics. Extraction of the battery was done if present. Stylet wires were used to stiffen leads for removal and minimising the chance of lead breakage during traction and assist sheath advancement, which results in the extraction of a greater amount of intact lead. *Simple traction* Technique was initially tried for all patients in which manipulation of the lead was done so that the lead exits the vasculature via the implant vein using tools typically supplied for the lead implant, with the addition of traction. If the simple traction failed, traction with the help of Locking stylets and Evolution mechanical dilator sheath was attempted. A locking stylet is a special type of a traction device, designed to hold onto the inside of the conductor coil along its length or near the distal stimulating electrode. It improves the tensile properties and prevents elongation of the lead body during traction. We used Liberator *(Cook Vascular)* locking stylet.

Finally, after extraction of all leads, complete removal of all granulation tissue and necrotic material was done with adequate haemostasis and closure of the wound by properly interrupted suture.

Patients were kept postoperatively in the Cardiac Care Unit (CCU) under strict monitoring of vital signs, haematocrit and other labs with the adequate replacement of any blood loss during or after the procedure. Post-procedure chest X-ray & TTE were done within a few hours to detect any complications of TLE, *e.g. pericardial effusion*. Proper antibiotic therapy was instituted, and revision of the need for CIED reimplantation was done.

Procedure success was classified as; (1) Complete procedural success: if all targeted leads and lead material were removed from the vascular space, (2) Clinical success: if all targeted leads and lead material was removed, but with retention of a small portion of the lead that does not negatively affect outcome goals, or (3) Failure: if neither complete procedural nor clinical success could be achieved[(7)]. Complications were classified into *Intraprocedural*: Any event related to the performance of the procedure that occurs or becomes evident in the Cath. Lab. *Postprocedural:* Any event related to the procedure that occurs or becomes evident within the 30 days following the procedure. Events are classified as major or minor according to severity: *Major complications:* Those that create a life-threatening situation or require a major intervention for their resolution or result in death, e.g. cardiac avulsion and vascular tear requiring surgical repair. Then *Minor complications:* not life-threatening, but require an intervention, such as medication, for their resolution, e.g. pericardial effusion not requiring pericardiocentesis.

The study was approved by the ethical committee of the critical care department, Cairo University. Written informed consent was signed by the patient or his/her family.

Statistical Analysis

Data were coded and entered using the statistical package SPSS (*Statistical Package for the Social Sciences*) version 24. Data were summarised using mean, standard deviation, median, minimum and maximum in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. Comparison between quantitative variables was made using the non-parametric Kruskal-Wallis and Mann-Whitney tests [8]. For

comparing categorical data, Chi-square (χ^2) test was performed. Exact test was used instead when the expected frequency is less than 5 [9]. P-values less than 0.05 were considered as statistically significant.

Results

In total, 52 patients were included in our study; the baseline characteristics were shown in Table 1. Thirty-one males (59.6%) and 21 females (40.4%) with a mean age of 53.5 ± 14.1 years, the mean left ventricular ejection fraction (LVEF) was 51.7 ± 13.4%. The mean Body Mass Index (BMI) was 26.04 ± 3.95. Indications for TLE were infective in 37 patients (71.15%) and non-infective in 15 patients (28.85%). A total of 110 leads were extracted via the venous entry approach from 52 patients with a mean number of leads extracted per patient was 2.1. They included 106 pacing leads (96.36%) and 4 shock leads with dual coils (3.64%).

Table 1: Demographic and descriptive characteristics

Item		Number (%)
Age (mean)		53.56 ± 14.12
Sex	Male	31 (59.6%)
	Female	21 (40.4%)
Left Ventricle EF%		51.73 ± 13.47
Body Mass Index (mean)		26.04 ± 3.95
Indications	Infective indications	37 (71.15%)
	Pocket infection	26 (50%)
	Infective Endocarditis	11 (21.15%)
	Non-infective indications	15 (28.85%)
	Lead malfunction	7 (13.46%)
	Lead displacement or extrusion	5 (9.61%)
	Upgrading	3 (5.76%)
Type of implanted device	DDD	33 (63.5%)
	CRT-P	10 (19.2%)
	CRT-D	4 (7.7%)
	VVI	5 (9.6%)
Leads implanted	Total	110
	Active fixation	88 (80%)
	Passive fixation	22 (20%)
	Atrial leads	44 (40%)
	RV leads	48 (43.64%)
	Coronary sinus	14 (12.73%)
	Shock leads	4 (3.63%)
Extraction Technique	Simple traction	34 (31%)
	Evolution traction	76 (69%)
Mean implant duration (years)		4.67 ± 3.61
Mean procedure duration (minutes)		81.73 ± 38.4

Fixation mechanisms did not affect the method of TLE and its outcome. The method of the lead extraction showed a statistically significant relationship with the lead implant duration *(P-value < 0.001)* as shown in Table 2, where simple traction was more successful with shorter lead age. Also, the longer the leads' age, the longer was the procedure duration with a *P-value of < 0.001*.

Table 2: Method of extraction vs lead age

	Method of extraction				P-value
	Simple Traction		Evolution		
	Mean	SD	Mean	SD	
Lead age (years)	1.53	1.80	6.48	3.11	< 0.001

In our study, complications of TLE occurred in 28 patients (53.8%) and most of them were minor complications and managed conservatively and only one major complication (subclavian vein tear) which required surgical repair by a vascular surgeon.

The most common complication was hematoma formation in the pocket site (21.1%) and managed by proper compression and medical treatment after vascular surgery consultation. Patients with mild and moderate pericardial effusion (26.9%) were managed conservatively without the need for tapping. In our study, the rate of occurrence of complications was more in older age with a *P-value 0.008* where the mean age in the complicated patients was 58.43 ± 11.1 years, and the mean age in the non-complicated patients was 47.88 ± 15.25 years. The rate of complications showed statistically significant difference according to the method of extraction where complications were more in Evolution method with a P-value 0.003, as shown in Table 3.

Table 3: Complications

Parameter		Complications		P
		yes	No	
Extraction indications	Pocket Infection N (%)	14 (53.8%)	12 (46.2%)	0.012
	Infective endocarditis N (%)	10 (90.9%)	1 (9.1%)	
	Upgrade to ICD N (%)	1 (50.0%)	1 (50.0%)	
	Upgrade to CRT-D N (%)	1 (100%)	0 (0%)	
	Not connected old Lead extrusion N (%)	0 (0%)	1 (100%)	
	Malfunctioning RV lead N (%)	1 (20.0%)	4 (80 %)	
	Malfunctioning atrial lead N (%)	0 (0%)	2 (100%)	
	Lead displacement N (%)	1 (25.0%)	3 (75.0%)	
Patient's Age (Mean± standard deviation)		58.43 ±11.19	47.88±15.25	0.008
Lead Age (Mean± standard deviation)		5.73 ± 3.47	3.44 ± 3.44	0.011
Method of extraction	Simple Traction N (%)	5 (26.3%)	14 (73.7%)	0.003
	Evolution Method N (%)	23 (69.7%)	10 (30.3%)	
Type of PPM	VVI N (%)	2 (40.0%)	3 (60.0%)	0.037
	DDD N (%)	14 (42.4%)	19 (57.6%)	
	CRT-P N (%)	8 (80.0%)	2 (20.0%)	
	CRT-D N (%)	4 (100.0%)	0 (0 %)	
No. of extracted leads	1 N (%)	4 (36.4%)	7 (63.6%)	0.041
	2 N (%)	11 (44.0%)	14 (56.0%)	
	3 N (%)	12 (80.0%)	3 (20.0%)	
	4 N (%)	1 (100%)	0 (0%)	

The number of extracted leads was directly proportional to the occurrence of complications with a *P-value 0.041*. There was a statistically significant difference regarding the occurrence of complications and the type of CIED and the extracted leads *(P-value 0.037)*, where the percentage of complications in the form of subclavian vein tear, pericardial effusion and hematoma formation was more with extraction of shock leads in CRT-D devices due to presence of large and thick fibrous bands at shock lead binding sites, e.g. SVC coil as shown in Table 3. Our study showed that longer lead implant duration (lead age) reflects a higher incidence of complications of TLE with a *P-value 0.011,* as shown in Table 3.

Regarding procedure success, clinical success was achieved in all patients (52 patients) while complete procedure success (radiological and clinical) was achieved in 48 patients (92.3%) where a retained part of RV lead occurred in 4 patients (7.7%) and did not affect the patient outcome.

Discussion

In a European survey conducted by the European Heart Rhythm Association (EHRA) in 2012, the success rates and complications of TLE techniques showed that TLE was still under-developed across European countries with divergent practices between centres [3], [10]. *In our Critical Care Department, Cairo University*, the rate of CIED implantation increased over the years. This increase in rate was associated with increased risk of complications requiring TLE, especially with multiple generator replacements.

Many studies have been done in the last 10 years on CIED lead extraction techniques. Regarding indications of TLE, the most common indications of extraction in our patients were infection in 71.15% of patients (50% local and 21.15 % lead vegetations) while indications were non-infective in 28.85% of patients. In agreement with our study, *Andrzej et al.,* [11], also found that the most common indication for TLE was an infection. Similarly, indications of extraction in *Stylianos P et al.,* [12] study was an infection in 69.4% of the patients and led dysfunction in 30.6% of the patients. While in the EHRA survey 2012, the infection was the indication in 70% of the patients and non-infective indications in 30% of the patients [10]. In ELECTRa, the infection was the indication in 52.8% of the patients and non-infective indications in 47.2% of the patients [10]. On the other hand, in *Oto et al.,* [13] study in Turkey, the indications for lead extraction were primarily due to lead malfunction in 65.2% of the patients, cardiac device infection only in 30.4% of the patients and lead displacement in 4.4% of the patients.

In our study, there was no effect of lead fixation mechanisms on the method of extraction or the outcome of the procedure, only the *Andrzej et al., the* study stated that modern leads with active fixation could be easily extracted by traction [11].

The method of lead extraction showed a statistically significant relationship with the age of the lead (P-value < 0.001). The more the age of lead implantation; the more the difficulty of extraction by simple traction technique and the leads were extracted by locking stylet and evolution dilator sheath.

In *Andrzej et al*., [11] study, 19.5% of leads were removed by simple traction while other leads (80.5 %) were removed using the Lead Extraction System with the rotational cutting force only, with no laser or RF energy. In three cases in which breakage of the lead occurred, the remaining part of the broken lead was removed via a femoral approach.

Byrd et al., [14] reported that incomplete / failed extraction was more likely to occur in younger patients as the extent and quality of the scar tissue formed around the leads are inversely related to the age. However, our study failed to express this relation as younger patients were not significantly represented in our cohort (P-value 0.171).

In our study, older leads were associated with significantly prolonged procedure time compared to other leads (P-value < 0.001) and associated with a higher incidence of complications of TLE with a *P-value 0.011* with no effect on patient outcome. However, *Stylianos et al., a* study [12], [15] noted that lead age per se did not hurt lead extraction success rate or occurrence of complications.

Regarding complications of TLE, they occurred in 28 patients (53.8%) and most of them were minor complications and managed by conservative measures, the most common was hematoma formation (21.15% of patients), mild and moderate pericardial effusion and retained parts of leads and only one major complication (1.9% of patients) in which subclavian vein tear occurred and required surgical repair by a vascular surgeon. In *Andrzej et al., a study* [11], major complications occurred in 4 % of patients in the form of massive pericardial effusion, massive pulmonary embolism and papillary muscle rupture. While in *Oto et al.,* [13] study, minor complications occurred in 4.3 % of patients in the form of a hematoma. While in the *EHRA survey* [10], all 81 centres revealed major complications in their experience, where 10% of the centres experienced a death rate of 0.5-2% of patients. Other major complications like massive pericardial effusion requiring pericardiocentesis, vascular avulsion and hemothorax requiring chest tube, occurred in about 1-5 % of patients in all centres. In our study, the rate of occurrence of complications showed a statistically significant relationship with the type of CIED and the extracted leads *(P-Value 0.037),* where complications in the form of moderate pericardial effusion, subclavian vein tear and hematoma formation were more with the extraction of shock leads in CRT-D devices.

Our procedure success rate was high and comparable to other centres and in comparison, with the other studies, our rate and type of complications were relatively less, in spite of the relatively new experience of different methods of extraction in our department and restricted budget. This was most probably attributed to the good patient preoperative preparation and the skilled operator technique who was trained in a large centre of extraction in Europe.

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References

1. Diemberger I, Mazzotti A, Biffi M, Massaro G, Martignani C, Ziacchi M, Reggiani ML, Battistini P, Boriani G. From lead management to implanted patient management: systematic review and meta-analysis of the last 15 years of experience in lead extraction. Expert review of medical devices. 2013; 10(4):551-73. https://doi.org/10.1586/17434440.2013.811837 PMid:23895081

2. Greenspon AJ, Patel JD, Lau E, Ochoa JA, Frisch DR, Ho RT, et al. Trends in permanent pacemaker implantation in the United States from 1993 to 2009: Increasing complexity of patients and procedures. J Am Coll Cardiol. 2012; 60(16):1540-5. https://doi.org/10.1016/j.jacc.2012.07.017 PMid:22999727

3. Bongiorni MG, Marinskis G, Lip GY, Svendsen JH, Dobreanu D, Blomström-Lundqvist C, conducted by the Scientific Initiative Committee, European Heart Rhythm Association. How European centres diagnose, treat, and prevent CIED infections: results of an European Heart Rhythm Association survey. Europace. 2012; 14(11):1666-9. https://doi.org/10.1093/europace/eus350 PMid:23104858

4. Bongiorni MG, Blomstrm-Lundqvist C, Kennergren C, Dagres N, Pison L, Svendsen JH, et al. Current practice in transvenous lead extraction: A European Heart Rhythm Association EP Network Survey. Europace. 2012; 14(6):783-6. https://doi.org/10.1093/europace/eus166 PMid:22622992

5. Deharo JC, Bongiorni MG, Rozkovec A, Bracke F, Defaye P, Fernandez-Lozano I, et al. Pathways for training and accreditation for transvenous lead extraction: A European Heart Rhythm Association position paper. Europace. 2012; 14(1):124-34. https://doi.org/10.1093/europace/eur338 PMid:22167387

6. Love CJ, Wilkoff BL, Byrd CL, Belott PH, Brinker JA, Fearnot NE, et al. Recommendations for Extraction of Chronically Implanted Transvenous Pacing and Defibrillator Leads: Indications, Facilities, Training. Pacing Clin Electrophysiol. 2000; 23(4):544-51. https://doi.org/10.1111/j.1540-8159.2000.tb00845.x PMid:10793452

7. Wilkoff BL, Love CJ, Byrd CL, Bongiorni MG, Carrillo RG, Crossley GH, et al. Transvenous Lead Extraction: Heart Rhythm Society Expert Consensus on Facilities, Training, Indications, and Patient Management. This document was endorsed by the American Heart Association (AHA). Hear Rhythm. 2009; 6(7):1085-104. https://doi.org/10.1016/j.hrthm.2009.05.020 PMid:19560098

8. Chan YH. Biostatistics 102: Quantitative Data - Parametric & Non-parametric Tests. Singapore Med J. 2003; 44(8):391-6.

9. Chan YH. Biostatistics 103: qualitative data-tests of independence. Singapore Med J. 2003; 44(10):498-503.

10. Bongiorni MG, Blomström-Lundqvist C, Kennergren C, Dagres N, Pison L, Svendsen JH, Auricchio A. Current practice in transvenous lead extraction: a European Heart Rhythm Association EP Network survey. Europace. 2012 Jun 1;14(6):783-6. https://doi.org/10.1093/europace/eus166 PMid:22622992

11. Kutarski A, Malecka B, Rucinski P, Zabek A. Percutaneous extraction of endocardial leads - A single centre experience in 120 patients. Kardiol Pol. 2009; 67(2):149-56.

12. Paraskevaidis S, Konstantinou D, Vassilikos V, Theofilogiannakos E, Mantziari L, Megarisiotou A, et al. Percutaneous extraction of transvenous permanent pacemaker/defibrillator leads. Biomed Res Int. 2014; 2014. https://doi.org/10.1155/2014/949785 PMid:24971363 PMCid:PMC4058177

13. Oto A, Aytemir K, Yorgun H, Canpolat U, Kaya EB, Kabakçi G, et al. Percutaneous extraction of cardiac pacemaker and implantable cardioverter defibrillator leads with evolution mechanical dilator sheath: A single-centre experience. Europace. 2011;13(4):543-7. https://doi.org/10.1093/europace/euq400 PMid:21084359

14. Byrd CL, Wilkoff BL, Love CJ, Sellers TD, Reiser C. Clinical study of the laser sheath for lead extraction: the total experience in the United States. Pacing Clin Electrophysiol. 2002; 25(5):804-8. https://doi.org/10.1046/j.1460-9592.2002.t01-1-00804.x PMid:12049372

15. Bongiorni MG, Kennergren C, Butter C, Deharo JC, Kutarski A, Rinaldi CA, et al. The European Lead Extraction ConTRolled (ELECTRa) study: A European Heart Rhythm Association (EHRA) Registry of Transvenous Lead Extraction Outcomes. Eur Heart J. 2017; 38(40):2995-3005. https://doi.org/10.1093/eurheartj/ehx080 PMid:28369414

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The Possible Associations of Nasal Septal Deviation with Mastoid Pneumatization and Chronic Otitis

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Abstract

BACKGROUND: The nasal septum deviation is the most common deformity of the nasal, and that can be congenital or acquired. Despite many studies exist about the impact of nasal septum deviation on chronic sinusitis and also association between chronic otitis and mastoid pneumatization; few studies exist about the impact of nasal septum deviation on chronic otitis and mastoid pneumatization.

AIM: The aim of this study was to evaluate the associations of nasal septum deviation and mastoid pneumatization and chronic otitis.

METHODS: In this study review, all CT scans of PNS and Mastoid View in the imaging section from Imam Ali hospital in 2016-2017 years and cases of nasal septum deviation were enrolled. The nasal septum deviation was recorded, and the degree of nasal septum deviation in the coronal plane that showed the maximum deviation of the nasal septum was recorded. The volume of the mastoid cells automatically and directly was calculated using three diameter measurements (2 coronal diameters and 1 axial diameter) by the program. The software of SPSS 22 was used for statistical analysis.

RESULTS: There was no relationship between nasal septum deviation severity and incidence of mastoid pneumatization in patients with nasal septum deviation ($P > 0.05$). There was relationship between nasal septum deviation severity and chronic otitis in patients with nasal septum deviation ($P < 0.05$). In patients with moderate and severe intensity of nasal septum deviation, the volume of mastoid air cells in deviation side was lower than the front side ($P < 0.05$).

CONCLUSION: Based on the results of the CT scan, in patients with moderate and severe nasal septum deviation intensity, the volume of mastoid air cells in deviation side was lower than the front side. Also, there was a relationship between nasal septum deviation severity and chronic otitis.

Introduction

Nasal septum deviation is the most common nasal deformity that can be congenital or acquired abnormality. The prevalence of nasal septal deviation based on computer tomography (CT scan) is reported to be 40% [1]. There was a *relatively high prevalence* of nasal deviation, and its possible complications include sinusitis, nasal obstruction, meningitis, cavernous sinus thrombosis (CST), etc.

Nasal septum deviation and its complications in the early stages are visible in the imaging and can be easily treated at this stage [2]. The temporal bone consists of three parts, and the bone segments are separated at birth, and then combined into a single bone. The mastoid is a part of the temporal bone that lies behind the ear and at birth is a cavity at the centre of the bone, but within 2 years, many air cells develop in the mastoid by a process termed pneumatization. Compression of mastoid cells begins at the thirty-third week of pregnancy and continues until the age of 9-8.

The *mastoid antrum* is an air space at the petrous part of the temporal bone, which reaches their adult maturity at the 35th week of pregnancy [3]. Full development of pneumatization can be divided into three stages: the period of childbirth from birth to two years, the transitional period from 2 to 5 years and adulthood [4]. There are two theories about the pneumatization of mastoid air cells. The first theory, “genetic theory”, emphasises on the genetical extent of pneumatization. The "environmental theory" is based on the fact that pneumatization of mastoid air

cells depends on postnatal pathologies and involvement of the middle ear, where the pneumatization can be decreased via middle ear inflammation or tubal dysfunction.

According to this theory, any factor that changes the pressure of the middle ear is effective in the level of pneumatization of mastoid air cells. For example, a deviation of nasal septum causes a change in the amount of air passing through the nose. Regarding the relationship of the nasopharynx to the middle ear through the Eustachian tube, this change in the airflow rate causes a change in the pressure of the middle ear and therefore affects the pneumatization of the mastoid [6], [7].

Due to the proximity of the mastoid with the middle ear, middle ear infections can easily inflammation mastoid and cause some degree of mastoiditis associated with middle ear infections. Several studies have been conducted to determine the relationship between middle ear disease and *mastoid air* cell *system*.

High air in the *mastoid air* cell *system* is a dangerous factor for the development of multiple diseases of the middle ear [8]. Ear diseases during infancy and early childhood can stop the normal flow of air into the mastoid. This process may cause problems in the *mucous* membrane of the *ear*, which will make it more susceptible to recurrent infections than normal ear. Most patients with chronic suppurative otitis have a small, cell-free *mastoid* in temporal bone radiography.

Chronic suppurative *otitis media* is one of the most common chronic infectious diseases all over the world, which affects not only developing societies but also industrial societies [9].

In spite of many studies on the effect of nasal septum deviation on chronic sinusitis and association of mastoid pneumatization with chronic otitis media, few studies have been conducted regarding the effect of nasal septum deviation on mastoid pneumatization or chronic otitis media, while its impact has not been proven yet.

Therefore, the current study was aimed to determine the effect of nasal septum deviation with pneumatization of mastoid cells and its possible association with chronic otitis media.

Material and Methods

This prospective cross-sectional study was conducted at Imam Ali Hospital in Zahedan between 2016 and 2017.

The study population included all paranasal sinuses (*PNS*) scans and mastoid views, which were archived in the imaging section. The inclusion criteria were the presence of *deviated septum* in radiography films. Exclusion criteria were: 1- Presence of history of nasal sinus, middle ear and mastoid surgery, 2- the presence of nasal polyp, 3- presence of cleft palate or other congenital anatomical disorders [12]. The sample size was determined as 50 people in each group. Finally, 150 people were enrolled in this study.

The *nasal deviation direction* and *the angle of septal deviation (ASD)* in coronal sections were recorded, which showed the maximum deviation of nasal septum. The *NSA* was defined as the angle between a line drawn from the crista Galli to the lower portion of the nasal septum in the maxillary spine and another line drawn from the upper nasal septum in the crista Galli to the most deviated point of the nasal septum. The patients were divided into 3 groups based on the *ASD*: 1- Mild (less than 9). 2-medium (between 15-9). 3. Severe (more than 15), [13].

The volume of mastoid cells was measured directly and automatically by measuring 3 diameters (two coronal diameters and one axial diameter) via certain program which is briefly called the 3DMPVR (*three-dimensional multiplanar rendering technique*), [14]. The size of the mastoid cells is defined as the volume of the air cells in the aditus and *antrum* located in the mastoid part of the temporal bone. Criteria for the definition of chronic otitis media in this study were: 1- bone destruction or sclerosis in the temporal bone, 2- massive fluid or structural changes in the temporal air cells [4].

Following the above-mentioned measures, the relationships between *ASD* and pneumatization of mastoid and chronic otitis were investigated.

In this study, data collection form was used to record variables. This form was completed on the basis of the information in the patient records. The data collection form included various variables such as age, sex, ASD, presence or absence of mastoid pneumatization, the volume of mastoid cells and chronic otitis media. Using *GE* Light Speed 16 Slice *CT Scanner*, PNS and mastoid View were visualised based on coronal and axial sections.

Data analysis

Statistical analysis was performed using *SPSS 22 software*. Descriptive statistics were used to calculate the mean and standard deviation of variables related to the characteristics of individuals and disease. Before analysing quantitative data, the normal distribution of data was assessed using KS test. The analysis was performed using Chi-square test for qualitative variables and T-test or variance analysis for quantitative variables. Similar nonparametric tests were used in the case of non-normal distribution. For all tests, a significant level of 0.05 was considered.

Results

In this study, 150 patients with nasal septal deviation were enrolled who referred to the radiology section of Imam Ali Hospital in Zahedan for CT scan. Fifty patients (33.3%) had mild nasal septum deviation, followed by moderate nasal septum deviation (50%, 33.3%), and severe nasal septum deviation (50%, 33.3%).

The age of the patients was determined to be between 18-72 years with a mean and standard deviation of 39.6 ± 14.07 years (Figure 1). Furthermore, 90 (60%) of the patients were male, and 60 (40%) were female. Also, *deviation* of *nasal septum* has been determined as to the right in 98 (65.3%) patients, and to the left in 52 (34.7%).

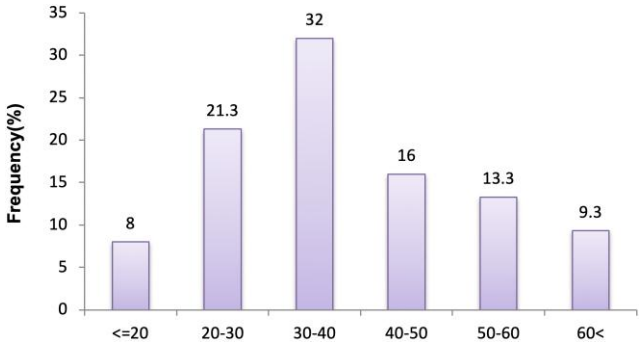

Figure 1: Distribution of patients' age

The *ASD* in patients was between 23.5 to 4.5 degrees with a mean and standard deviation of 12.27 ± 5.39 degrees (Figure 2).

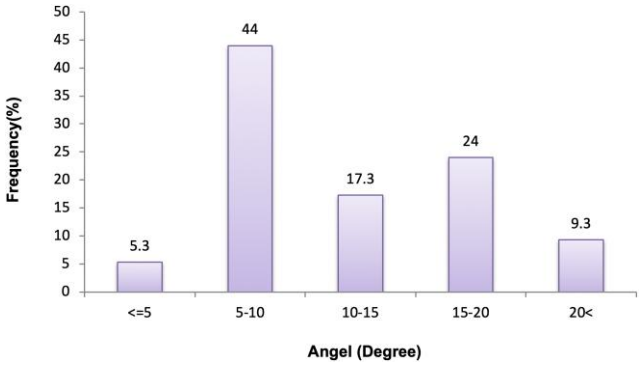

Figure 2: Distribution of the angle of the septal deviation (ASD) in patients

Our findings revealed that 146 (97.3%) patients had mastoid pneumatization and 4 (2.7%) had no mastoid pneumatization. Moreover, the direction of mastoid pneumatization was bilateral in 148 (98.7%), and two (1.7%) was unilateral. Additionally, 98 (65.3%) patients did not show chronic otitis media, and 52 (34.7%) revealed chronic otitis media.

Descriptive features and comparison of age, gender and direction of nasal septal deviation in 3 groups with the severity of nasal septal deviation are summarized in Table 1.

As indicated in Table 1, age, sex, and direction of deviation of the nasal septum in patients in 3 groups did not show a significant association with severity of nasal deviation (mild, moderate and severe), (P < 0.05). The frequency of gender profiles in patients with nasal septum deviation and the frequency of deviation.

Table 1: Descriptive characteristics and comparison of age, gender and direction of nasal septal deviation with the severity of nasal septal deviation

Property	Severity			P-Value
	Mild	Moderate	Severe	
Age (Year) Standard deviation ± Mean	42.28±13.58	39.44±11.41	37.08±16.78	0.289
Sex	26(% 52)	30(%60)	34(% 68)	0.513
Male	24(48%)	20(%40)	16(% 32)	
Female				
Direction for deviation of nasal	36(% 72)	28(%56)	34(%68)	0.465
Right	14(%28)	22(%44)		
Left			16(% 32)	

The occurrence of mastoid pneumatization in patients with a deviated septum

In order to compare the incidence of mastoid pneumatization in patients suffering from *deviated septum* with the severity of the nasal septal deviation, chi-square test was used (Table 2).

Based on the results of Table 2, there was no significant difference between the incidences of mastoid pneumatization in the three groups with severity nasal septum deviation.

Table 2: Frequency and comparison of the incidence of mastoid pneumatization with *septal deviation*

Property	The severity of nasal septal deviation		
	Mild Frequency (%)	Moderate Frequency (%)	Severe Frequency (%)
Mastoid pneumatization			
Yes	50 (100%)	50 (100%)	46 (92%)
No	0 (0%)	0 (0%)	4 (8%)

Frequency of chronic otitis media

Chi-square test showed that the severity of septal deviation was significantly correlated with chronic otitis media in patients with nasal septum deviation (P < 0.05, Table 3).

Table 3: Comparison of chronic otitis media with nasal *septal deviation* in 3 groups

Property	The severity of nasal septal deviation			Test statistic	P-Value
	Mild Frequenc y (%)	Moderate Frequenc y (%)	Severe Frequency (%)		
Chronic otitis				7/18	0/028
No	42 (84%)	32 (64%)	24 (48%)		
Yes	8 (16%)	18 (36%)	26 (52%)		

The difference in the volume of mastoid air cells on the side of the deviation and opposite side ***of patients***

Chi-square test showed that the volume of mastoid air cells in the side of the deviation and the

opposite side had a significant relationship with the severity of nasal septal deviation (mild, moderate and severe) ($P < 0.05$; Table 4). Therefore, it can be said that in patients with moderate and severe deviations, the volume of airborne mastoid cells on the side of the deviation is less than the opposite side.

Table 4: Comparison of the differences in the volume of mastoid air cells on the side of the deviation and the opposite side with the severity of nasal *septal deviation*

the volume of mastoid air cells	The severity of nasal septal deviation			Test statistic	P-Value
	Mild Frequency (%)	Moderate Frequency (%)	Severe Frequency (%)		
				6.98	0.03
Side deviation is less than the opposite side	24 (48%)	38 (76%)	40 (80%)		
Side deviation is more than the opposite side	26 (52%)	12 (24%)	10 (20%)		

Discussion

Our study revealed that there was no correlation between the severity of nasal septum deviation and the occurrence of mastoid pneumatization in patients with nasal septum deviation. Furthermore, there was a relationship between the severity of nasal septal deviation and chronic otitis media. In patients with moderate and severe deviation of nasal septum, the volume of mastoid cells in the side of the deviation is less than the opposite side. Consistent with our findings, Kapusuz Gencer et al. reported that there is a relationship between nasal deviation on both sides and chronic otitis media [4].

Chronic otitis exerted on the side with a deviation of septal nose. The results also showed that the mastoid air cell volumes tend to be larger at the contralateral side of the severe septum deviations [4]. Lee et al. evaluated the association pf nasal septal deviation with the volume of mastoid air cell pneumatization is a pediatric subject. They indicated that mastoid air cell volume of the deviated side was smaller in comparison with contralateral side, indicating nasal septal deviation is capable of influencing both aerations [15], this finding is in agreement with our results.

In a study by Raman et al., 2016, a significant correlation of septal spur with mastoiditis was found on the same side. Mastoiditis is significantly associated with sinonasal pathologies, and mastoiditis is linked to sinonasal pathologies, which this result was in line with our study [17].

Other study indicated a moderate but remarkable negative association between the ASD and volumes of ethmoid cell. Ethmoid cell volume on the ipsilateral side tends to be reduced by increasing the degree of nasal septal deviation compared with the contralateral side [18]. This can be due to the lack of development of the secondary mastoid air cells to the severe deviation of the nasal septum. Nasal septal deviation, concha bullosa, and Haller's cells do not show to play a key role in sinus's inferior pneumatization [16], which was in line with our findings.

During the development of paranasal sinuses, there are two active pneumatizations, first between birth and age 4, and second between 8 and 12 years. The stages of growth are amid the two stages [19]. Similar to the development of paranasal sinuses, the mastoid cell system has three stages. The first stage is rapid pneumatization during the first year of life, the second stage is linear pneumatization between 1 and 6 years old, and the third stage is a slow stage until puberty [20].

Some researchers have reported the relationship between paranasal sinuses and mastoid and found that the development of the ethmoid, mastoid and sphenoid occurs at the age of 3 to 14 years [21]. Another study indicated that maxillary sinus volumes increased in the contralateral side of the severe septum deviations. Furthermore, maxillary sinusitis on ipsilateral to the severe septum deviation has been found to be remarkably enhanced [22]. CT scan is a gold standard for evaluating chronic sinusitis. Awareness of various anatomical deviations for surgeons and radiologists is essential to avoid possible complications and to improve the success of therapeutic strategies [23]. In addition, CT scan images can be used to reconstruct the *axilla* designs for data collection. This method is quicker and less costly than the MRI, which provides more information about soft tissue than the bone. CT scans information is capable of showing more detail in the posterior sinuses [24]. One of the strengths of this study is to investigate the relationship between nasal septum deviation and mastoid pneumatization. However, a small number of similar studies have limited the possibility of comparing our study with other studies. Therefore, it is suggested that further studies be developed in the future. Finally, it seems that there was no relationship between the severity of the nasal deviation and the incidence of mastoid pneumatization based on CT scan in patients with nasal septum deviation.

There was a correlation between the severity of nasal deviation and chronic otitis media in these patients. In patients with moderate and severe deviation, the volume of mastoid cells in the side of the deviation is less than the opposite side. Therefore, it can be concluded that as the severity of septal deviation increases, the pneumatization of mastoid air cells showed decreasing trend. These air cells act on gas reserves so that the middle ear is less dependent on the eustachian tube to maintain a balance of pressure. Reducing their de-*airing* disturbs the ear mucus, and thus the affected ear becomes more susceptible to recurrent infections than the normal

ear.

In conclusion, regarding the high prevalence of nasal septum deviations and its complications and sometimes the effects on the inner and outer ear and auditory nerves, more attention should be given to this issue. Based on the finding presented herein, increasing the severity of nasal septal deviation is capable of increasing the likelihood of chronic otitis at a higher deviation side. There is a significant relationship between the severity of nasal septum deviation and the development of chronic otitis media and the percentage of the mastoid pneumatization. Early surgical repair of nasal septal deviation can prevent chronic otitis and its many complications particularly in cases where the severity of this deviation is moderate to severe. A careful study of the severity of nasal septum deviation in PNS CT scans can help ENT specialists to decide for early surgical repair of nasal septum deviation and to prevent the onset of chronic otitis media and many other complications.

References

1. Uygur K, Tuz M, Dogru H. The correlation between septal deviation and concha bullosa. Otolaryngol Head Neck Surg. 2003;129(1):33-6. https://doi.org/10.1016/S0194-5998(03)00479-0

2. Dewees S. Nose and paranasal sinuses. In: Cummings CHW, editor. Otolaryngology - head and neck surgery. Philadelphia : Mosby, 1998: 89-100.

3. Koc A, Karaaslan O, Ko T. Mastoid air cell system. Otoscope. 2004; 144:54.

4. Kapusuz Gencer Z, Ozkiriz M, Okur A, Karaoavus S, Saydam S. The Possible Associations of Septal Deviation on Mastoid Pneumatization and Chronic Otitis. Otology & Neurotology. 2013; 34(6):1052-57. https://doi.org/10.1097/MAO.0b013e3182908d7e PMid:23820794

5. Doyle W. The mastoid as a functional rate-limiter of middle ear pressure change. Int J Pediatr Otorhinolaryngol. 2007; 71:393-402. https://doi.org/10.1016/j.ijporl.2006.11.004 PMid:17174408 PMCid:PMC2905545

6. Sade j. The correlation ear aeration with mastoid pneumatization. the mastoid az a pressure buffer. Eur Arch Otorhinolaryngol. 1992; 249:301-4. https://doi.org/10.1007/BF00179376 PMid:1418937

7. Todd NW. Mastoid pneumatization in patients with unilateral aural atresia. Eur Arch Otorhinolaryngol. 1994;251:196-8. https://doi.org/10.1007/BF00628422 PMid:7917250

8. Mey K, SLrensen M, HomLe P. Histomorphometric estimation of air cell development in experimental otitis media. Laryngoscope. 2006; 116:1820-3. https://doi.org/10.1097/01.mlg.0000233540.26519.ba PMid:17003720

9. Sheikhi M, Yasrebi M, Torkzadeh A. Evaluation of the effect of nasal septum deviation on chronic sinusitis. J Isfahan Den Sch. 2011; 6(5):568-73.

10. Majidi M, Taheri A. Correlation between location of lateral sinus and sever of otitis media The Iranian Journal of Otorhinolaryngology. 2007; 18(46):175-80.

11. Tos M, Stangerup S, Andreassen U. Size of the mastoid air cells and otitis media. Ann Otol Rhinol Laryngol. 2000; 94(4):386-92.

12. Lee DH, Jin KS. Effect of nasal septal deviation on pneumatization of the mastoid air cell system: 3D morphometric analysis of computed tomographic images in a pediatric population. The Journal of International Advanced Otology. 2014 Oct 1;10(3):251-5. https://doi.org/10.5152/iao.2014.276

13. Koc A, Ekinci G, Bilgili AM, Akpinar H, Yakut H, Han T. J Laryngol Otol. 2003; 117(8):595-8. https://doi.org/10.1258/002221503768199906 PMid:12956911

14. Lee DH, Jin KS. Effect of Nasal Septal Deviation on Pneumatization of the Mastoid Air Cell System: 3D Morphometric Analysis of Computed Tomographic Images in a Pediatric Population. Journal of International Advanced Otology. 2014; 10(3):251-5. https://doi.org/10.5152/iao.2014.276

15. Göçmen G, Borahan MO, Aktop S, Dumlu A, Pekiner FN, Göker K. Effect of Septal Deviation, Concha Bullosa and Haller's Cell on Maxillary Sinus's Inferior Pneumatization; a Retrospective Study. Open Dent J. 2015; 9:282-286. https://doi.org/10.2174/1874210601509010282 PMid:26464596 PMCid:PMC4598377

16. Raman R, Murthy N, Galag S, Diwakar S. Mastoiditis and Sinonasal Pathologies on Cranial Computed Tomography Imaging: A Correlative Study. Int J Sci Stud. 2016; 4(1):165-168.

17. Firat AK, Miman MC, Firat Y, Karakas HM, Ozturan O, Altinok T. Effect of nasal septal deviation on total ethmoid cell volume. J Laryngol Otol. 2006; 120(3):200-4. https://doi.org/10.1017/S0022215105007383 PMid:16372990

18. Adibelli ZH, Songu M, Adibelli H. Paranasal sinus development in children: a magnetic resonance imaging analysis. American journal of rhinology & allergy. 2011; 25(1):30-5. https://doi.org/10.2500/ajra.2011.25.3552 PMid:21711972

19. Cinamon U. The growth rate and size of the mastoid air cell system and mastoid bone: a review and reference. European Archives of Oto-Rhino-Laryngology. 2009; 266(6):781-6. https://doi.org/10.1007/s00405-009-0941-8 PMid:19283403

20. O'Tuama LA, Swanson MS. Development of paranasal and mastoid sinuses: a computed tomographic pilot study. Journal of child neurology. 1986; 1(1):46-9. https://doi.org/10.1177/088307388600100107 PMid:3598107

21. Gencer ZK, Özkırış M, Okur A, Karaçavuş S, Saydam L. The effect of nasal septal deviation on maxillary sinus volumes and development of maxillary sinusitis. European Archives of Oto-Rhino-Laryngology. 2013; 270(12):3069-73. https://doi.org/10.1007/s00405-013-2435-y PMid:23512432

22. Gupta S, Gurjar N, Mishra HK. Computed tomographic evaluation of anatomical variations of paranasal sinus region. International Journal of Research in Medical Sciences. 2016; 4(7):2909-13. https://doi.org/10.18203/2320-6012.ijrms20161975

23. Kumar P, Rakesh BS, Prasad R. Anatomical variations of sinonasal region: a CT scan study. IJCMR. 2016; 3(6):2601-4.

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Clinical Science

Effectiveness of Lung Ultrasound in Comparison with Chest X-Ray in Diagnosis of Lung Consolidation

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Keywords: Lung ultrasound; Chest X-ray; Community-acquired pneumonia

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Abstract

BACKGROUND: Lung ultrasound (US) is an available and inexpensive tool for the diagnosis of community-acquired pneumonia (CAP); it which has no hazards of radiation and can be easily used.

AIM: To evaluate the efficacy of lung ultrasound in the diagnosis and follow-up of CAP.

PATIENTS AND METHODS: 100 patients aged from 40 to 63 years with a mean age of 52.3 ± 10 years admitted to the Critical Care Department, Cairo University with pictures of CAP. Lung US was performed for all patients initially, then a plain chest X-ray (CXR) was performed. Another lung ultrasound was performed on the 10th day after admission.

RESULTS: Initial chest X-ray was correlated with the initial chest ultrasound examination in CAP diagnosis (R-value = 0.629, P < 0.001). Cohen's κ was run to determine if there is an agreement between the findings of the initial chest X-ray findings and those of the initial chest ultrasound in CAP diagnosis. A moderate agreement was found where κ = .567 (95% CI, 0.422 to 0.712) and P < 0.001. Upon initial examination, the CXR diagnosed CAP in 48.0% of patients, while lung US diagnosed the disease in 70% of patients. Moreover, lung US was more sensitive than CXR (P-value < 0.001). Compared to the accuracy of computed tomography (CT) chest (100%) which is the gold standard for CAP diagnosis, the accuracy of lung US was 95.0%, while the accuracy of CXR was 81.0%.

CONCLUSION: This study proved the effectiveness of lung ultrasound in CAP diagnosis.

Introduction

Acute pneumonia is considered as a fatal infectious disease in the Western world which frequently leads to sepsis and septic shock.

Nowadays, lung ultrasound (US) can be used in the diagnosis of many chest diseases such as pneumothorax, cases of pneumonia, pleural effusions, and pulmonary contusions [4], [5], [6].

The current study aimed to evaluate the efficacy of lung ultrasound in diagnosing community-acquired pneumonia (CAP) in comparison with chest X-ray (CXR).

Patients and Methods

This study is a prospective observational study which was conducted on 100 consecutive patients with suspected CAP who were admitted to the Critical Care Department at Cairo University. The study was conducted after the approval of the ethical committee of the Faculty of Medicine, Cairo University.

All patients were admitted from the emergency department to the hospital in the period from December 2014 to January 2016.

Inclusion criteria

Patients whose age > 18 years and those who were presenting with symptoms of chest infection (e.g. Dyspnea, cough, expectoration, fever, tachypnea and tachycardia) were included in the study.

All patients upon admission are subjected to:

- Detailed history taking

- Screening of the symptoms of dyspnea, cough, expectoration and fever

- Demographic data collection including age and gender

- Clinical examination for fever, rales, wheezes, heart rate, and respiratory rate. In this study, fever is considered significant if temperature > 38 c, tachycardia is considered significant if the heart rate > 100 bpm, and tachypnea is considered significant if the respiratory rate > 30 bpm

- Routine labs including liver functions where liver impairment is considered significant if the liver enzymes elevated more than 3 folds or the liver functions are impaired, and kidney functions are considered impaired if creatinine > 2gm/l or the patient is oliguric (urine output < 0.5 ml per hour)

- Complete blood count where anaemia is considered significant if Hb < 10gm/dl, leukocytosis is considered significant if WBCs > 11000/cc, and C-reactive protein (CRP) where it is considered significant if above 10

- Electrocardiogram (ECG)

Chest X-ray

Plain CXR was done in anteroposterior and lateral views using a portable machine most of the time.

The presence of air bronchogram or localised opacity in the lung field was considered positive for CAP.

Lung Ultrasound

A 3.5 to 5.0 MHz transducer with a convex sector design was used.

We used an ultrasound machine manufactured by Philips Affinity 70 in most of the cases and other cases, and we used cardiac transducer. Hypoechogenic lung lesions, bronchogram sign (i.e. hyperechoic area within the consolidation), and the lung respiratory mobility impairment (absence or decrease of “lung sliding”) all help to diagnose lung consolidation.

Pleura was taken into consideration, if it exists, in diagnosing parapneumonic effusion.

We measured the hypoechoic lung lesion, both longitudinally and sagittally.

- High-resolution computed tomography (CT) chest without contrast was performed only if the chest X-ray was negative, and the lung ultrasound was positive for consolidation.

Statistical methods

Data were statistically described in terms of mean and standard deviation for quantitative data and terms of frequencies (number of cases) and relative frequencies (percentages) for qualitative data. Comparison of quantitative variables was made using the unpaired t-test. For comparing categorical data, Chi-square ($\chi 2$) test was performed. Exact test was used instead when the expected frequency is less than 5. Logistic regression was done to identify models for detecting the cardiac disease as a cause of dyspnea using ultrasound, modified Boston criteria, and pet CO_2. Predicted values by different models were calculated and compared with the actual state using receiver operator characteristic (ROC) curves. Area-under-the curve (AUC) and 95% confidence interval were used to determine the model accuracy. Sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), positive likelihood ratio (LR+), and negative likelihood ratio (LR-) were estimated in every model. ROC curves and AUC analysis were used to get the best cut-off values for detecting the cause of dyspnea in numerical data. Odds ratios (ORs) and 95% confidence interval (CIs) were calculated to examine the risk of a cardiac cause of dyspnea. A probability value (P-value) less than 0.05 was considered statistically significant. Cohen k is a value that measures the agreement between both sides. All statistical calculations were done using SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 21.

Results

All patients were subjected to full history taking, full clinical examination, lab tests including WBCs and CRP, plain CXR, and Lung US. Only selected cases were subjected to CT chest. Accordingly, patients proved to have CAP were categorised as Group 1, while patients without CAP were categorized as Group 2. All the study patients (100) were subjected to initial (on admission) plain CXR. Consolidation was detected in 48 patients; 25 of them had the consolidation in the Rt lung, 16 in the Lt lung, and 7 patients had bilateral lung consolidation, as shown in Table 1.

Table 1: Site and percentage of consolidation

Patients with Consolidation	Number	Percentage
Rt side	25	52.1 %
Lt side	16	33.3%
Bilateral	7	14.6%
Total	48	100 %

There was a significant difference between the number of males and that of females whose chest X-rays were positive for consolidation where the number of affected males was 17, and the number of affected males were 31 with P-value = 0.049. Moreover, there was a significant difference between diabetic and non-diabetic patients whose chest X-rays were positive for consolidation where the number of diabetic patients having consolidation was 19, and the number of non-diabetic patients having consolidation was 29 with P-value = 0.036.

Table 2: Correlation between positive initial CXR and demographic data

	Positive	Negative	P-value
Gender (female)	31(56.4%)	17 (37.8%)	0.049
Diabetic	19 (63.3%)	29 (41.4%)	0.036
Smoker	3 (25%)	45 (51.1%)	0.081

I – Lung ultrasound

Lung ultrasound was done for all patients irrespective of the chest X-ray result. It was found that lung US was positive for consolidation in 70 patients where it shows consolidation in the Rt side in 39 patients, Lt side in 18 patients, and bilateral in 13 patients.

Table 3: Number and percentage of patients with positive lung ultrasound

Positive Initial Lung US	Number	Percentage
Rt side	39	55.7
Lt side	22	31.4
Bilateral	9	12.8
Total	70	100%

There was a significant difference between males and females in terms of the proportion of cases with a positive finding in lung US suggestive of consolidation. This difference is shown in Table 3.

There was a significant difference between diabetic and non-diabetic patients in terms of the proportion of cases with a positive finding in the lung US suggestive of consolidation. This difference is shown in Table 2.

Furthermore, there was a significant difference between smokers and non-smokers in terms of the proportion of cases having a positive finding in the lung US suggestive of consolidation. This difference is shown in Table 4.

Table 4: Correlation between positive initial lung ultrasound and demographic data

	Positive	Negative	P value
Gender (female)	45 (81.8%)	25 (55.6%)	0.004
Diabetic	26 (86.7%)	44 (62.9%)	0.013
Smoker	12 (100.0%)	58 (65.9%)	0.010

II- CT Chest

High-resolution CT chest was done for cases with positive lung US and negative CXR for consolidation. This was done for 22 patients, and it proved consolidation (CAP) in 17 patients, while the other 5 patients had negative CT chest for consolidation and hence not having CAP as shown in Table 5.

Table 5: High-resolution CT chest

CT Chest showing consolidation	Number	Percentage
Positive	17	77.3%
Negative	5	22.7%

CAP and imaging

Initial imaging data (chest X-ray and lung ultrasound) were correlated with the diagnosis of CAP. The following table shows the correlation between the initial chest X-ray, initial chest ultrasound examination, and the diagnosis of CAP. Lung ultrasound showed a strong correlation with CAP diagnosis, while a chest X-ray showed a moderate correlation with CAP diagnosis, as shown in Table 6.

Table 6: Correlation between initial CXR, lung US and CAP

Community-acquired Pneumonia		
CXR (initial)	Correlation coefficient	0.663
	P value	< 0.001
Lung US (initial)	Correlation coefficient	0.892
	P value	< 0.001

Initial chest X-ray was correlated with the initial chest ultrasound examination in diagnosing CAP (R-value 0.629, P-value < 0.001). Cohen's κ was run to determine if there is an agreement between the findings of initial chest x-ray and those of the initial chest ultrasound in the diagnosis of CAP. A moderate agreement was found where κ = .567 (95% CI, 0.422 to 0.712) and P-value < 0.001.

Table 7: Chest X-ray vs Lung Ultrasound

	Chest X-ray	Lung Ultrasound
Sensitivity	72.3%	100.0%
Specificity	97.1%	85.7%
PPV*	97.9%	92.9%
NPV**	65.4%	100.0%
Accuracy	81.0%	95.0%

*PPV: positive predictive value; **NPV: negative predictive value.

Initial CXR diagnosed CAP in 48.0% of patients, while the initial lung US diagnosed CAP in 70% of patients. Lung US outperformed the CXR in diagnosing and excluding CAP upon initial examination. Besides, it was more sensitive than CXR (P-value < 0.001).

Table 8: Chest X-ray vs Lung Ultrasound with demographic data

		Chest x-ray	lung ultrasound
Gender(female)	Correlation coefficient	-0.185	-0.285
	P value	0.033	0.002
Diabetes	Correlation coefficient	0.201	0.238
	P value	0.023	0.009
Smoking status	Correlation coefficient	-0.170	0.242
	P value	0.045	0.008

Compared to CT chest accuracy (100%), which is the gold standard in diagnosing CAP, the lung US accuracy was 95.0%, while the CXR accuracy was 81.0%.

It is noteworthy that both initial CXR and lung US assessments were correlated with diabetes status, female gender, and smoking status.

Follow-up

Follow-up chest X-ray had a strong correlation with the follow-up chest ultrasound (P-value < 0.001). Besides, the follow-up chest X-ray and chest ultrasound were correlated with the clinical picture (P-value < 0.001).

Table 9: Chest X-ray vs lung ultrasound after 10 days follow up

CAP after 10 days		
CXR (follow-up)	Correlation coefficient	0.896
	P value	< 0.001
Lung US (follow-up)	Correlation coefficient	0.896
	P value	< 0.001

Wilcoxon signed ranks test showed that there was a significant difference between the repeated chest X-ray assessment and the baseline one (P < 0.001). Moreover, Wilcoxon signed ranks test showed that there was a significant difference between the repeated lung ultrasound and the initial one (P < 0.001).

Multivariate Regression for Diagnosing Community-Acquired Pneumonia (CAP)

Reviewing the significant correlations with CAP, multivariate regression showed that the initial lung ultrasound findings suggestive of CAP and CRP elevation were significant predictors of CAP.

The superiority of the lung US findings over CXR findings could be explained by the high sensitivity of Lung US in diagnosing CAP. However, the high specificity of CXR according to our results is due to depending on CXR in our methodology as a cornerstone in diagnosing CAP.

Discussion

CAP is a leading cause of death. Effective treatment can markedly decrease mortality, which can be caused by this serious disease. However, the issue is that CAP can-not be easily diagnosed at presentation.

Using the lung ultrasound in the emergency department increase the efficacy and accuracy of CAP diagnosis.

Early and correct CAP diagnosis helps to start early and effective treatment. Hence, we can solve this serious issue or at least decrease the morbidity related to it.

In this study, we analysed the characteristic ultrasonography findings of CAP but concentrating only on consolidation for the diagnosis of the disease. Also, we compared the diagnostic sensitivity, specificity, and accuracy of ultrasonography with those of chest X-ray using CT chest as the gold standard in diagnosing pneumonia in the case of –ve CXR and +ve lung US.

In the current study, we found that lung US has a sensitivity of 100% and accuracy of 95% in CAP diagnosis compared to the sensitivity and accuracy of 72.3% and 81%, respectively for CXR.

The same fact was proved by Cortellaro et al., [6] and Parlamento et al., [8].

The results of our study go hand by hand with Emilia et al. 2016, and the same conclusion was achieved by Mengetal, 2014.

However, some studies have shown substantial variability in the interpretation of chest radiographs18 as well as the risk of cancer development after exposure to radiation in early life.

Reissig et al., [12] reported the first prospective study of CAP diagnosis in adults using ung US with an excellent sensitivity of 94% and specificity of 98%.

Recently published papers confirm the high efficacy and sensitivity of lung US.

In our study, we concentrated only on lung consolidation as the diagnostic finding in lung US to detect CAP although CAP can be detected from the interstitial infiltrate, which appears as ground-glass opacity on CT chest.

Lichtenstein et al., [26] concentrated on dynamic air bronchogram as a pathognomonic finding in lung US for CAP diagnosis.

Zhang M et al., [21] has found that the lung US is a rapid way to diagnose pneumothorax. These findings are along with Advanced Trauma Life Support (ATLS). Although lung ultrasound can detect pneumothorax, fluids, and lung contusion, still it is operator dependent.

We found that the assessment of the lung using ultrasound is an easy and rapid way to diagnose various lung diseases.

Although lung US was a new technique at the time of the study, it gained popularity later on and became an everyday practice in our department.

Currently, all physicians can perform lung US, especially after the advancement of critical care ultrasound which became a mandatory skill for every critical care physician.

A study was performed by Chaves et al., [20] on how it is easy to learn and practice lung US.

Follow-up

Follow-up after 10 days of admission CXR and lung US were correlated with the clinical picture.

The improvement in symptoms and signs were correlated with the improvement in CXR and lung US (correlation coefficient is 0.896 and P = 0.001).

We found a strong correlation between lung US and CXR during the follow-up.

The same results were achieved by Meng et al. 2014 [17], [20], [21].

In conclusion, our study proved that lung ultrasound is highly effective in the diagnosis and follow-up of lung consolidation. We recommend that lung ultrasound should be available in (ED) and that all doctors should be trained on how to deal with it easily.

References

1. Shah VP, Tunik MG, Tsung JW. Prospective evaluation of point-of-care ultrasonography for the diagnosis of pneumonia in children and young adults. JAMA pediatrics. 2013; 167(2):119-25. https://doi.org/10.1001/2013.jamapediatrics.107 PMid:23229753

2. Peden M, Oyegbite K, Ozanne-Smith J, Hyder AA, Branche C, FazlurRahman AK, et al. World Report on Child Injury Prevention, World Health Organization: Geneva, Switzerland, 2008:1e211.

3. Clark JE, Hammal D, Hampton F, Spencer D, Parker L. Epidemiology of community-acquired pneumonia in children seen in hospital. Epidemiology & Infection. 2007; 135(2):262-9. https://doi.org/10.1017/S0950268806006741 PMid:17291362 PMCid:PMC2870565

4. Senstad AC, Surén P, Brauteset L, Eriksson JR, Høiby EA, Wathne KO. Community-acquired pneumonia (CAP) in children in Oslo, Norway. Acta Paediatrica. 2009; 98(2):332-6. https://doi.org/10.1111/j.1651-2227.2008.01088.x PMid:19006533

5. Ma Y, Guo S, Wang H, Xu T, Huang X, Zhao C, Wang Y, Scherpbier RW, Hipgrave DB. Cause of death among infants in rural western China: a community-based study using verbal autopsy. The Journal of pediatrics. 2014; 165(3):577-84. https://doi.org/10.1016/j.jpeds.2014.04.047 PMid:24929335

6. C Grijalva CG, Nuorti JP, Zhu Y, Griffin MR. Increasing incidence of empyema complicating childhood community-acquired pneumonia in the United States. Clinical Infectious Diseases. 2010; 50(6):805-13. https://doi.org/10.1086/650573 PMid:20166818 PMCid:PMC4696869

7. Rudan I, Boschi-Pinto C, Biloglav Z, Mulholland K, Campbell H. Epidemiology and etiology of childhood pneumonia. Bulletin of the world health organization. 2008; 86:408-16B. https://doi.org/10.2471/BLT.07.048769 PMid:18545744 PMCid:PMC2647437

8. Harris M, Clark J, Coote N, Fletcher P, Harnden A, McKean M, Thomson A. British Thoracic Society guidelines for the management of community acquired pneumonia in children: update 2011. Thorax. 2011; 66(Suppl 2):ii1-23. https://doi.org/10.1136/thoraxjnl-2011-200598 PMid:21903691

9. Bowen SJ, Thomson AH. British Thoracic Society Paediatric Pneumonia Audit: a review of 3 years of data. Thorax. 2013; 68(7):682-3. https://doi.org/10.1136/thoraxjnl-2012-203026 PMid:23291351

10. Palafox M, Guiscafré H, Reyes H, Muñoz O, Martínez H. Diagnostic value of tachypnoea in pneumonia defined radiologically. Archives of disease in childhood. 2000; 82(1):41-5. https://doi.org/10.1136/adc.82.1.41 PMid:10630911 PMCid:PMC1718193

11. Shah S, Bachur R, Kim D, Neuman MI. Lack of predictive value of tachypnea in the diagnosis of pneumonia in children. The Pediatric infectious disease journal. 2010; 29(5):406-9. https://doi.org/10.1097/INF.0b013e3181cb45a7 PMid:20032805

12. Reissig A, Gramegna A, Aliberti S. The role of lung ultrasound in the diagnosis and follow-up of community-acquired pneumonia. European journal of internal medicine. 2012; 23(5):391-7. https://doi.org/10.1016/j.ejim.2012.01.003 PMid:22726366

13. Reissig A, Copetti R, Mathis G, Mempel C, Schuler A, Zechner P, Aliberti S, Neumann R, Kroegel C, Hoyer H. Lung ultrasound in the diagnosis and follow-up of community-acquired pneumonia: a prospective, multicenter, diagnostic accuracy study. Chest. 2012; 142(4):965-72. https://doi.org/10.1378/chest.12-0364 PMid:22700780

14. Viera AJ, Garrett JM. Understanding interobserver agreement: the kappa statistic. Fam med. 2005; 37(5):360-3.

15. Reali F, Papa GF, Carlucci P, Fracasso P, Di Marco F, Mandelli M, Soldi S, Riva E, Centanni S. Can lung ultrasound replace chest radiography for the diagnosis of pneumonia in hospitalized children?. Respiration. 2014; 88(2):112-5. https://doi.org/10.1159/000362692 PMid:24992951

16. Esposito S, Papa SS, Borzani I, Pinzani R, Giannitto C, Consonni D, Principi N. Performance of lung ultrasonography in children with community-acquired pneumonia. Italian journal of pediatrics. 2014; 40(1):37. https://doi.org/10.1186/1824-7288-40-37 PMid:24742171 PMCid:PMC4012508

17. Pereda MA, Chavez CC, Hooper-Miele RH, Gilman MC, Steinhoff LE, Ellington, et al. Lung ultrasound for the diagnosis of pneumonia in children:a meta-analysis. Pediatrics. 2015; 135:714-722. https://doi.org/10.1542/peds.2014-2833 PMid:25780071

18. Chavez MA, Naithani N, Gilman RH, Tielsch JM, Khatry S, Ellington LE, Miranda JJ, Gurung G, Rodriguez S, Checkley W. Agreement between the World Health Organization algorithm and lung consolidation identified using point-of-care ultrasound for the diagnosis of childhood pneumonia by general practitioners. Lung. 2015; 193(4):531-8. https://doi.org/10.1007/s00408-015-9730-x PMid:25921013

19. Ho MC, Ker CR, Hsu JH, Wu JR, Dai ZK, Chen IC. Usefulness of lung ultrasound in the diagnosis of community-acquired pneumonia in children. Pediatrics & Neonatology. 2015; 56(1):40-5. https://doi.org/10.1016/j.pedneo.2014.03.007 PMid:25034957

20. Caiulo VA, Gargani L, Caiulo S, Fisicaro A, Moramarco F, Latini G, Picano E, Mele G. Lung ultrasound characteristics of community-acquired pneumonia in hospitalized children. Pediatric pulmonology. 2013; 48(3):280-7. https://doi.org/10.1002/ppul.22585 PMid:22553150

21. Copetti R, Cattarossi L. Ultrasound diagnosis of pneumonia in children. La radiologia medica. 2008; 113(2):190-8. https://doi.org/10.1007/s11547-008-0247-8 PMid:18386121

22. Iuri D, De Candia A, Bazzocchi M. Evaluation of the lung in children with suspected pneumonia: usefulness of ultrasonography. La radiologia medica. 2009; 114(2):321-30. https://doi.org/10.1007/s11547-008-0336-8 PMid:18956148

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Clinical Science

Evaluation of Relation between HbA1c Level with Cognitive Disorders and Depression in Type 2 Diabetes Mellitus Patients

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Abstract

BACKGROUND: The role of HbA1c level in cognition decline and depression in type 2 diabetic patients is reported in some studies.

AIM: We evaluated the possible significant relationship between HbA1c level and cognition decline and depression in type 2 diabetic patients.

MATERIAL AND METHODS: This descriptive-analytic study was performed on 512 patients with a different HbA1c level and ages range. All subjects were administered a structured clinical interview. Cognitive functions and depressive disorders were assessed through the Mini-Mental Status Exam (MMSE) and Patient Health Questionnaire-9 (PHQ-9) respectively. Chi-square test was used for relationships between variables.

RESULTS: HbA1c mean in all patients was 7.58%. MMSE score mean in total was 27.28. 83.3% of patients had a depressive disorder, and 8.59% of patients had an MMSE score < 24. There was no significant relationship between HbA1c level and cognitive problems, but there was a significant relationship between recent memory declines with the level of HbA1c (P = 0.03). Also, there was no significant relationship between attention-deficit with HbA1c level.

CONCLUSION: Our finding provides that even though there is no significant difference between HbA1c level and cognitive problems and depression, recent memory state in these patients are more affected than the normal population and these patients have a worse state of depressive disorders.

Introduction

Diabetes mellitus is a chronic long-term illness. All types of diabetes are a global public health problem, particularly type 2 diabetes [1]. The prevalence of diabetes is increasing in many countries around the world. By the year 2025, approximately 380 million people will be diagnosed with type 2 diabetes [1], [2]. In Iran, in 2011, the prevalence of diabetes in the adult population was 11.4% in 2011, with a 35% increase compared to the 2005 reports. It is estimated that by the year 2030, 9.2 million people in Iran will have diabetes [3]. Diabetes mellitus is associated with the poor cognitive function of the brain. Total grey-matter and hippocampus volume affects these associations and reduce it by almost 50% when adjusted, but there was no correlation for white-matter volume [4], [5]. Type 2 diabetes is a known risk factor for the development of depression.

Symptoms and disorders of depression in patients with type 2 diabetes are twice as probable as the general population [6]. A possible relationship between diabetes and cognitive problems has been suggested since the discovery of insulin [7]. It's a controversy about how diabetes can affect cognition and make cognitive problems in patients. Changes in glucose level as hyper or hypoglycemia, an increase in insulin level of resistance to it, oxidative stress and inflammatory cytokine effects and deposition of beta-amyloid are possible mechanisms [8]. Recently, HbA1c has been used as a diagnostic test for diabetes. This is a preferred test for defining glycemic

control in people with diabetes. After using HbA1c, as a demonstration of the glycemic condition of diabetic patients, an inverse correlation has been noted between any cognitive measures and depression with HbA1c levels, which proposing that inadequate glycemic control could be associated with worse cognitive function [9], [10].

The purpose of this study was the use of this marker for evaluating glycemic index in diabetic patients. We performed this study in type 2 diabetic patients referring to the Tehran city (Capital of Iran) hospitals.

Material and Methods

Study design

In a retrospective study, the data were from 632 patients referring to Tehran city hospitals with diagnosed Type 2 diabetes mellitus already. Diagnosed type 2 diabetes was the only inclusion criteria. Patients were recruited between July 2017 to March 2018. Five hundred and twelve adults, 219 males and 293 females with diagnosed type 2 diabetes by their primary care physicians. Exclusion criteria were: dementia, CNS diseases, unstable medical diseases, another psychiatric disease (including bipolar disorder), dependency on drug or alcohol, or history of head trauma. Subjects were not admitted if they were taking anti-depressant or psychotropic drugs. All diabetic patients were taking combinations of insulin and oral hypoglycemic agents for blood sugar control.

Data collection tools

All subjects were administered a structured clinical interview. Patient Health Questionnaire-9 (PHQ-9) which is a 9-question instrument given to patients in a primary care setting to screen for the presence and severity of depression. Cognitive functioning was assessed through the MMSE (Mini-Mental Status Exam). The MMSE's purpose has been not, on its own, to provide a diagnosis for any particular neurological entity [8]. It takes 5 to 10 minutes to administrate the test, and the test examines attention, registration (repeating named prompts), and calculation, language, recall, ability to follow simple commands and orientation [9]. Originally it was designed to differentiate organic from functional psychiatric patients [10], [11].

Procedures

The study was performed following the policies of the Human Subject Protection Committees and approved by the University Institutional Review Board. Written informed consent was obtained from all participants after the procedures had been successfully explained. All subjects received an in-person or telephone introductory interview, followed by an in-person psychiatric evaluation. This evaluation included psychiatric and medical history, health and functioning status. All patients were referred to laboratories, and after taking a blood sample, HbA1c was analysed. This subject was investigated by University Ethics group, and the Ethics Code is IR.AJAUMS.REC.1396.79.

Statistics

All data obtained were recorded in tests checklists, and the SPSS version 24 (Chicago, Illinois, USA) for Windows was used to perform a chi-square test for relationships between variables.

Results

Of 512 patients with type 2 diabetic disease, 219 (42.77%) were male, and 293 (57.23%) were female. The age range of patients was 33 to 90 years old with an average of 61.84. HbA1c mean in all patients was 7.58%. The mean of HbA1c in male was 7.54% and in female was 7.61%. MMSE score mean in total was 27.28 of 30 (scores from 14 Min and Max to 30). PHQ-9 scores mean in all participants was 9.58 of 27. The rate of different factors, according to gender, is shown in Table 1.

Table 1: The rate of different factors in 512 patients with diabetic disease

Patients	Age (mean)	HbA1c (%)	MMSE (of 30 scores)	PHQ-9 (of 27 score)
Male 219 (42.77%)	61.93	7.54	27.52	9.14
Female 293 (57.23%)	61.77	7.61	27.11	9.92
Total 512	61.84	7.58	27.28	9.58

MMSE: Mini Mental Status Exam; PHQ-9: Patient Health Questionnaire-9; HbA1c: Hemoglobin A1c.

This study showed that the depression rate was 83.3% among all patients. It was 84.6% and 81.7% among female and male respectively. There was a significant relationship between age and depression (P = 0.022). There was no significant statistical association between sex and depression (P > 0.05). According to PHQ-9 scores, 184 patients have a score between 5-10 which suggesting a mild depression. Due to PHQ-9 scores, 160 patients have a score between 10-14 which suggesting a moderate depressive disorder. Sixty-three patients have a PHQ-9 score 15-19 reminding of moderately severe depression. Twenty patients have a score higher or equal to 20 which suggesting a severe depression. Other factors related to patients participating in the study are presented in Table 2.

Table 2: Overview of PHQ-9 test results of 512 patients with diabetic disease

PHQ-9 score range (Type of depression)	Male (%)	Female (%)	Age (mean)	HbA1c (%)	MMSE mean (Of 30 scores)	PHQ-9 mean (Of 27 scores)
≤ 4 (normal)	40 (47.1%)	45 (52.9%)	61.18	7.56	27.74	2.62
5-10 (mild)	82 (44.6%)	102 (55.4%)	61.13	7.60	27.41	7.06
10-14 (moderate)	66 (41.3%)	94 (58.7%)	61.94	7.75	27.16	11.8
15-19 (moderately severe)	22 (34.9%)	41 (65.1%)	63.14	7.60	26.95	16.87
≥ 20 (severe)	9 (45%)	11 (55%)	66.3	7.52	26.25	21.6
Total	219	293	61.94	7.57	27.16	11.8

MMSE: Mini Mental Status Exam; PHQ-9: Patient Health Questionnaire-9; HbA1c: Hemoglobin A1c.

Chi-square test showed no significance between HbA1c level and PHQ-9 (P = 0.276). An overview of the MMSE test in patients is shown in Table 3.

Table 3: Overview of MMSE test results of 512 patients with diabetic disease

Single cutoff method of MMSE	Male	Female	Age (mean)	HbA1c (%)	MMSE mean (Of 30 scores)	PHQ-9 mean (Of 27 scores)
MMSE < 24	14 (31.8%)	30 (68.2%)	69.27	7.74	21.43	10.27

MMSE: Mini Mental Status Exam; PHQ-9: Patient Health Questionnaire-9; HbA1c: Hemoglobin A1c.

In this research, 44 subjects have a score of less than 24 on the MMSE test which means a possible cognitive problem (Table3). Of this, 14 were men (31.8%) and 30 (68.2%) were women. The mean of age, HbA1c, MMSE score, PHQ-9 score was 69.27, 7.74, 21.41 and 10.27 respectively. Statistic test showed that there was no significant relationship between MMSE score and HbA1c level (P > 0.05). Chi-square test showed that there was no significant association between sex and age with MMSE score less than 24 (P > 0.05) (Table 3). Also, there were no significant differences between sex, age and MMSE score less than 24 (P > 0.05). Chi-square test showed that there was no significant association between MMSE score and HbA1c level in the 33+ (33-49, 50-69, 70-90) age groups (p > 0.05). Also, the significant correlation was not observed between PHQ-9 score and HbA1c level in all age groups (p > 0.05). Also, the mean level of HbA1c is higher in clinically depressed patients in contrast with not-depressed participants (7.60 and 7.56 respectively), but there was no significant relationship between depression severity and HbA1c level. But there was a significant relationship between MMSE score and PHQ-9 score with each other in the analysis of the entire subjects (P = 0.013).

In a partial correlation analysis of age groups, there was no significant relation between MMSE score and PHQ-9 score in the first (33-49) and last (70-90) age groups (p > 0.05). But there was a significant correlation between those two with 50-69 years old group (P < 0.05). After controlling ageing and sex, there was no significant relationship between an HbA1c level and MMSE score or PHQ-9 score and MMSE score and PHQ-9 score in all three age groups of male and female except a significant relationship between MMSE score and PHQ-9 score in 50-69 years old group (P < 0.05). We considered Question 4 (spelling backward) of MMSE for checking attention and question 5 (recall 3 words in 5 minutes) for checking recent memory. Mean of scores of question 4 was 4.03 of 5, in male it was 4.35 and there was no significant relation with an HbA1c level (P = 0.997), In females the score mean was 3.78 but there was no significant relationship with an HbA1c level (P = 0.921) Also there was no significant relation between attention deficit and sex (P = 0.130) and age (P = 0.235). Mean scores of questions 5 were 1.66 0f 3. In male score mean was 1.41 and there was a significant relation between HbA1c level and recent memory impairment (P = 0.03). In females, the mean score was 2.10, and there was a significant relationship between HbA1c levels and memory impairment (P = 0.01). Also, there was a significant relation between recent memory impairment and sex (P = 0.005). But there was no significant relationship between age and recent memory impairment (P = 0.231).

Discussion

This report aimed to provide the role of HbA1c level in cognition decline and depression in type 2 diabetic patients. The data of 512 patients with Type 2 diabetes mellitus who referred to Tehran city hospitals between July 2017 to March 2018 were collected. This study showed that both sexes were susceptible to the disease, and most of the patients (83.3%) had a depressive disorder in different grades. An investigation in Iran reported similar findings. This study showed a high level of depression (85.3%) among diabetic patients [12]. IN another study, researchers reported that depression is more common among diabetic patients and 77% of the subjects had a severe kind of depressive disorder [13]. The results of a study in Iran showed that type 2 diabetic patients had a more severe level of depression and depression incidence was 72% among them [14]. Our findings were consistent with the results of these studies. Our study showed that there was a significant difference between age and depression, but there was no relation between sex and depression. Mahmoudi et al. reported a significant relationship between age and sex with depression [13]. In some studies, researchers in foreign countries reported similar results. Ronny et al. showed a 75% rate of depression in rural older African Americans, Native Americans, and whites' diabetic patients [15]. This is close to our findings. We found a higher prevalence of type 2 diabetic (84.6%) among female patients. In another study, researchers reported a higher prevalence of depression among type 2 diabetic patients and they also reported that depression has a higher prevalence among female patients [16]. Other investigations in the Netherlands, US, Bangladesh and Iran continually report the raised prevalence of depression in diabetic patients, in a range between 5% to 71.8% [17], [18], [19], [20], [21]. These studies show a higher level of depressive disorders among type 2 diabetic patients,

but the prevalence percentage is lower than ours because of the lower incidence of depression in those countries. Another study in Finland reported no significant relationship between cognitive function and diabetes. There was no significant difference in cognitive scores of the MMSE test; however, the female had better performance [22]. The results of this study have concordance with our results in Tehran. On the other hand, the English physician Thomas Willis suggested that in diabetic patients, the mental function might not be the same as others. Currently, cognition is an important issue in diabetic patient's health, especially for postmenopausal women.

In a large study, researchers used Telephone Interview of Cognitive Status (TICS) and suggested an effect of diabetes on cognition state. In this study, subjects with diabetes scored lower on mental health and energy indices [23]. Also many studies suggest positive relationship between diabetes and one or more cognitive domains declines, including attention/concentration [24], [25], [26], [27], [28], [29] which is opposed to our findings. In another study, the possible relation between HbA1c level and memory decline was evaluated [30]. In this study diabetes was related to a 10% faster rate of memory decline and higher HbA1c was associated with memory decline. A relative biological mechanism that describes the association between type 2 diabetes and cognition is chronic hyperglycemia as well as hyperinsulinemia and deficiency of insulin in the brain. Also, other risk factors of memory decline such as severe hypoglycemic events and depression are more common among type 2 diabetic patients and could explain the possible association [30].

In this study, there was no significant relationship between HbA1c level and cognitive disorders and depression, but diabetic patients' recent memory and depression level had been influenced. We suggest that these patients go through regular memory and depressive state surveys.

References

1. Cheung N, Mitchell P, Wong TY. Diabetic retinopathy. Lancet. 2010; 376(9735):124-36. https://doi.org/10.1016/S0140-6736(09)62124-3

2. Darvishi M, Nazer MR, Alipour MR. Investigating the end of patients suffering from diabetic foot hospitalized in Be'sat hospital of IRIAF from 2009 to 2014. Biomedical Research. 2017; 28(10):4630-4633.

3. Esteghamati A, Larijani BMF, Kermanchi J, Shahrami A, et al. Diabetes in Iran: Prospective Analysis from First Nationwide Diabetes Report of National Program for Prevention and Control of Diabetes (NPPCD-2016). Scientific Reports. Published. 2017. https://doi.org/10.1038/s41598-017-13379-z PMid:29044139 PMCid:PMC5647418

4. Busko M. Gray-matter atrophy may drive cognitive decline in diabetes. Medscape Medical News, WebMD, LLC. 2013.

5. Moran C, Phan TG, Chen J, Blizzard L, Beare R, Venn A, Münch G, Wood AG, Forbes J, Greenaway TM, Pearson S, Srikanth V. Diabetes Care. 2013; 36(12):4036-42. https://doi.org/10.2337/dc13-0143 PMid:23939539 PMCid:PMC3836136

6. Nouwen A, Winkley K, Twisk J, Lloyd CE, Peyrot M, Ismail K, Pouwer F. European Depression in Diabetes (EDID) Research Consortium. Type 2 diabetes mellitus as a risk factor for the onset of depression: a systematic review and meta-analysis. Diabetologia. 2010; 53(12):2480-6. https://doi.org/10.1007/s00125-010-1874-x PMid:20711716 PMCid:PMC2974923

7. Strachan MW, Frier BM, Deary IJ. Cognitive assessment in diabetes: the need for consensus. Diabet Med. 1997; 14(6):421-2. https://doi.org/10.1002/(SICI)1096-9136(199706)14:6<421::AID-DIA382>3.0.CO;2-F

8. Haan MN. Therapy Insight, type 2 diabetes mellitus and the risk of late-onset Alzheimer's disease. Nat Clin Pract Neurol. 2006; 2(3):159-66. https://doi.org/10.1038/ncpneuro0124 PMid:16932542

9. Kövari E, Herrmann FR, Bouras C, Gold G. Amyloid deposition is decreasing in aging brains: an autopsy study of 1,599 older people. Neurology. 2014; 82:326. https://doi.org/10.1212/WNL.0000000000000069 PMid:24363129

10. Grasset L, Brayne C, Joly P, et al. Trends in dementia incidence: Evolution over a 10-year period in France. Alzheimers Dement. 2016; 12:272. https://doi.org/10.1016/j.jalz.2015.11.001 PMid:26693893

11. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975; 12(3):189-98. https://doi.org/10.1016/0022-3956(75)90026-6

12. Taheri N, Hojjati H, Mousavi M, Afra A, Dehghan B. The Survey of Anxiety and Depression Prevalence in Diabetic Patient Referred to Abadan Taleghani and Khorramshahr Valiasr Hospitals in 2011. Journal of Diabetes Nursing. 2014; 1(2):21-31.

13. Mahmodi A, Sharifi A. Comparison of the prevalence and depression factors in patients with diabetes and non-diabetic. Journal of Urmia Nursing and Midwifery Faculty. 2008; 6(2):88-93.

14. NoriNehad Q, Bostani H, Nemat Poor S, Behroozian F. Compare depression in diabetics and non-diabetics. Jundishapur Scientific Medical Journal. 2006; 5(1):38595.

15. Bell RA, Smith SL, Arcury TA, Snively BM, Stafford JM, Quandt SA. Prevalence and correlates of depressive symptoms among rural older African Americans native Americans, and whites with diabetes. Diabetes Care. 2005; 28(4):823-9. https://doi.org/10.2337/diacare.28.4.823 PMid:15793180 PMCid:PMC1592640

16. Roy T, Lloyd CE. Epidemiology of depression and diabetes: a systematic review.J Affect Disord. 2012; 142 Suppl:S8-21. https://doi.org/10.1016/S0165-0327(12)70004-6

17. Pouwer F, Geelhoed-Duijvestijn PH, Tack CJ, Bazelmans E, Beekman AJ, Heine RJ, Snoek FJ. Prevalence of comorbid depression is high in out-patients with Type 1 or Type 2 diabetes mellitus. Results from three out-patient clinics in the Netherlands. Diabet Med. 2010; 27(2):217-24. https://doi.org/10.1111/j.1464-5491.2009.02903.x PMid:20546267

18. Li C, Ford ES, Strine TW, Mokdad AH. Prevalence of depression among U.S. adults with diabetes: findings from the 2006 behavioral risk factor surveillance system. Diabetes Care. 2008; 31(1):105-7. https://doi.org/10.2337/dc07-1154 PMid:17934145

19. Asghar S, Hussain A, Ali SM, Khan AK, Magnusson A. Prevalence of depression and diabetes: a population-based study from rural Bangladesh. Diabet Med. 2007; 24(8):872-7. https://doi.org/10.1111/j.1464-5491.2007.02136.x PMid:17403122

20. Holt RI, Phillips DI, Jameson KA, Cooper C, Dennison EM, Peveler RC. Hertfordshire Cohort Study Group. The relationship between depression and diabetes mellitus: findings from the Hertfordshire Cohort Study. Diabet Med. 2009; 26(6):641-8. https://doi.org/10.1111/j.1464-5491.2009.02742.x PMid:19538241

21. Khamseh ME, Baradaran HR, Rajabali H. Depression and diabetes in Iranian patients: a comparative study. Int J Psychiatry Med. 2007; 37(1):81-6. https://doi.org/10.2190/FP64-82V3-1741-842V PMid:17645200

22. Vanhanen M, Kuusisto J, Koivisto K, Mykkänen L, Helkala EL, Hänninen T, et al. Type-2 diabetes and cognitive function in a non-demented population. Acta Neurol Scand. 1999; 100(2):97-101. https://doi.org/10.1111/j.1600-0404.1999.tb01045.x PMid:10442450

23. Grodstein F, Chen J, Wilson RS, Manson JE. Nurses' Health Study. Type 2 diabetes and cognitive function in community-dwelling elderly women. Diabetes Care. 2001; 24(6):1060-5. https://doi.org/10.2337/diacare.24.6.1060 PMid:11375371

24. Meuter F, Thomas W, Grüneklee D, Gries FA, Lohmann R. Psychometric evaluation of performance in diabetes mellitus. Horm Metab Res Suppl. 1980; 9:9-17.

25. Tun PA, Perlmuter LC, Russo P, Nathan DM. Memory self-assessment and performance in aged diabetics and non-diabetics. Exp Aging Res. 1987; 13(3):151-7. https://doi.org/10.1080/03610738708259317 PMid:3691586

26. Reaven GM, Thompson LW, Nahum D, Haskins E. Relationship between hyperglycemia and cognitive function in older NIDDM patients. Diabetes Care. 1990; 13(1):16-21. https://doi.org/10.2337/diacare.13.1.16 PMid:2298111

27. Jagusch W, Cramon VDY, Renner R, Hepp KD. Cognitive function and metabolic state in elderly diabetic patients. Diabetes Nutr Metab. 1992; 5(4):265-274.

28. Biessels GJ, Kappelle AC, Bravenboer B, Erkelens DW, Gispen WH. Cerebral function in diabetes mellitus. Diabetologia. 1994; 37(7):643-50. https://doi.org/10.1007/BF00417687 PMid:7958534

29. Dey J, Misra A, Desai NG, Mahapatra AK, Padma MV. Cognitive function in younger type II diabetes. Diabetes Care. 1997; 20(1):32-5. https://doi.org/10.2337/diacare.20.1.32 PMid:9028690

30. Marden JR, Mayeda ER, Tchetgen Tchetgen EJ, Kawachi I, Glymour MM. High Hemoglobin A1c and Diabetes Predict Memory Decline in the Health and Retirement Study. Alzheimer Dis Assoc Disord. 2017; 31(1):48-54. https://doi.org/10.1097/WAD.0000000000000182 PMid:28225507 PMCid:PMC5325158

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Clinical Science

Association between Dyslipidemia and Peritoneal Dialysis Technique Survival

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Abstract

BACKGROUND: A large body of research has investigated the effects of pro-atherogenic lipid profile on cardiovascular diseases (CVD) in peritoneal dialysis (PD) patients. However, there is a general lack of research on the association between atherogenic dyslipidemia and PD technique survival.

AIM: The study aimed to define the association between dyslipidemia and PD technique survival.

METHODS: It was a prospective single-centre observational study involving 40 outpatients on continuous ambulatory PD treatment for more than 3 months between 2010 and 2016 in a single centre in Ukraine. There were 27 males and 13 females. The mean age of the participants was 49.3 ± 12.2 years. The primary outcome measures were all-cause technique failure.

RESULTS: Atherogenic dyslipidemia was identified in 28/40 (70 %) patients and correlated with PD adequacy parameters. During the 36-month- follow-up period technique failure occurred in 2/12 (16.6 %) patients with atherogenic dyslipidemia compared with 12 / 28 (42.9 %) patients without atherogenic dyslipidemia ($\chi 2 = 2.5$; $p = 0.12$). In the univariate Cox regression model, atherogenic dyslipidemia at baseline was significantly associated with a higher risk of all-cause PD technique failure (HR 4.5; 95% CI 1.6 to 12.9; $\chi 2 = 5.5$, $p = 0.019$).

CONCLUSION: The presence of atherogenic dyslipidemia was significantly associated with a higher risk of technique failure in PD patients. This is an important issue for future research. Further well-designed clinical trials are needed to determine the impact of dyslipidemia on PD adequacy and technique survival.

Introduction

End-stage renal disease (ESRD) is a worldwide public health problem. The number of patients diagnosed with ESRD continues to increase considerably from year to year [1], [2]. Peritoneal dialysis (PD) is a well-established treatment modality for ESRD patients. Currently, in Ukraine, approximately 12 % of ESRD patients are maintained on PD [3]. Although Ukrainian government and healthcare providers continue to focus on increasing incident PD use, less attention is focused on the effect of time spent on PD therapy and financial resources associated with the development of PD technique failure. Also, despite the significant progress in technique survival, the duration of PD therapy is still limited [4], [5].

There are several factors affecting PD technique survival: age, the presence of diabetes, residual renal function (RRF), glucose degradation products, peritonitis, chronic inflammation, cardiovascular diseases (CVD) [5], [6]. Previous studies have demonstrated a higher risk *of* mortality 2 years after PD treatment compared to the hemodialysis **(**HD) population [7], [8], [9], [10]. CVD due to the traditional and non-traditional risk factors are the most common causes of death in PD patients [9], [11].

Dyslipidemia is a traditional risk factor of cardiovascular events in the general population and the HD population [12], [13], [14]. High total cholesterol (TC), high levels of low-density lipoprotein cholesterol (LDL), very low-density lipoprotein cholesterol (VLDL), triglyceride and low level of high-density lipoprotein cholesterol (HDL) are all well-known risk factors of developing CVD [13], [14]. It has been suggested that patients on PD have differences in the lipid profile compared with patients on HD. The lipid profile in PD patients is more atherogenic with more altered dyslipidemia when compared with those in HD patients. This condition may be associated with glucose absorption from the peritoneal dialysate, peritoneal protein loss and a decrease in residual renal function (RRF) [11], [12], [15]. Also, PD treatment leads to body weight gain, and it can also be a potential risk factor for accelerated atherosclerosis [16], [17], [18].

A large body of research *has* investigated the effects of pro-atherogenic lipid profile on CVD in PD patients [11], [12], [19], [20]. However, in reviewing the literature, no data was found on the association between atherogenic dyslipidemia and PD technique survival and other clinical outcomes including parameters *of* dialysis adequacy in PD patients. It is still not clear whether dyslipidemia is associated with PD technique survival**.** We hypothesised that if all factors mentioned above associated with dyslipidemia in PD patients were the predictors of loss of peritoneal function*,* then dyslipidemia itself could not be associated with PD technique survival.

Therefore, we initiated this prospective observational study to determine whether dyslipidemia was associated with PD technique failure.

Patients and Methods

Study Design and Subjects

The prospective single-centre observational study was carried out between January 2010 and September 2016 at State Institution "Institute of Nephrology of the National Academy of Medical Sciences of Ukraine" in Kyiv, Ukraine. The study was conducted following the Declaration of Helsinki. The study protocol was confirmed by the Ethics Committee of the Institute, and all patients provided their written informed consent to participate in the study. Informed consent was obtained from all subjects participating in the study.

Forty outpatients with ESRD on PD (27 males, 13 females) were included in the study. All patients were treated with continuous ambulatory PD (CAPD) for more than 3 months. They were observed to determine the impact of atherogenic lipid profile on PD technique survival *during* the *36-month-* follow-up *period*.

All recruited PD patients received 4 exchanges daily. The diabetics and the patients with a history of peritonitis or significant illness/hospitalisation were excluded in the previous 3 months.

Methods

Patient demographics data including age, gender, comorbid conditions, hypertension and dyslipidemia were obtained from medical records.

Whole blood samples were collected from the patients after an overnight fast during a routine outpatient visit. The blood samples were processed immediately after sampling.

Routine biochemical parameters including blood and daily dialysate concentration of urea and creatinine, serum albumin, C-reactive protein (CRP), glucose, electrolytes and lipid profile were carried out using a Flexor Junior Chemistry Analyzer (Vital Scientific, Dieren, Netherlands*)*. Blood lipid profile parameters included triglyceride (TG), TC, HDL, LDL and VLDL. Atherogenic index of plasma (AIP) was calculated from plasma triglyceride and HDL (log [TG / HDL]). Body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters.

Haematological parameters of blood were determined using an ABX Micros-60 (ABX Diagnostics, Montpelier, France).

The adequacy of dialysis was determined by measuring the total weekly creatinine clearance (CrCl) (it was normalized to 1.73 m^2 of the body surface area) and total weekly urea clearance (Kt / V) using the Watson formula for calculating body water [21]. Peritoneal Kt / V, plasma and renal Kt / V were calculated separately. The dialysate/plasma creatinine ratio (D / P) was calculated from the concentrations of creatinine in 24-h dialysate and plasma [22].

Statistical analysis

The statistical analysis and all graphs were performed using MedCalc (Belgium). The average means (M) and the standard deviations (SD) or the median (Me) and the interquartile ranges [Q25-Q75] were calculated according to the standard normal distribution. For the statistical analysis, we used the student's t-test and the nonparametric (U-test) Mann-Whitney U test.

Categorical variables were expressed as proportions. The Chi-square test was used to

compare *2* groups. Pearson's correlation test was used to evaluate relationships between lipid profile parameters and PD adequacy variables.

The Kaplan-Meier analysis was used to estimate technique survival. The log-rank test was used to evaluate the difference in technique survival rate according to atherogenic dyslipidemia status.

The primary endpoint of this study was the 3-year PD technique survival rate dichotomised according to atherogenic dyslipidemia status. PD technique failure was defined as discontinuation of PD due to uncontrolled volume overload with 2.5% dextrose solution or a decrease in total weekly Kt / V less than 1.7. Death and kidney transplantation were censored.

Atherogenic dyslipidemia was defined as the combination of elevated triglycerides ≥ 2.26 mmol/L (≥ 200 mg/dL) and HDL levels ≤ 0.9 mmol/L (≤ 80 mg/dL) in men and ≤ 1.15 mmol/L (≤ 102 mg/dL) in women.

The duration of technique survival was calculated from the date of study entry. For this analysis, the patients were categorised into 2 groups according to atherogenic dyslipidemia presence at baseline. Group I consisted of 28 patients with atherogenic dyslipidemia and Group II included 12 patients without atherogenic dyslipidemia.

The Cox proportional hazards model of univariate analysis was used to evaluate the association between atherogenic lipid profile and time to PD technique failure as well as to estimate the Hazard ratios (HR) with 95% Confidence Interval (CI). The Cox model was used without adjustment for a priori defined confounding variables including age, gender, BMI, blood pressure, history of CVD and other parameters. P values were calculated, and the null hypothesis was rejected if the P-value was < 0.05.

Results

Association between dyslipidemia and baseline characteristics

Dyslipidemia defined as an increase in atherogenic lipoprotein fractions and inhibition of HDL-cholesterol levels was identified in 28/40 (70%) patients. Table 1 shows the baseline characteristics of study participants according to the presence of atherogenic dyslipidemia. All these data were obtained during routine clinical practice immediately after enrolling the patients in the study.

As shown in Table 1, the subjects without atherogenic dyslipidemia at baseline were younger and less obese. Also, they had lesser systolic blood pressure and better glucose control compared with those with atherogenic dyslipidemia at baseline. Moreover, BMI ratios were directly correlated with VLDL levels (r = 0.42, p = 0.007).

Table 1: Baseline characteristics of the study participants according to atherogenic dyslipidemia status

Clinical parameters	All (n = 40)	Absence of atherogenic dyslipidemia at baseline (n = 12)	Presence of atherogenic dyslipidemia at baseline (n = 28)	P
Clinical parameters				
Male gender, n (%)	27 (67.5%)	8 (66.6 %)	19 (67.8 %)	0.9
Age, years	49.3 ± 12.2	44.6 ± 8.2	52.3 ± 10.4	0.028
Charlson Comorbidity Index, points	5.57±1.5	4.83 ± 0.8	5.89 ± 1.75	0.06
BMI, kg/m^2	25.3 ± 4.3	23.03 ± 1.3	25.07 ± 2.54	0.01
Serum albumin, g/L	37.1 [34-39]	37.8 [36-40.5]	37 [31.9-39]	0.12
CRP, mg/L	9.8 [4.3 - 17.2]	9.8 [6.0-21]	10.8 [9.7-17.2]	0.26
Systolic blood pressure, mm Hg	129 ± 14.2	119 ± 10.2	129.3 ± 14.8	0.03
Diastolic blood pressure, mm Hg	78 ±12.4	75 ±11.2	79 ± 13.2	0.38
Hb, g/L	101.8 ±17.6	105.8 [99-124]	104 [94-121]	0.65
Glucose, mmol/L	5.4 ± 2.1	5.2 [4.8-5.5]	5.9 [4.8-5.1]	0.02
Ferritin, ng/ml	533 [338.5-832.7]	523 [348.5-822.1]	542 [382-785]	0.58
Calcium, mmol/L	2.18 [2.0 - 2.33]	2.25 [2.0 - 2.33]	2.11 [2.0-2.27]	0.33
Phosphorus, mmol/L	1.8 ± 0.5	1.7 ± 0.6	1.8 ± 0,4	0.9
iPTH, ng/L	225 [123 - 389]	215 [113 - 329]	253 [124-468]	0.11
Peritoneal dialysis parameters				
Time on PD, months	29 [18.5-37]	17.4 [15.0 – 31.3]	56.0 [32.0-68.1]	<0.0001
Urine volume, mL/24 h	570 [320-1200]	920 [680-1300]	450 [400-750]	0.002
D/P creatinine ratio	0.79 ± 0.08	0.71 ± 0.12	0.78 ± 0.18	0.03
Low-average transporters, n (%)	11 (27.5%)	4 (33.3%)	7 (25.0%)	0.59
High-average transporters,n (%)	11 (27.5%)	5 (41.6%)	6 (21.4%)	0.19
High transporters,n (%)	18 (45.0%)	3 (25.0%)	15 (53.6%)	0.09
Icodextrin, n (%)	5 (12.5%)	1 (8.3%)	4 (14.3%)	0.60
Renal weekly Kt/V	0.87 [0.44 - 1.43]	0.91 [0.52 - 1.33]	0.69 [0.22-0.94]	0.06
Plasma weekly Kt/V	1.46 [1.12 - 1.68]	1.69 [1.32-1.96]	1.35 [1.06-1.62]	0.004
Total Kt/V	2.26 [1.62-2.84]	2.1 [1.35-2.62]	1.78 [1.6-1.91]	0.11
CrCl, L/1.73 m^2	48.2±18.7	49.2 ± 11.05	47.4 ± 7.09	0.02
Daily peritoneal ultrafiltration, ml	600 [400-830]	600 [400-900]	400 [200-650]	0.01
Lipid profile parameters				
TC, mmol/L	4.95 [4.1-6.1]	4.9 [4.1-5.8]	5.2 [4.1-6.1]	0.3
TG, mmol/L	1.58 [1.07 - 2.82]	1.02 [0.85-1.94]	2.45 [1.08-3.32]	0.004
LDL, mmol/L	2.7 [2.4 - 3.3]	2.6 [2.1-2.9]	2.8 [2.4-3.7]	0.04
VLDL, mmol/L	0.63 [0.44 - 0.9]	0.7 [0.43-0.8]	1.14 [0.57-1.7]	0.0005
HDL, mmol/L	1.16 [0.9 - 1.56]	1.25 [0.98-1.3]	0.81 [0.71-1.0]	0.0003
AIP	3.6 [2.3 - 5.3]	2.9 [2.5-3.15]	4.8 [4.2-5.8]	<0.0001
Medications, n (%)				
ACE inhibitors / RAAS blockers	21 (52.5 %)	6 (50.0%)	15 (53.6%)	0.83
Iron supplementation	19 (47.5 %)	5 (41.6%)	14 (50.0%)	0.63
Erythropoietins	26 (65.0 %)	7 (58.3%)	19 (67.9%)	0.56
Beta-blockers	16 (40.0 %)	3 (25%)	13 (46.4%)	0.21
Calcium channel blockers	28 (70.0 %)	8 (66.6%)	20 (71.4%)	0.76
Diuretics	15 (37.5 %)	4 (33.3%)	11 (39.3%)	0.72
Lipid-lowering therapy	12 (30 %)	2 (16.6%)	10 (35.7%)	0.23

The values are expressed as mean ± standard deviation (M ± SD) or as the median and interquartile range (Me [Q25-Q75]). The values are compared between the groups by the Chi-square test, t-test and the Mann–Whitney U test as appropriate. *Abbreviations: AIP, atherogenic index of plasma; ACE, angiotensin-converting enzyme; BMI, body mass index; CrCl, creatinine clearance; CRP, C-Reactive Protein; Hb, hemoglobin; HDL-C, high-density lipoprotein cholesterol; iPTH, intact parathyroid hormone; LDL, low-density lipoprotein cholesterol; RAAS, renin-angiotensin-aldosterone system; TC, total cholesterol; total Kt / V, total weekly Kt / V urea; VLDL, very low density lipoprotein cholesterol.*

Besides, both groups showed significant differences in duration of PD therapy, daily urine output, dialysate to plasma ratio of creatinine (D/P creatinine), plasma weekly Kt/V, weekly creatinine clearance and daily peritoneal ultrafiltration.

Correlation analysis of baseline lipid profile with PD adequacy parameters

Pearson's correlation analysis was performed to investigate the correlation between lipid profile and PD adequacy variables. It was found that such levels as VLDL and HDL and, accordingly, AIP were significantly associated with duration of PD treatment: r = 0.4, p = 0.009; r = -0.34, p = 0.03 and r = 0.51, p < 0.0001 (Figure 1), respectively.

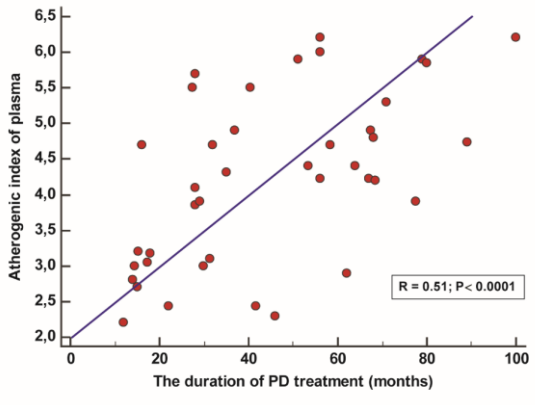

Figure 1: The correlation between AIP level and duration of PD treatment in PD patients

In addition, total weekly Kt/V was negatively correlated with serum level of plasma atherogenic index (r = -0.35, p = 0.02) and positively correlated with HDL (r = 0.35, p = 0.027) in PD patients (Figure 2).

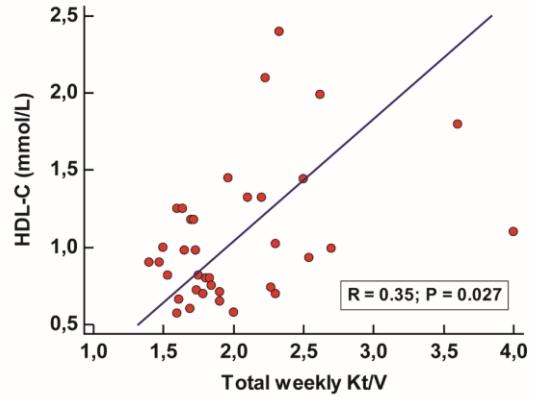

Figure 2: The correlation between HDL levels and total weekly Kt / V in PD patients

The other associations between baseline lipid profile and PD adequacy parameters are listed in Table 2.

Table 2: The correlations between lipid profile markers and PD adequacy parameters

PD adequacy parameters	Baseline lipid profile					
	TC	Triglyceride	LDL	VLDL	HDL	AIP
Duration of PD therapy (months)	r = 0.16 p = 0.29	r = -0.1 p = 0.53	r = 0.54 p = 0.0008	r = 0.4 p = 0.009	r = -0.34 p = 0.03	r = 0.51 p < 0.0001
Urine volume	r = -0.16 p = 0.15	r = 0.18 p = 0.14	r = -0.31 p = 0.01	r = 0.22 p = 0.21	r = 0.31 p = 0.007	r = 0.15 p = 0.19
D / P creatinine ratio	r = 0.13 p = 0.29	r = 0.06 p = 0.65	r = 0.5 p = 0.0001	r = 0.18 p = 0.16	r = -0.15, p = 0.23	r = 0.26 p = 0.04
Total Kt / V	r = -0.13 p = 0.27	r = -0.01 p = 0.93	r = -0.16 p = 0.19	r = -0.13 p = 0.03	r = 0.35 p = 0.027	r = -0.35 p = 0.02
Peritoneal Kt / V	r = -0.32 p = 0.004	r = -0.05 p = 0.67	r = -0.18 p = 0.15	r = -0.02 p = 0.85	r = 0.4 p = 0.0005	r = -0.17 p = 0.15
Renal Kt / V	r = -0.19 p = 0.09	r = -0.03 p = 0.78	r = -0.17 p = 0.17	r = -0.09 p = 0.44	r = 0.27 p = 0.02	r = -0.04 p = 0.69
Peritoneal CrCl	r = -0.33 p = 0.005	r = -0.09 p = 0.94	r = -0.25 p = 0.04	r = -0.05 p = 0.69	r = 0.33 p = 0.006	r = -0.04 p = 0.75
Daily peritoneal ultrafiltration	r = -0.27 p = 0.01	r = -0.26 p = 0.02	r = -0.12 p = 0.35	r = -0.23 p = 0.053	r = 0.05 p = 0.97	r = -0.19 p = 0.11

Abbreviations: AIP, atherogenic index of plasma; CrCl, creatinine clearance; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; total Kt / V, total weekly Kt / V urea; VLDL-C, very low-density lipoprotein cholesterol.

There was no correlation between triglyceride level and PD adequacy parameters, excluding daily peritoneal ultrafiltration volume. However, in contrast, baseline HDL level was positively correlated with urine output, total weekly Kt/V, peritoneal and renal Kt / V. Also, HDL level was negatively correlated with duration of PD therapy and D/P creatinine ratio.

Causes of PD technique failure

Fourteen of forty (35.0 %) patients experienced PD technique failure during the 36-month- follow-up period*:* 9/14 (64.3 %) participants who were transferred to hemodialysis and 5 patients (35.7%) who considered being “technique failures”. However, these 5 patients continued PD treatment for various reasons: inability of adequate vascular access formation-3/5 (60.0%) patients, refusal of HD treatment-1/5 (20.0 %) patients, decompensated heart failure-1 (20.0%) patient. The main reasons for technique failure were the following: dialysis inadequacy-7/14 (50.0%) patients, ascribed to peritonitis-5/14 (35.7%) patients and non-compliance-2/14 (14.3%) patients.

The incidence of all-cause PD technique failure was 2 times higher in the patients with atherogenic dyslipidemia compared with the patients without dyslipidemia: 2/12 (16.6 %) versus 12/28 (42.9 %) (Figure 3). However, we did not determine a statistically significant difference between the groups (χ^2 = 2.5; p = 0.12), which might be associated with small sample size.

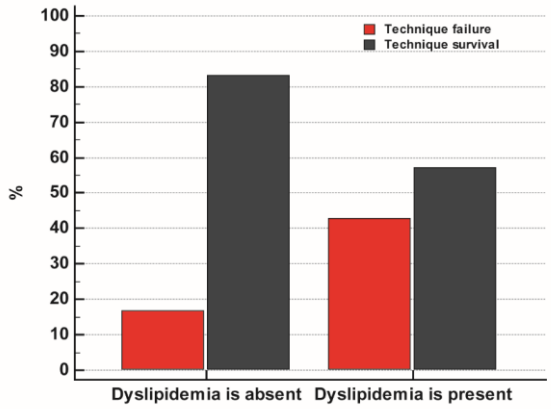

Figure 3: The incidence of all-cause PD technique failure stratified according to atherogenic dyslipidemia status

The effect of atherogenic dyslipidemia on all-cause technique failure

Figure 4 shows the results of the Kaplan-Meier analysis used to estimate technique survival. We observed a highly significant difference in technique failure rate in the patients with atherogenic dyslipidemia compared with the patients without dyslipidemia (log-rank p = 0.02). In the univariate Cox regression model, the presence of atherogenic

dyslipidemia was significantly associated with a higher risk of technique failure (HR 4.5; 95% CI 1.6 to 12.9; χ^2 = 5.5, p = 0.019).

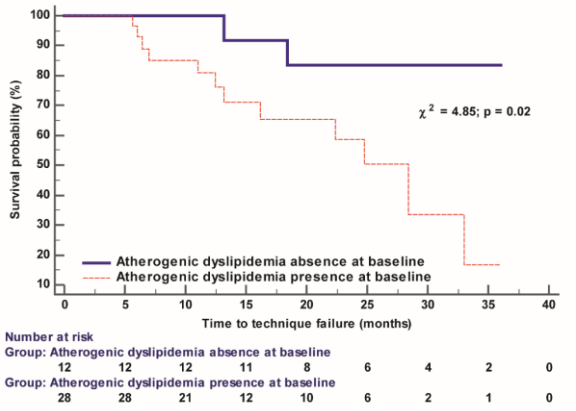

Figure 4: The Kaplan-Meier technique survival curves in PD patients dichotomised according to atherogenic dyslipidemia status

Discussion

Association between dyslipidemia and mortality in PD patients has been well characterized [12], [23], [24], [25]. However, there have been no reports on the association between pro-atherogenic lipid profiles and PD technique survival. To our knowledge, this study is the first prospective cohort study to compare technique survival in PD patients according to atherogenic dyslipidemia status.

One of the more significant findings to emerge from this study is that we found a strong association between lipid profile and PD adequacy parameters. Moreover, this study also found a significantly higher all-cause PD technique failure rate in patients with atherogenic dyslipidemia at baseline.

The effect of dyslipidemia on clinical outcomes in PD patients has not been carefully addressed. Also, the exact mechanism by which dyslipidemia affects a higher risk of all-cause technique failure remains unclear. On the other hand, the association between pro-atherogenic lipid profile and longer PD duration [26], a decrease in RRF [20], [27] and glucose absorption from the dialysis fluid [28], [29] can be a possible explanation of a higher risk of all-cause technique failure.

Cho et al. reported a statistically significant increase in serum TG, AIP and VLDL levels and a decrease in serum HDL levels were associated with longer PD treatment time while the type of PD solution received had no significant effect on these levels [26]. Consistent with this notion, we found a strong positive correlation between the duration of PD therapy and an increase in LDL, VLDL and AIP levels, whereas LDL level decreased over the PD treatment time.

Chen et al. demonstrated different longitudinal changes in lipid profiles in PD patients with different RRF. They also showed an association between a decrease in HDL and deterioration of RRF [20]. LinYC et al. analysed the data from a nationwide population-based cohort study and concluded that the effect of lipid profiles on mortality was modified by RRF [30]. In the present study, we did not analyse RRF in the patients. However, the current study supports the results of the findings mentioned above and confirms the idea of a positive association between urine volume and HDL level. Moreover, we observed a significant decrease in residual diuresis in dyslipidemia conditions. Additionally, fluid and weight control problems are common amongst PD patients and may be *accompanied* by dyslipidemia [16], [20], [25], [29], [31]. Rincón et al. found the mean increase in fat mass of 3.0 ± 3.2 kg/year during the 26-month-follow-up period. Changes in fat mass were directly associated with an inverse correlation between fat mass and HDL in PD patients [28]. Besides, they concluded that changes in body composition in patients undergoing PD might affect other parameters, namely, alteration in lipid profile [28]. We did not determine the fat mass in this study. On the other hand, we observed an increase in BMI which was significantly associated with high levels of VLDL.

Glucose absorption from the dialysis fluid can be considered as an important risk factor for increased insulin resistance, enhanced hepatic synthesis and secretion of VLDL-C [31]. As a result, administration of glucose-based PD solutions leads to structural and functional changes in the peritoneal membrane resulting in fluid overload, impaired glucose control, altered lipid profiles and peritoneal dialysis technique failure [23], [32], [33]. This research supports previous findings. Thus, we found a direct correlation between D/P creatinine ratio and LDL levels and an inverse correlation between daily peritoneal ultrafiltration and triglyceride level (Table 2). We reported total weekly Kt/V urea was associated with high levels of LDL and AIP (Table 2).

It is almost certain that further investigation is needed to determine the role of atherogenic dyslipidemia in the pathogenesis of PD technique failure. Furthermore, inflammation and malnutrition can affect lipid levels and PD technique survival [34]. However, these factors were not included in the study. Our investigation demonstrated a significant association between atherogenic dyslipidemia and a higher risk of technique failure in PD patients. Thus, it becomes possible to hypothesise that lipid-lowering therapy can reduce not only CVD risk but also improve technique survival in PD patients.

Finally, several important limitations need to be considered

First, it was a small sample size study performed in a single-centre study. Therefore, our

findings revealed only associations. Further study with a larger sample size from multiple dialysis centres is needed to confirm our results. Second, only baseline data were used for this analysis. The current study was limited by baseline data. Third, we did not investigate the effect of changes in lipid profiles on the development of CVD in PD patients during the observation period.

Moreover, dialysis-related, patient-related and systemic factors could affect both the development of atherosclerosis and PD technique failure. However, these factors were not considered in our study. Despite the limitations mentioned above, the strong association between dyslipidemia and PD technique survival observed in the present study indicated the important role of dyslipidemia in predicting PD technique failure. The larger-scale studies are needed for further confirmation of our findings.

In conclusion, the presence of atherogenic dyslipidemia in PD patients was significantly associated with a higher risk of technique failure. The results of the present study demonstrated that dyslipidemia in PD patients could be considered not only as a traditional risk factor for CVD but also as a predictor of PD technique survival. Further well-designed clinical trials are needed to determine the impact of dyslipidemia on PD adequacy and technique survival.

Authors' Contribution

NS**:** the concept, design; analysed and interpreted the patient data; a major contributor in writing the manuscript. OB**:** performed the data collection, the manuscript preparation.

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References

1. Barone R, Cámpora M, Gimenez N, Ramirez L, Panese S, Santopietro M. Peritoneal Dialysis as a first versus second option after previous haemodialysis: a very long-term assessment. Int J Nephrol. 2014; 2014:693670. https://doi.org/10.1155/2014/693670 PMid:25505992 PMCid:PMC4258321

2. Jain AK, Blake P, Cordy P, Garg AX. Global trends in rates of peritoneal dialysis. J Am Soc Nephrol. 2012; 23(3):533-44. https://doi.org/10.1681/ASN.2011060607 PMid:22302194 PMCid:PMC3294313

3. Kolesnyk I, Noordzij M, Kolesnyk M, Kulyzky M, Jager K. Renal replacement therapy in Ukraine: epidemiology and international comparisons. Clin Kidney J. 2014; 7(3):330-5. https://doi.org/10.1093/ckj/sfu037 PMid:25852905 PMCid:PMC4377756

4. Velloso MS, Otoni A, de Paula Sabino A, de Castro WV, Pinto SW, Marinho MA, et al. Peritoneal dialysis and inflammation. Clin Chim Acta. 2014; 430:109-14. https://doi.org/10.1016/j.cca.2013.12.003 PMid:24333488

5. Cho Y, Hawley CM, Johnson DW. Clinical causes of inflammation in peritoneal dialysis patients. Int J Nephrol. 2014; 2014:909373. https://doi.org/10.1155/2014/909373 PMid:24895536 PMCid:PMC4033334

6. Baroni G, Schuinski A, de Moraes T, Meyer F, Pecoits-Filho R. Inflammation and the peritoneal membrane: causes and impact on structure and function during peritoneal dialysis. Mediators Inflamm. 2012; 2012:912595. https://doi.org/10.1155/2012/912595 PMid:22547910 PMCid:PMC3323921

7. Termorshuizen F, Korevaar JC, Dekker FW, Van Manen JG, Boeschoten EW, et al. Hemodialysis and peritoneal dialysis: comparison of adjusted mortality rates according to the duration of dialysis: analysis of The Netherlands Cooperative Study on the Adequacy of Dialysis 2. J Am Soc Nephrol. 2003; 14(11):2851-60. https://doi.org/10.1097/01.ASN.0000091585.45723.9E PMid:14569095

8. Jaar BG, Coresh J, Plantinga LC, Fink NE, Klag MJ, et al. Comparing the risk for death with peritoneal dialysis and hemodialysis in a national cohort of patients with chronic kidney disease. Ann Intern Med. 2005;143(3):174-83. https://doi.org/10.7326/0003-4819-143-3-200508020-00003 PMid:16061915

9. Wu C-L, Wu H-M, Chiu P-F, Liou HH, Chang CB, Tarng DC, et al. Associations between the duration of dialysis, endotoxemia, monocyte chemoattractant protein-1, and the effects of a short-dwell exchange in patients requiring continuous ambulatory peritoneal dialysis. Stover CM, ed. PLoS One. 2014; 9(10):e109558. https://doi.org/10.1371/journal.pone.0109558 PMid:25286027 PMCid:PMC4186838

10. Tonelli MA, Wanner C. Kidney disease: improving global outcomes (KDIGO) lipid work group. KDIGO clinical practice guideline for lipid management in chronic kidney disease. Kidney International Supplements. 2013; 3(3):1-315.

11. Chiu YW, Mehrotra R. Can we reduce the cardiovascular risk in peritoneal dialysis patients? Indian J Nephrol. 2010; 20(2):59-67. https://doi.org/10.4103/0971-4065.65296 PMid:20835317 PMCid:PMC2931134

12. Mikolasevic I, Žutelija M, Mavrinac V, Orlic L. Dyslipidemia in patients with chronic kidney disease: etiology and management. Int J Nephrol Renovasc Dis. 2017; 10:35-45. https://doi.org/10.2147/IJNRD.S101808 PMid:28223836 PMCid:PMC5304971

13. Omran J, Al-Dadah A, Dellsperger KC. Dyslipidemia in patients with chronic and end-stage kidney disease. Cardiorenal Med. 2013; 3(3):165-77. https://doi.org/10.1159/000351985 PMid:24454313 PMCid:PMC3884190

14. Yang W-L, Zhu X-Y, Zhu N, Chun-Yan Su, Qing-Feng Han, Tao Wang, et al. What's the optimal lipids level for dialysis patients? A cohort study from a Chinese dialysis center in a university hospital. PLoS One. 2016; 11(12):e0167258. https://doi.org/10.1371/journal.pone.0167258 PMid:27992532 PMCid:PMC5161355

15. Shurraw S, Tonelli M. Statins for treatment of dyslipidemia in chronic kidney disease. Perit Dial Int. 2006; 26:523-39.

16. Rincón Bello A, Bucalo L, Abad Estébanez S, Barraca Núñez

D, Yuste Lozano C, Pérez de José A, et al. Fat tissue and inflammation in patients undergoing peritoneal dialysis. Clinical Kidney Journal. 2016; 9(3):374-80. https://doi.org/10.1093/ckj/sfw007 PMid:27274820 PMCid:PMC4886903

17. de Mattos AM, Ovidio PP, Jordão AA, da Costa JA, Chiarello PG. Association of body fat with inflammation in peritoneal dialysis. Inflammation. 2013; 36(3):689-95. https://doi.org/10.1007/s10753-013-9593-3 PMid:23321723

18. Fernström A, Hylander B, Moritz A, Jacobsson H, Rössner S. Increase of intra-abdominal fat in patients treated with continuous ambulatory peritoneal dialysis. Perit Dial Int. 1998; 18(2):166-71.

19. Harmankaya O, Akalin N, Akay H, Okuturlar Y, Erturk K, Kaptanogullari H, et al. Comparison of risk factors for cardiovascular disease in hemodialysis and peritoneal dialysis patients. Clinics. 2015; 70(9):601-605. https://doi.org/10.6061/clinics/2015(09)01

20. Chen H-Y, Tsai W-C, Chiu Y-L, Hsu SP, Pai MF, Yang JY, et al. Triglyceride to high-density lipoprotein cholesterol ratio predicts cardiovascular outcomes in prevalent dialysis patients. Medicine. 2015; 94(10):e619. https://doi.org/10.1097/MD.0000000000000619 PMid:25761189 PMCid:PMC4602469

21. Ronco C. Adequacy of peritoneal dialysis is more than Kt/V. Nephrol Dial Transplant. 1997; 12(1):68-73.

22. Heimburger O. How should we measure peritoneal dialysis adequacy in the clinic. Contrib Nephrol. 2009; 163:140-6. https://doi.org/10.1159/000223792 PMid:19494607

23. Liao CT, Kao TW, Chou YH, Wu MS, Chen YM, Chuang HF, et al. Associations of metabolic syndrome and its components with cardiovascular outcomes among non-diabetic patients undergoing maintenance peritoneal dialysis. Nephrol Dial Transplant. 2011; 26(12):4047-54. https://doi.org/10.1093/ndt/gfr175 PMid:21565947

24. Habib AN, Baird BC, Leypoldt JK, Cheung AK, Goldfarb-Rumyantzev AS. The association of lipid levels with mortality in patients on chronic peritoneal dialysis, Nephrol Dial Transplant, 2006; 21(10):2881-92. https://doi.org/10.1093/ndt/gfl272 PMid:16735386

25. Lin YC, Lin YC, Peng CC, Chen KC, Chen HH, Fang TC, et al. Effects of cholesterol levels on mortality in patients with long-term peritoneal dialysis based on residual renal function. Nutrients. 2018; 10(3):300. https://doi.org/10.3390/nu10030300 PMid:29510483 PMCid:PMC5872718

26. Cho Y, Büchel J, Steppan S, et al. Longitudinal trend in lipid profile of incident peritoneal dialysis patients is not influenced by the use of biocompatible solutions. Perit Dial Int. 2016; 36(2):146-153. https://doi.org/10.3747/pdi.2014.00291 PMid:26429421 PMCid:PMC4803359

27. Liu X, Dai C. Advances in understanding and management of residual renal function in patients with chronic kidney disease. Kidney Dis. 2017; 2(4):187-196. https://doi.org/10.1159/000449029 PMid:28232935 PMCid:PMC5260570

28. Rincón Bello A, Bucalo L, Abad Estébanez S, Vega Martínez A, Barraca Núñez D, Yuste Lozano C, et al. Fat tissue and inflammation in patients undergoing peritoneal dialysis. Clin Kidney J. 2016; 9(3):374-380. https://doi.org/10.1093/ckj/sfw007 PMid:27274820 PMCid:PMC4886903

29. Guo Q, Lin J, Li J, Yi C, Mao H, Yang X, et al. The effect of fluid overload on clinical outcome in southern chinese patients undergoing continuous ambulatory peritoneal dialysis. Perit Dial Int. 2015; 35(7):691-702. https://doi.org/10.3747/pdi.2014.00008 PMid:26152580 PMCid:PMC4690624

30. Lin YC, Lin YC, Peng CC, et al. Effects of Cholesterol Levels on Mortality in Patients with Long-Term Peritoneal Dialysis Based on Residual Renal Function. Nutrients. 2018; 10(3):300. https://doi.org/10.3390/nu10030300 PMid:29510483 PMCid:PMC5872718

31. Tsimihodimos V, Mitrogianni Z, Elisaf M. Dyslipidemia associated with chronic kidney disease. Open Cardiovasc Med J. 2011; 5:41-48. https://doi.org/10.2174/1874192401105010041 PMid:21643500 PMCid:PMC3106357

32. Holmes CJ. Reducing cardiometabolic risk in peritoneal dialysis patients: role of the dialysis solution. J Diabetes Sci Technol. 2009; 3(6):1472-1480. https://doi.org/10.1177/193229680900300629 PMid:20144403 PMCid:PMC2787049

33. Mehrotra R, Devuyst O, Davies SJ, Johnson DW. The current state of peritoneal dialysis. . J Am Soc Nephrol. 2016; 27(11):3238-3252. https://doi.org/10.1681/ASN.2016010112 PMid:27339663 PMCid:PMC5084899

34. Liu Y, Coresh J, Eustace JA, Longenecker JC, Jaar B, Fink NE, Tracy RP, Powe NR, Klag MJ. Association between cholesterol level and mortality in dialysis patients: role of inflammation and malnutrition. Jama. 2004; 291(4):451-9. https://doi.org/10.1001/jama.291.4.451 PMid:14747502

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Pulse Pressure Variation-Guided Fluid Therapy during Supratentorial Brain Tumour Excision: A Randomized Controlled Trial

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Keywords: Goal-directed fluid therapy; Neurosurgical operations; Pulse pressure variation; Supratentorial mass excision

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Abbreviations: ANOVA: Analysis of variance; BRS: brain relaxation scale; CVP: central venous pressure; MAP: mean arterial pressure; GDT: goal-directed fluid therapy; PPV: pulse pressure variation; SPSS: Statistical package for social science

Abstract

BACKGROUND: Goal-directed fluid therapy (GDFT) improved patient outcomes in various surgical procedures; however, its role during mass brain resection was not well investigated.

AIM: In this study, we evaluated a simple protocol based on intermittent evaluation of pulse pressure variation for guiding fluid therapy during brain tumour resection.

METHODS: Sixty-one adult patients scheduled for supratentorial brain mass excision were randomized into either GDFT group (received intraoperative fluids guided by pulse pressure variation) and control group (received standard care). Both groups were compared according to the following: brain relaxation scale (BRS), mean arterial pressure, heart rate, urine output, intraoperative fluid intake, postoperative serum lactate, and length of hospital stay.

RESULTS: Demographic data, cardiovascular data (mean arterial pressure and heart rate), and BRS were comparable between both groups. GDFT group received more intraoperative fluids {3155 (452) mL vs 2790 (443) mL, P = 0.002}, had higher urine output {2019 (449) mL vs 1410 (382) mL, P < 0.001}, and had lower serum lactate {0.9 (1) mmol versus 2.5 (1.1) mmol, P = 0.03} compared to control group.

CONCLUSION: In conclusion, PPV-guided fluid therapy during supratentorial mass excision, increased intraoperative fluids, and improved peripheral perfusion without increasing brain swelling.

Introduction

Neurosurgical operations are characterised by major fluid shift, frequent use of diuretics, and prolonged operative time. The role of fluid therapy in these patients is very critical; hypovolemia might decrease cerebral perfusion; while, fluid over-infusion might swell the brain. Thus, fluid management in these procedures complex and challenging. Evidence on the optimum protocol for intraoperative fluid management in neurosurgical patients is still lacking.

Adequate intracranial volume management is considered a key factor that would overcome the tumour bulk and the surrounding vasogenic oedema facilitating surgical access [1]. Thus, a relaxed brain is one of the targets of intraoperative fluid management during craniotomy [1], [2]. The slack brain would allow proper surgical retraction and consequently, reduces brain retractor ischemia. Brain relaxation scale (BRS) had shown a good correlation with intracranial pressure [3]; thus, an increasing interest was paid to BRS as a simple surrogate for intracranial pressure [1], [2], [4].

Goal-directed hemodynamic therapy (GDT) in the operating room is a term used to describe the use of defined hemodynamic targets to guide intravenous fluid and inotropic therapy [5]. Pulse pressure variation (PPV) is one of the robust dynamic indices of fluid responsiveness which is based on heart-lung interactions [6]. GDT had been frequently investigated in the operating room in high-risk patients especially in major surgery [7], [8], [9]. However, the impact of GDT

on patient outcomes, especially BRS, is not well evaluated in brain surgery. In this study, we evaluated PPV-guided fluid management compared to standard fluid management in patients undergoing supratentorial mass excision. We hypothesised that in these procedures, GDT might restrict intraoperative fluid volume, improve brain relaxation, and provide stable patient hemodynamics.

Methods

A randomised, controlled study was conducted in Cairo University hospitals between March 2017 and January 2018, after being approved by the research ethics board (MD-4-2016). Written informed consent was obtained from all subjects who participated in this trial. The study was registered before patient enrolment at clinicaltrials.gov registry system (NCT03033706, principal investigator: Ahmed Hasanin, date of registration: January 27th, 2017).

Sixty-one patients scheduled for supratentorial mass excision were enrolled in the study. Patients less than 18 years old, patients with arrhythmias, pulmonary hypertension, impaired cardiac contractility, impaired liver or kidney function, and patients with body mass index above 40 were excluded. Randomisation was achieved using a computer-generated sequence. Opaque envelopes were used to assign patients in one of the two study groups.

Management of anaesthesia

Patients were pre-medicated with ranitidine (50 mg) and ondansetron (4 mg). Anaesthesia was induced using Propofol 2 mg/kg, atracurium 0.5 mg/Kg, and fentanyl 2 µg/Kg, and maintained using isoflurane (1-1.5%) and atracurium 0.5 mg/Kg/h. After induction of anaesthesia, arterial and right internal jugular central venous catheters were inserted. The endotracheal tube was inserted, and mechanical ventilation was adjusted at a tidal volume of 8 mL/Kg, no PEEP, respiratory rate was adapted to maintain end-tidal CO_2 between (30-35 mmHg). Patients' monitors included: continuous electrocardiograph, continuous invasive arterial pressure monitor, pulse oximetry, end-tidal CO_2 monitor, central venous pressure (CVP), and non-invasive blood pressure monitor. Mannitol (0.5 gm/Kg) was administered shortly after induction. Phenytoin (15 mg/Kg) was administered for loading if the patient was not previously loaded. Maintenance bolus of phenytoin (5 mg/Kg) was administered for previously loaded patients. Additional doses of fentanyl (50 µg/dose) were titrated with skin incision, and bur-hole keep means arterial pressure (MAP) and heart rate within 25% of the baseline reading. By the end of the operation; isoflurane was discontinued, and the residual neuromuscular blocking agent was reversed by neostigmine 0.05 mg/Kg and atropine 0.02 mg/Kg. Patients were extubated and admitted to the surgical intensive care unit.

Fluid therapy

All patients received a fluid bolus of 5 ml/kg Tetrastarch (Voluven: Hydroxyethyl starch 130/0.4 in isotonic sodium chloride solution manufactured by Fresenius Kabi Germany) after induction of anaesthesia, then fluid management was as follows:

Control group: in this group, patients received standard fluid management of 4 ml/Kg/hr ringer solution. If MAP decreased by 20% without obvious bleeding, the patient received a rescue fluid bolus of 3 mL/Kg Ringer solution over 5 minutes if CVP was lower than 4 mmHg, and received ephedrine bolus of 9 mg if CVP was higher than 4 mmHg.

GDT group: in this group, patients received a restricted fluid protocol of 1 ml/Kg/hr with concomitant PPV monitoring. The fluid bolus of 3 ml/Kg of ringer solution was administrated whenever PPV was higher than 13%. If the MAP decreased by 20% without obvious bleeding, the patient received ephedrine bolus of 9 mg.

PPV was assessed using invasive blood pressure monitor (GE solar 8000M/I monitor) every 15 minutes. PPV was calculated using the following equation [6]:

$$PPV(\%) = \frac{(PPmax - PPmin)}{\frac{(PPmax + PPmin)}{2}} \times 100$$

Maximal PP (PP_{max}) and minimal PP (PP_{min}) were obtained from the same respiratory cycle. PPV was calculated as the average of measurements obtained over three consecutive respiratory cycles.

In both groups, urine output was calculated and replaced using Ringer's solution. Blood loss was replaced by tetrastarch solution in the ratio of 1:1. Packed RBCs were transfused if blood haemoglobin was less than 7 g/dL.

Outcomes

Our primary outcome was brain Relaxation scale (BRS). BRS was assessed by the neurosurgeon at 3-time intervals; with dural opening, after two hours, and before dural closure. A 4-point scale [2] was performed as follows: grade 1, perfectly relaxed; grade 2, satisfactorily relaxed; grade 3, firm brain; grade 4, bulging brain. The surgeon was blinded to the study group. The end-tidal CO_2 was tightly controlled at 30-35 mmHg during the assessment of BRS.

In addition to demographic data (age, gender,

weight, ASA class, incidence of disturbed conscious level "defined as Glasgow Coma Scale below 15", and incidence of preoperative neurological deficit "defined as as upper and/or lower limb weaknesses in contralateral side of the tumor; the presence of dysphasia, visual field defects were considered as neurological deficits as well."), other outcomes included: Intraoperative fluid requirements, patient position during the operation, arterial-jugular oxygen saturation difference, arterial-Jugular lactate difference, urine output, vital signs (heart rate and MAP) arterial blood gases, intraoperative ephedrine consumption, postoperative electrolytes (Na, K, Mg, and Ca), postoperative serum lactate, postoperative 24-hour urine output, postoperative hemoglobin, and length of hospital stay.

Statistical analysis and sample size calculation

Our primary outcome was brain relaxation scale. A previous study [2] had assumed that a difference in mean BRS of 1 ± 1 is a clinically significant difference. We used a more conservative assumption to detect the same mean difference in BRS with a higher study power (95%). A minimum number of 26 patients per group was calculated Using MedCalc Software version 14.10.2 (MedCalc Sofware bvba, Ostend, Belgium), the minimum calculated number of participants to have a study power of 95% and an alpha error of 0.05 was 27 patients per group.

Statistical package for social science (SPSS) software, version 15 for Microsoft Windows (SPSS inc., Chicago, iL, USA) was used for data analysis. Continuous data were checked for normality using the Shapiro-Wilk test and was presented as mean (standard deviation) or median (interquartile range) as appropriate. Continuous data were analysed using unpaired t-test or Mann Whitney test as appropriate. Categorical data were presented as frequency (%) and analysed by chi-squared test. Repeated measures were analysed using analysis of variance (ANOVA) for repeated measures with post-hoc pairwise comparisons using the Bonferroni test. A P value less than 0.05 was considered statistically significant.

Results

Seventy-five patients were screened for eligibility. Fourteen patients were excluded (10 patients were excluded from meeting one of the exclusion criteria, and 4 patients declined to participate in the study). Sixty-one patients were available for final analysis (Figure 1).

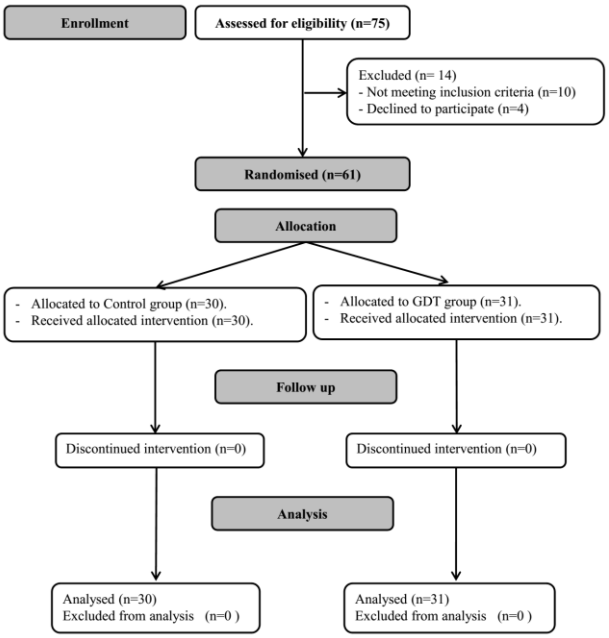

Figure 1: Consort chart showing patient recruitment; GDT: Goal-directed fluid therapy

Both groups were comparable according to demographic data and baseline characteristics (Table 1).

Table 1: Demographic data and patient characteristics. Data are presented as mean (standard deviation), and frequency (%)

	Control group (n = 30)	GDT group (n = 31)	P value
Age (years)	39 (13)	41 (12)	0.43
Weight (Kg)	77 (12)	76 (10)	0.85
Male gender	12 (40%)	16 (52)	0.4
ASA class (I/II)	20/10	19/12	0.87
DCL	1 (3%)	2 (7%)	1
Increased ICP	2 (7%)	4 (13%)	0.7
Neurological deficit	3 (10%)	2 (7%)	0.7
Convulsions	3 (10%)	2 (7%)	0.6
Tumour type (%)			0.93
Meningioma	50%	46%	
Glioma	25%	18%	
Intraventricular tumour	8%	14%	
Craniopharyngioma	8%	5%	
Other lesions	9%	17%	
Largest tumour diameter	5.9 (3)	5.4 (1.7)	0.42
Surgical duration (hours)	5.3 (1.1)	5.5 (0.9)	0.48
Baseline heart rate (bpm)	82 (7)	83 (7)	0.56
Baseline MAP (mmHg)	88 (9)	92 (10)	0.12

ASA: American society of anesthesiology classification; DCL: disturbed conscious level; ICP: intra-cranial pressure; MAP: mean arterial pressure.

All patients were operated in a supine, head-up position. The GDT group received a greater number of fluid boluses and had higher total fluid consumption compared to the control group (Table 2).

Table 2: Fluid management and urine output. Data are presented as mean (standard deviation), and median (quartiles)

	Control group (n = 30)	GDT group (n = 31)	P value
Infused fluids (mL)			
Total		3155 (452) *	0.002
Crystalloids	2790 (443)	2775 (423) *	0.01
Colloids	2408 (418)	380 (51)	0.85
	383 (61)		
Urine output (mL)	1410 (382)	2019 (449) *	< 0.001
Blood loss (mL)	897 (430)	887 (377)	0.93
Blood transfusion (mL)	885 (363)	773 (334)	0.45
Number of fluid boluses	2 (1.2)	5 (5.6) *	< 0.001
Number of vasopressor boluses	0 (0.1)	0 (0.1)	0.093

*denotes statistical significance compared to the control group.

Higher urine output and lower postoperative serum lactate were reported in the GDT group compared to the control group (Table 2, and 4).

Table 3: Brain relaxation scores. Data are presented as mean (standard deviation), and median (quartiles)

	Control group (n = 30)	GDT group (n = 31)	P value
BRS-1			0.15
Mean (SD)	1.8 (0.7)	1.54 (0.7)	
Median (quartiles)	2 (1.2)	1 (1.2)	
BRS-2			0.66
Mean (SD)	1.9 (0.6)	1.8 (0.4)	
Median (quartiles)	2 (2.2)	2 (2,2)	
BRS-3			0.34
Mean (SD)	1.7 (0.4)	1.58 (0.5)	
Median (quartiles)	2 (1.2)	2 (1.2)	

BRS: brain relaxation score, SD: standard deviation.

No significant differences were reported between both groups in BRS (Table 3), intraoperative mean arterial pressure (Figure 2), nor heart rate.

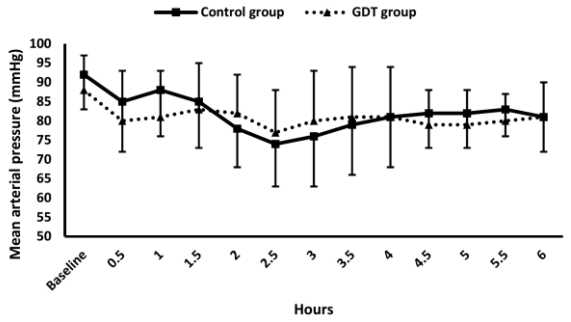

*Figure 2: Mean arterial pressure; Markers are means, error bars are standard deviations; * denotes statistical significance compared to the baseline reading within the control group. † Denotes statistical significance compared to the baseline reading within the GDT group; GDT: Goal-directed fluid therapy*

Postoperative laboratory investigations (haemoglobin, coagulation profile, and electrolytes), postoperative intensive care unit stay and postoperative hospital stay were comparable between both groups (Table 4).

Table 4: Postoperative data. Data are presented as mean (standard deviation), and median (quartiles)

	Control group (n = 30)	GDT group (n = 31)	P value
PH	7.38 (0.09)	7.39 (0.06)	0.56
Lactate (mmol/L)	2.5 (1.1)	0.9 (1) *	0.03
ScVo2 (%)	75 (6.3)	74 (5.2)	0.69
Arterio-jugular Spo2 difference (%)	23.6 (7.6)	25.5 (5.1)	0.26
Arterio-jugular lactate difference (mmol/L)	0.5 (0.2,0.6)	0.4 (0.2,0.5)	0.25
Postoperative urine output (mL)	1152 (261)	1277 (269)	0.07
ICU stay (days)	2.1 (1.3)	2.1 (1.2)	0.93
Hospital stay (days)	5.7 (1.5)	5.2 (1.3)	0.13

Scvo2: central venous oxygen saturation; Spo2: arterial oxygen saturation; * denotes statistical significance compared to the control group.

Discussion

We found that the GDT group received more fluids compared to the control group without impacting BRS. Postoperative serum lactate was lower in the GDT denoting better peripheral organ perfusion compared to the control group. Patients undergoing craniotomy receive brain dehydrating measures; thus, they are vulnerable to intravascular volume depletion. Using PPV as a marker of volume status allowed accurate and early detection of hypovolemia; thus, more fluids were infused in the GDT group compared to the standard therapy group. The relatively better peripheral perfusion in the GDT group is most probably due to adequate and early restoration of intravascular volume using an accurate and reliable hemodynamic target before the occurrence of hypotension. The use of CVP as a measure for fluid responsiveness is declining; however, it is still conventionally used by many physicians [10], [11]. PPV had been reported to be a more accurate index for fluid responsiveness especially in mechanically ventilated patients [6].

In our study, we tried to answer two questions: 1- Would the PPV-guided therapy impact BRS? 2- Would PPV-guided therapy improve the hemodynamic profile and peripheral perfusion? We hypothesised that using GDT might avoid unnecessary excessive fluids; thus, we designed our study to provide a baseline restrictive fluid infusion rate (1 mL/Kg/hour) in the GDFT group with additional fluid boluses according to the PPV values. Surprisingly, the GDT group required more fluids than the control group due to the higher number of fluid boluses.

In our patients, better peripheral perfusion was reported in the GDT group. This was represented by the lower postoperative serum lactate level in addition to higher fluid volume and a smaller number of vasopressor boluses in the GDT group. Serum lactate is an important marker of tissue perfusion [12]. Postoperative serum lactate is a commonly used marker of peripheral perfusion during craniotomy [2], [4], [13], [14]. Elevated serum lactate level is usually associated with poor outcomes [14], especially in patients undergoing craniotomy [16]. Our results are in line with two recent randomised controlled studies. In the first study, Wu et al., [13] had recently compared two-stroke volume variation-based fluid protocols in neurosurgical procedures. Wu et al. had reported that the more liberal fluid protocol (targeting lower stroke volume variation) was associated with lower postoperative serum lactate compared to the restrictive protocol (targeting higher stroke volume variation). In the second study, Sundaram et al. compared PPV-directed therapy to CVP-guided therapy and found that the patients in PPV-guided therapy group received more fluids and had more stable hemodynamic profile compared to the CVP-guided group [17]. Our study showed similar results to the two mentioned studies concerning the higher fluid requirements and the better peripheral perfusion. Furthermore, we had also reported that the higher fluid requirements in the GDT group were not

associated with brain swelling.

In a randomised controlled study, Luo et al. had investigated stroke volume variation-guided fluid therapy in brain surgeries [18]. Unlike our study, Luo et al. had given less intraoperative fluids in their study group compared to the control group. Two differences between our study and Luo study might account to the different findings: 1-Their fluid protocol in the control group was not well clarified. 2-They had used higher baseline fluid infusion rate (3 mL/Kg/hour) in their study group compared to our baseline infusion rate (1 mL/Kg/hour).

Two previous studies had evaluated the impact of GDT on BRS; however, both studies aimed to compare colloid and crystalloid solutions in neurosurgical patients [4], [19]; thus, they used GDT in both study groups and did not compare GDT to standard care. Our study is the first to evaluate the impact of GDT, compared to standard care, on BRS in addition to intraoperative fluid requirements and peripheral perfusion.

Usually, the neurosurgical patient is kept as dry as possible. However, to which extent should we restrict fluids? This question has not been adequately answered. The relationship between perioperative fluid administration and patient outcome is U-shaped. Under-transfusion results in cerebral, myocardial, and renal ischemia; while, fluid overload leads to lung congestion, brain oedema, and delayed wound healing [20]. Neurosurgical procedures are characterised by difficult assessment of blood loss under the drapes especially in the presence of irrigating fluids. The frequent use of diuretics makes urine output unreliable index for evaluation of volume status. Thus, it is necessary to use reliable parameters for guiding fluid management. It is highly believed that fluid administration should target clear hemodynamic variables rather than targeting traditional, fixed infusion rate. We chose PPV as a target for fluid management in our patients as it is a simple, accurate dynamic method of fluid responsiveness in mechanically ventilated patients [6]. Based on our findings, we found that using PPV for guiding fluid management in neurosurgical patients is feasible and has promising results. Using PPV would be also beneficial for patients whom anesthesiologists do not prefer central venous line insertion. Our findings might draw attention to the under-determined fluid needs during mass brain resection.

One of the limitations for implementing GDT in the operating room is the need for either complicated algorithms or sophisticated equipment. Introduction of simple protocols for GDT would enable wider application in different settings. Our study has the advantage of the use of a simple GDT protocol based on optimisation of PPV which needs only an arterial line. Most of other GDT protocols are based on either complicated algorithms or sophisticated equipment.

Our study had some limitations: 1-It is a single centre study. 2-All our operations were elective operations. 3-We had a low incidence of disturbed conscious level and increased intracranial pressure; thus, extrapolation of our findings in these groups of patients' needs more research.

In conclusion, guiding fluid therapy using manual, intermittently calculated PPV during supratentorial mass excision increased intraoperative fluid administration and improved peripheral perfusion without impacting brain relaxation. More research is warranted to reach the optimum strategy and the best cut-off targets.

Ethical approval and consent to participate

Ethical approval from Cairo university hospitals research committee was obtained (MD-4-2016)**.** Written informed consents were obtained from participants before inclusion.

References

1. Quentin C, Charbonneau S, Moumdjian R, et al. A comparison of two doses of mannitol on brain relaxation during supratentorial brain tumor craniotomy: A randomized trial. Anesth Analg. 2013; 116(4):862-868. https://doi.org/10.1213/ANE.0b013e318282dc70 PMid:23354336

2. Rozet I, Tontisirin N, Muangman S, et al. Effect of equiosmolar solutions of mannitol versus hypertonic saline on intraoperative brain relaxation and electrolyte balance. Anesthesiology. 2007; 107(5):697-704. https://doi.org/10.1097/01.anes.0000286980.92759.94 PMid:18073543

3. Todd MM, Warner DS, Sokoll MD, et al. A prospective, comparative trial of three anesthetics for elective supratentorial craniotomy. Propofol/fentanyl, isoflurane/nitrous oxide, and fentanyl/nitrous oxide. Anesthesiology. 1993; 78(6):1005-1020. https://doi.org/10.1097/00000542-199306000-00002 PMid:8512094

4. Xia J, He Z, Cao X, et al. The brain relaxation and cerebral metabolism in stroke volume variation-directed fluid therapy during supratentorial tumors resection: crystalloid solution versus colloid solution. J Neurosurg Anesthesiol. 2014; 26(4):320-327. https://doi.org/10.1097/ANA.0000000000000046 PMid:24487733

5. Bisgaard J, Gilsaa T, Rønholm E, Toft P. Optimising stroke volume and oxygen delivery in abdominal aortic surgery: a randomised controlled trial. Acta Anaesthesiol Scand. 2013; 57(2):178-188. https://doi.org/10.1111/j.1399-6576.2012.02756.x PMid:22897633

6. Hasanin A. Fluid responsiveness in acute circulatory failure. J intensive care. 2015; 3:50. https://doi.org/10.1186/s40560-015-0117-0 PMid:26594361 PMCid:PMC4653888

7. Hasanin A, Mourad KH, Farouk I, et al. The Impact of Goal-Directed Fluid Therapy in Prolonged Major Abdominal Surgery on Extravascular Lung Water and Oxygenation: A Randomized Controlled Trial. Open Access Maced J Med Sci. 2019; 7(8):1276-

1281. https://doi.org/10.3889/oamjms.2019.173 PMid:31110569 PMCid:PMC6514339

8. Benes J, Giglio M, Brienza N, Michard F. The effects of goal-directed fluid therapy based on dynamic parameters on post-surgical outcome: a meta-analysis of randomized controlled trials. Crit Care. 2014; 18(5):584. https://doi.org/10.1186/s13054-014-0584-z PMid:25348900 PMCid:PMC4234857

9. Pearse RM, Harrison DA, MacDonald N, et al. Effect of a perioperative, cardiac output-guided hemodynamic therapy algorithm on outcomes following major gastrointestinal surgery: a randomized clinical trial and systematic review. JAMA. 2014; 311(21):2181-2190. https://doi.org/10.1001/jama.2014.5305 PMid:24842135

10. Kastrup M, Markewitz A, Spies C, et al. Current practice of hemodynamic monitoring and vasopressor and inotropic therapy in post-operative cardiac surgery patients in Germany: results from a postal survey. Acta Anaesthesiol Scand. 2007; 51(3):347-358. https://doi.org/10.1111/j.1399-6576.2006.01190.x PMid:17096667

11. Bignami E, Belletti A, Moliterni P, Frati E, Guarnieri M, Tritapepe L. Clinical practice in perioperative monitoring in adult cardiac surgery: is there a standard of care? Results from an national survey. J Clin Monit Comput. 2016; 30(3):347-365. https://doi.org/10.1007/s10877-015-9725-4 PMid:26089166

12. Hasanin A, Mukhtar A, Nassar H. Perfusion indices revisited. J Intensive Care. 2017; 5(1):24. https://doi.org/10.1186/s40560-017-0220-5 PMid:28331621 PMCid:PMC5351209

13. Wu CY, Lin YS, Tseng HM, et al. Comparison of two stroke volume variation-based goal-directed fluid therapies for supratentorial brain tumour resection: A randomized controlled trial. Br J Anaesth. 2017; 119(5):934-942. https://doi.org/10.1093/bja/aex189 PMid:28981592

14. Hernández-Palazón J, Fuentes-García D, Doménech-Asensi P, Piqueras-Pérez C, Falcón-Araña L, Burguillos-López S. A comparison of equivolume, equiosmolar solutions of hypertonic saline and mannitol for brain relaxation during elective supratentorial craniotomy. Br J Neurosurg. 2016; 30(1):70-75. https://doi.org/10.3109/02688697.2015.1109061 PMid:26571037

15. Fuller BM, Dellinger RP. Lactate as a hemodynamic marker in the critically ill. Curr Opin Crit Care. 2012; 18(3):267-272. https://doi.org/10.1097/MCC.0b013e3283532b8a PMid:22517402 PMCid:PMC3608508

16. Brallier JW, Dalal PJ, McCormick PJ, Lin H-M, Deiner SG. Elevated Intraoperative Serum Lactate During Craniotomy Is Associated With New Neurological Deficit and Longer Length of Stay. J Neurosurg Anesthesiol. 2017; 29(4):388-392. https://doi.org/10.1097/ANA.0000000000000332 PMid:27438799

17. Sundaram SC, Salins SR, Kumar AN, Korula G. Intra-Operative Fluid Management in Adult Neurosurgical Patients Undergoing Intracranial Tumour Surgery: Randomised Control Trial Comparing Pulse Pressure Variance (PPV) and Central Venous Pressure (CVP). J Clin Diagnostic Res. 2016; 10(5):UC01-UC05. https://doi.org/10.7860/JCDR/2016/18377.7850 PMid:27437329 PMCid:PMC4948505

18. Luo J, Xue J, Liu J, Liu B, Liu L, Chen G. Goal-directed fluid restriction during brain surgery: a prospective randomized controlled trial. Ann Intensive Care. 2017; 7(1):16. https://doi.org/10.1186/s13613-017-0239-8 PMid:28211020 PMCid:PMC5313491

19. Lindroos A-CB, Niiya T, Silvasti-Lundell M, Randell T, Hernesniemi J, Niemi TT. Stroke volume-directed administration of hydroxyethyl starch or Ringer's acetate in sitting position during craniotomy. Acta Anaesthesiol Scand. 2013; 57(6):729-736. https://doi.org/10.1111/aas.12105 PMid:23550716

20. Holte K, Sharrock NE, Kehlet H. Pathophysiology and clinical implications of perioperative fluid excess. Br J Anaesth. 2002; 89(4):622-632. https://doi.org/10.1093/bja/aef220 PMid:12393365

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Role of Intraoperative Transesophageal Echocardiography in Cardiac Surgery: an Observational Study

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Keywords: Intraoperative TEE; Valve surgery

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Abstract

AIM: This study is based on the hypothesis that the routine use of transesophageal echocardiography in cardiac surgery will influence the surgical decision taken by the surgeon intra-operatively in Kasr-Alainy hospitals.

METHODS: Patients were examined with intraoperative transesophageal echocardiography (TEE) before and after cardiopulmonary bypass. Complete and comprehensive intraoperative TEE examinations will be performed by TEE certified cardiac anesthesiologists. Data that will be collected from the intraoperative examination and will be compared with preoperative transthoracic echocardiography, and the surgical decision that was taken preoperatively will be revised again with the cardiothoracic surgeon before the start of surgery. Also, TEE will be used again after weaning from bypass for revision and assessment of our decision.

RESULTS: We examined the utility of TEE in 100 patients undergoing different types of cardiac procedures in Kasr Al-Ainy hospital. This prospective clinical investigation found that the pre- and post-CPB TEE examinations influenced surgical decision making in 10% of all evaluated patients.

CONCLUSION: Intraoperative TEE has the potential to influence clinical decision making for cardiac surgical patients significantly. It is useful in surgical planning, guiding various hemodynamic interventions, and assessing the immediate results of surgery. Thus, IOTEE should be used routinely in all patients undergoing all types of cardiac surgeries.

Introduction

Transesophageal echocardiography (TEE) is a relatively recent development in imaging. The major innovations in TEE have all occurred since 1970. Early workers had both Doppler and M-mode technology available for use via the oesophagal route, but the most significant development was the rigid, mechanical, two-dimensional, echocardiographic transesophageal endoscopy in 1977 [1], [2].

The establishment of TEE in perioperative cardiac anaesthetic care has resulted in a significant change in the role of the anaesthetist who, using TEE can provide new information which may change the course and the outcome of surgical procedures [3].

The accuracy of perioperative transesophageal echocardiography in the diagnosis of structural abnormalities is quite high, producing results that are reliable and reproducible. The information obtained from TEE influences important therapeutic decisions in valvular surgery, coronary artery surgery, and thoracic aortic surgery [4].

Advances in intraoperative monitoring techniques aid the cardiovascular surgeon in patient management and, possibly, contribute to improved outcomes. Although the usefulness of TEE in influencing clinical decisions during valvular heart surgery is well documented, the clinical utility of routine TEE in patients undergoing all types of cardiac surgery remains unresolved [5].

Patients and Methods

Population Selection

This study was conducted in Kasr Al-Ainy Hospital, Cairo University in the period from September 2018 to May 2019 after approval from Ethics and research committee of anaesthesia department, faculty of medicine, Cairo University

The study included 100 adult patients with different types of cardiac procedures in Kasr Al-Ainy hospital. The patients were informed, and written consent was obtained. Patients with contraindication to using TEE were excluded from the study as oesophagal pathology including stricture, trauma, active upper GI bleeding or recent upper GI surgery

Our phased array probe is only suitable for adults, and as such, the cases evaluated in this study include adult operations only. The patients were informed before the operation about the procedure with a written, formatted paper and consent was taken only after questioning regarding swallowing difficulty and other oesophagal abnormalities the patient may have experienced.

Echocardiography

All studies were performed with a TEE probe (ATL, HDI 5000, CV, and Bothell, WA) which is a 5 MHz phased array multiplane transducer that permits Doppler Color Flow imaging either with pulsed or continuous wave facilities. Complete and comprehensive intraoperative TEE examinations were performed by TEE certified cardiac anesthesiologists. Results of the examinations were discussed with the attending cardiac surgeon. Data that was collected from the intraoperative examination was compared with preoperative transthoracic echocardiography, and the surgical decision that was taken preoperative was revised again with the cardiothoracic surgeon before the start of surgery. Also, TEE was used again after weaning from bypass for revision and assessment of our decision.

Standard images of all patients included a long-axis view of the left atrium, left ventricle and the mitral and aortic valves; a transgastric short-axis view of the left ventricle at the papillary muscle level; a four-chamber view visualizing the two atrioventricular valves and all four chambers; and an interatrial septal view.

Some images were taken as warranted by clinical circumstances. Doppler colour imaging was performed using enhanced maps with an optimal Doppler signal obtained by angulation of the transducer tip to interrogate blood flow in multiple planes in all views.

Left ventricular function was evaluated by comparison of transgastric short-axis imaging at the midpapillary level and midesophageal 2-chamber or 4-chamber images before bypass and at the end of the operation. Decreased segmental wall motion or a global decrease in contractility was defined as a decreased ventricular function.

Valve function was assessed by comparison of pre- and final post-bypass images. Cusp mobility and morphology were evaluated by two-dimensional echocardiography before use of Doppler colour flow imaging. Valve insufficiency was graded as first, second, third- and fourth-degree insufficiency, with first-degree insufficiency representing mild and second- and third-degree insufficiencies defining moderate, and finally, fourth-degree insufficiency describing severe regurgitant flow.

Results

This study showed that out of 100 TEE examinations, there was 56 females (56%) and 44 males (44%). Age ranges between 15 and 50.

Out of 100 patients 25 cases (25%) were mitral valve operations, 35 cases (35%) were aortic valve operations, 9 cases (9%) were tricuspid valve operations, 5 cases (5%) were VSD operations, 6 cases were ASD operations, 20 cases (20%) were CABG operations.

Table 1: Different types of operations done and their percentages

Operation	Mitral	*25*	*25.0%*
	Aortic	*35*	*35.0%*
	Tricuspid	*9*	*9.0%*
	VSD	*5*	*5.0%*
	ASD	*6*	*6.0%*
	CABG	*20*	*20.0%*

Results of our study showed that there were changes in findings between pre-operative trans-thoracic echocardiography and intra-operative trans-esophageal echocardiography in the mitral valve, aortic valve and tricuspid valve. Also, there was a change in the surgical decision by the post bypass transesophageal echocardiography in 2 mitral valve operations.

Table 2: Differences between findings of pre-operative TTE and findings of intra & post-operative TEE

	Preoperative transthoracic echo			*TEE*		
Mitral	Normal	*73*	*73.0%*	Normal	*65*	*65.0%*
	Abnormal	*27*	*27.0%*	Abnormal	*35*	*35.0%*
Aortic valve	Normal	*62*	*62.0%*	Normal	*59*	*59.0%*
	Abnormal	*38*	*38.0%*	Abnormal	*41*	*41.0%*
Tricuspid	Normal	*91*	*91.0%*	Normal	*87*	*87.0%*
	Abnormal	*9*	*9.0%*	Abnormal	*13*	*13.0%*
Septal Abn.	Normal	*80*	*80.0%*	Normal	*80*	*80.0%*
	Abnormal	*20*	*20.0%*	Abnormal	*20*	*20.0%*
Aorta	Normal	*88*	*88.0%*	Normal	*88*	*88.0%*
	Abnormal	*12*	*12.0%*	Abnormal	*12*	*12.0%*
RWMA	Normal	*75*	*75.0%*	Normal	*78*	*78.0%*
	Abnormal	*25*	*25.0%*	Abnormal	*22*	*22%*

Pre-pump events

Out of 100 TEE examinations, there were only 8 cases that the surgical decision was changed in them, 4 were females, and 4 were males. Thirty percent of the altered surgical management involved the mitral valve, and 40% involved the tricuspid valve, and 10% involved the aortic valve.

Post-pump events

The post-CPB IOTEE revealed unexpected findings requiring immediate surgical correction in 2 patients following mitral valve repair where one case showed a severe paravalvular leak and the second case showed the systolic anterior motion of mitral valve with a high-pressure gradient across the left ventricular outlet.

Aortic valve

Of the 35 cases undergoing aortic valve replacement, One of the cases in which TEE changed the surgical decision intra-operatively was 30 years old female patient, her pre-operative trans-thoracic echocardiography showed sever aortic stenosis with pressure gradient = 80 mmHg, while intra-operative TEE showed subaortic membrane and the valve was normal, so the decision was the only removal of the membrane, and the pressure gradient became 15 mmHg.

Mitral valve

Of the 25 cases undergoing mitral valve surgery, 20 cases were assigned for mitral valve replacement, and 5 for mitral valve repair.one of the cases that were assigned for repair showed severe calcification of subvalvular apparatus, so, the surgical decision was changed for mitral valve replacement. Two of the cases that were assigned for coronary artery bypass grafting showed severe ischemic mitral regurgitation that required mitral valve repair in addition to coronary revascularization.

Tricuspid valve

The surgical decision was changed in 4 cases where the preoperative echo showed mild tricuspid regurge and the pre bypass IOTEE showed severe tricuspid regurge that required tricuspid valve repair.

CPB-Weaning

An example for medical decision change in a male patient 45 years old, his post bypass TEE examination showed poor RV contractility and elevated pulmonary artery pressure, so the decision was to add milrinone (primacor) to improve contractility of the right ventricle.

Discussion

Our prospective study showed that routine use of intraoperative TEE in patients undergoing cardiac surgery revealed new cardiac pathology in 20% of patients is in concordance with previous clinical observations. The new TEE information altered surgical management in 10% of patients. 50% of the altered surgical management involved the mitral valve, and 40% involved the tricuspid valve, and 10% involved the aortic valve. The pre bypass IOTEE affected the surgical decision in 8 of our cases while the post bypass IOTEE affected 2 of our cases.

The finding that new intraoperative TEE information altered surgical management in 25% of patients is the highest percentage reported to date. Although the clinical study by Savage et al., [6] published in 1997 reported altered surgical management in 33% of patients, this relatively small (82 patients) study evaluated only “high-risk” patients undergoing isolated myocardial revascularisation.

The pre-CPB TEE examination allows the cardiac surgeon to confirm the preoperative indication for surgery, and therefore to avoid an unnecessary intervention with its associated morbidity. Newly recognised pathologic findings can change the planned procedure, and avoid an additional surgical procedure in the future. This wide range of new findings reported in the literature (10-40%) reflects the great variation in study design and patient populations evaluated (retrospective/prospective, CABG surgery only, valve surgery only, mixed, etc.). Similarly, in literature, the new intraoperative TEE information altered surgical management in a wide range of patients by5-33%. Also, the most frequent pre-CPB finding was either undetected valve dysfunction or a change in the preoperatively diagnosed valve pathology [7].

In a small series of 75 patients, Bergquist et al. analysed the impact of IOTEE on intraoperative anaesthetic management during routine CABG. They found that 17% of intraoperative clinical interventions (such as the need for fluid boluses, need for vasopressors or dilators) were influenced predominately by the IOTEE although its contribution to surgical interventions was only 3% [8]. Mishra et al., in a large IOTEE series, reported a subset of patients having only CABG surgery. In this nonconsecutive series with IOTEE based on probe availability and high-risk, IOTEE impacted on the surgical plan in 27% of patients [9]. According to Rosenhek et al., over one-fifth (21.5%) of perioperative TEE alterations were related to mitral valve pathology, mostly of regurgitant lesions. There is a decrease in the severity of MR under anaesthesia, due to systemic vasodilatation and offloading of the left ventricle. Before commenting on the severity of the MR under anaesthesia, steps were taken to restore the preinduction heart rate and mean arterial blood pressure, but this study was performed on patients undergoing valve surgeries only [10].

Tasoglu I et al. performed a study on a large group of patients (466) that undergone different heart surgery types. TEE examinations performed before

and after the cardiopulmonary bypass influenced surgical decisions by 14.8% and 9.0% respectively, that was a relatively high percentage, especially when we know that the new findings in intraoperative TEE regarding the mitral valve, for example, were 77 cases (60.6%) [11]. The use of post-CPB TEE imaging to evaluate surgical results is very important. The post-CPB TEE examination can provide a direct and immediate assessment of the surgical procedure, and therefore can expedite the decision to return to CPB when necessary. In recently published prospective studies, the incidence of new post-CPB findings that prompted a second CPB run was 2-6% [12].

In our study, immediate surgical correction was required in 2%, a case of severing MR for MVR, operated and a new finding by post-CPB examination was suture dehiscence resulting in hemodynamic instability. Another case of mitral valve repair showed post bypass systolic anterior motion of mitral valve with a significant gradient across the left ventricular outlet and the surgical decision was to replace the mitral valve with a mechanical bileaflet valve.

Some of the previously published studies assessing the use of TEE in cardiac surgical patients have detailed how information from TEE simplified/altered hemodynamic management. There is no doubt that TEE provides valuable information regarding myocardial contractility and preload, thus clarifying the proper treatment of hemodynamic instability. The authors chose to focus solely on how TEE affected surgical management, realising that essentially, all patients undergoing cardiac surgery have their hemodynamic management altered via information obtained from TEE [6].

Minhaj et al. is the first study to assess the effect of routine intraoperative TEE in a patient population that includes OPCAB. In this study, intraoperative TEE information influenced decisions regarding use/nonuse of CPB in 8 patients (3%). Such decisions (use/nonuse of CPB) should be made only after thoughtful contemplation of the risks and benefits of each approach (on the pump or off-pump) in each particular patient. Because of the well-known risks of initiating CPB (neurologic dysfunction, renal dysfunction, pulmonary dysfunction, hematologic abnormalities), such decisions also may profoundly affect patient outcome. With the increased use of OPCAB by cardiac surgeons (20% of the present patient population was scheduled for OPCAB), the importance of intraoperative TEE information in guiding surgical management and perhaps influencing the patient outcome may play an even greater role shortly [7].

Limitations in our study; the number of cases was small due to the short time of the study. Despite operating in a large facility, our study had a shortage of information about CABG patients as TEE is not used as much as in valvular heart diseases.

In conclusion, intraoperative TEE has the potential to influence clinical decision making for cardiac surgical patients significantly. It is useful in surgical planning, guiding various hemodynamic interventions, and assessing the immediate results of surgery. Thus, IOTEE should be used routinely in all patients undergoing all types of cardiac/aortic surgery. It is generally believed that the use of TEE in “higher-risk” populations will yield a higher incidence of new findings.

References

1. Frazin L, Talano JV, Stephanides L, et al. Esophageal echocardiography. Circulation. 1976; 54:102-8. https://doi.org/10.1161/01.CIR.54.1.102 PMid:1277411

2. Hisanaga K, Hisanaga A, Nagata K, et al. A newtransesophageal real-time two-dimensionalechocardiographic system using a flexible tube and its clinical application. Proc Jpn Soc Ultrason Med. 1977; 32:43-4.

3. Klein AA, Snell A, Nashef SA, et al. The impact of intra-operative transesophageal echocardiography on cardiac surgical practice. Anesthesia. 2009; 64:947-952. https://doi.org/10.1111/j.1365-2044.2009.05991.x PMid:19686478

4. Skinner HJ, Mahmoud A, Uddin A, et al. An investigation into the causes of unexpected intra-operative trans esophageal echocardiography findings. Anesthesia. 2012; 67:355-360. https://doi.org/10.1111/j.1365-2044.2011.07022.x PMid:22409793

5. Sugeng L, Shernan SK, Salgo IS, et al. Live 3D transesophageal Echocardiography initial experience using the fully-sampled matrix array probe. J Am CollCardiol. 2008; 52:446-9. https://doi.org/10.1016/j.jacc.2008.04.038 PMid:18672165

6. Savage RM, Lytle BW, Aronson S, et al. Intraoperative echocardiography is indicated in high-risk coronary artery bypass grafting. Ann Thorac Surg. 1997; 64:368 -373. https://doi.org/10.1016/S0003-4975(97)00612-7

7. Minhaj M, Patel K, Muzic D, et al. The effect of routine intraoperative transesophageal echocardiography on surgical management. J Cardiothoracic Vasc. Anesth. 2007; 21:800-4. https://doi.org/10.1053/j.jvca.2007.04.012 PMid:18068055

8. Bergquist BD, Bellows WH, Leung JM. Transesophageal echocardiography in myocardial revascularization (II. Influence on intraoperative decision making). Anesth Analg. 1996; 82:1139-1145. https://doi.org/10.1097/00000539-199606000-00007 PMid:8638781

9. Mishra M, Chauhan R, Sharma KK, et al. Real-time intraoperative transesophageal echocardiography-how useful? Experience of 5,016 cases. J Cardiothoracic Vasc Anesth. 1998; 12:625-632. https://doi.org/10.1016/S1053-0770(98)90232-4

10. Rosenhek R, Binder T, Maurer G. Intraoperative transesophageal echocardiography in valve replacement surgery. Echocardiography. 2002; 19:701-07. https://doi.org/10.1046/j.1540-8175.2002.00701.x PMid:12487644

11. Tasoglu I, Imren Y, et al. Impact of intraoperative transesophageal echocardiographyon surgical decisions in the cardiovascular operating room. Arch Turk Soc Cardiol. 2012; 40(3):242-250. https://doi.org/10.5543/tkda.2012.75725 PMid:22864320

12. Sutton DC, Kluger R. Intraoperative transoesophageal echocardiography (impact on adult cardiac surgery). Anaesth Intensive Care. 1998; 26:287-293. https://doi.org/10.1177/0310057X9802600310 PMid:9619224

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Clinical Science

Intra-articular Platelet-Rich Plasma Injections for Treating Knee Pain Associated with Articular Cartilage and Degenerative Meniscal Lesions

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Keywords: Platelet-rich plasma; Articular cartilage; Degenerative meniscal lesions; Safety method; Intra-articular application

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Abstract

BACKGROUND: Platelet-rich plasma (PRP) is an autologous concentration of platelets that contain a large number of growth factors. These growth factors play a role in the regeneration, repair, and acceleration of the biochemical process, thereby reducing the pain associated with injuries of the articular cartilage and meniscus

AIM: The purpose of this study is to evaluate the effect of the PRP method in the treatment of knee joint cartilage injuries and degenerative meniscus lesions as well as pain relief.

MATERIAL AND METHODS: The process of obtaining PRP begins by taking 15 ml of blood from the patient with a special system called Arthrex Double Syringe system. The test tube is centrifuged at 4000 rpm for 5 minutes. From the separated plasma, 5-6 ml PRP is taken and prepared for application. PRP is administered intra-articularly.

RESULTS: At the Jan Mitrev Clinic in Skopje in 2018, PRP procedures were performed on 126 patients, 56 (44.4%) of whom were male, and 70 (55.6%) were female. The patients were evaluated by the Tegner Lysholm Knee Scoring Scale (TLKSS) before applying 3 doses of PRP for 7 days as well as 3 and 6 months after the application of PRP. The results showed considerable improvement 3 months after the PRP application, and 6 months after the application the results remained approximately identical.

CONCLUSION: The application of PRP in the field of medicine is widely applied, and it will continue to be because the understanding of PRP therapy is increasingly refined. This therapy represents a potential and latest method in short-term pain reduction, but additional studies are needed to prove its long-term effectiveness.

Introduction

The most recent research and findings in the treatment of knee pain in articular cartilage injuries and degenerative meniscus lesions are increasing the need for biology and its association with the inflammatory process. The normal function and preservation of articular cartilage and meniscus morphology depend primarily on the balance between aggressive and protective factors [1]. Biology and its use are extremely important for the balance between these factors [2], [3], [4]. Platelet-rich plasma (PRP) is increasingly used in articular cartilage injuries and degenerative meniscus lesions. PRP is an autologous concentration of platelets that contain a large number of growth factors [5], [6], [7], [8]. These growth factors play a role in the regeneration, repair, and acceleration of biochemical processes, thereby reducing the pain associated with injuries of the articular cartilage and meniscus [9]. The latest world research shows excellent results in reducing pain by using PRP [10], [11], [12], [13], [14]. In the Republic of Macedonia, the PRP application has been used for the last decade.

The purpose of this study is to evaluate the effect of the PRP method in the treatment of knee joint

cartilage injuries and degenerative meniscus lesions as well as pain relief.

Material and Methods

A prospective six-month study involved 15 patients treated at the Zan Mitrev Clinic in Skopje in 2018. All patients underwent a clinical examination, X-ray examinations and magnetic resonance imaging of the knee. They all received 3 doses of PRP applied over 7 days.

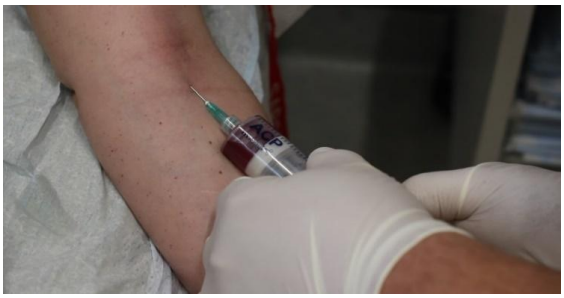

Figure 1: Taking 15 ml blood sample

The process of obtaining PRP begins by taking 15 ml of blood from the patient (Figure 1) with a special system called Arthrex Double Syringe system (Figure 2).

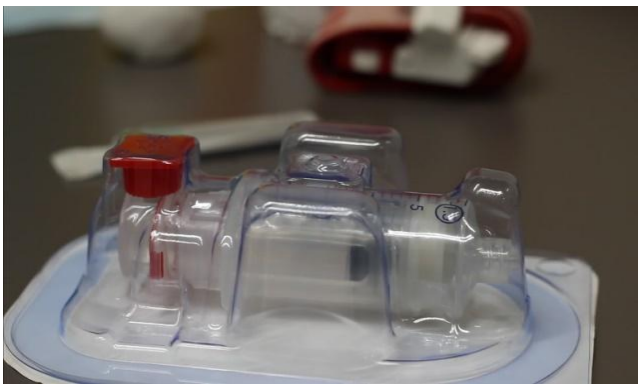

Figure 2: Arthrex Double Syringe System

The test tube is centrifuged at 4000 rpm for 5 minutes (Figure 3).

Figure 3: Centrifuge at 4000 rpm

From the separated plasma, 5-6 ml PRP is taken and prepared for application (Figure 4). PRP is administered intra-articularly (Figure 5). The Tegner Lysholm Knee Scoring Scale was used for evaluating the patients at the first and sixth month after the treatment with PRP.

Figure 4: PRP ready for application

All patients included in the study signed a consent to participate in the study, as well as consent for publishing the results.

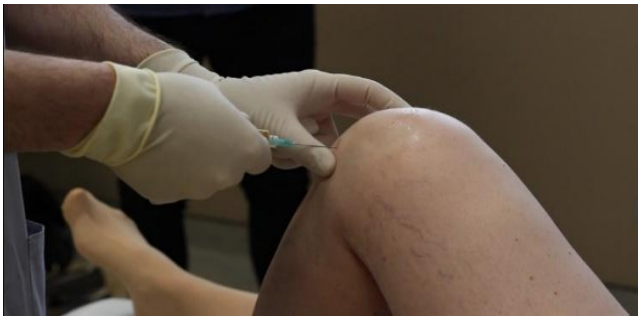

Figure 5: Intra-articular application of PRP

Statistical method

The statistical series, according to the defined variables of interest, are tabulated and graphically presented. The distribution of the numerical statistical series (correct/incorrect) was tested with Kolmogorov Smirnov test, Lilliefors test and Shapiro-Wilk's W test. The numerical series structure was analysed with the central tendency (mean) and dispersion measures (standard deviation). The significance test of the difference between two arithmetic means in the independent samples was performed with the parametric Student-t-test. The significance test of the difference between the two-arithmetic means in the dependent samples was performed with the nonparametric Wilcoxon matched-pairs test.

Testing the significance of the differences between the three arithmetic meanings in the dependent samples was performed with Friedman ANOVA, the significance level for $p < 0.05$ at Cl = 95% is considered statistically significant. The database is analysed with the statistical program STATISTICA 12 for Windows.

Results

At the Zan Mitrev Clinic in Skopje in 2018, PRP procedures were performed on 126 patients, 56 (44.4%) of whom were male, and 70 (55.6%) were female. From the 126 patients, 15 were examined and evaluated in this study, 10 of them (66.7%) were female, and 5 (33.3%) were male. The average age of the total number of respondents was 49.3 ± 6.3 years.

Table 1: Mean Age Value of Examined Patients by Sex

Sex	Mean	Std. Dev.	Min	Max
Females	49.1	5.8	38	60
Males	49.8	7.8	36	55
Total	49.3	6.3	36	60

The average age of the female respondents was 49.1 ± 5.8, and for males, it was 49.8 ± 7.8 years. There was no significant difference in age between the subjects of both sexes (There was no significant difference in age between the subjects of both sexes (Student-t-test: t = - 0.196, p = 0.8475) (Table 1 and Figure 6).

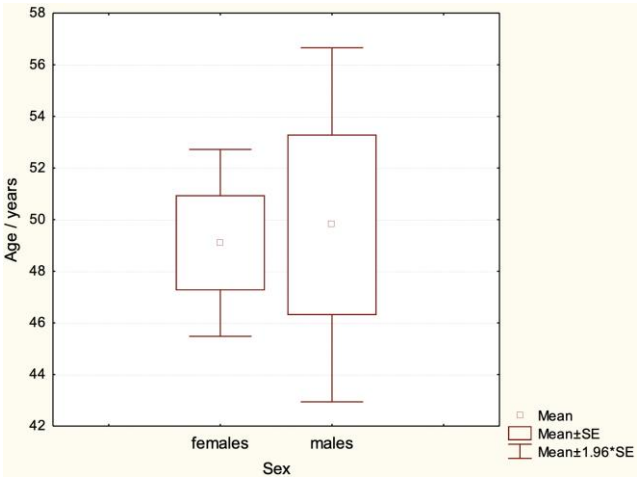

Figure 6: Mean Age Value of Examined Patients by Sex

The patients were evaluated by the TLKSS (Tegner Lysholm Knee Scoring Scale) before applying 3 doses of PRP for 7 days as well as 3 and 6 months after the application of PRP. The results showed considerable improvement 3 months after the PRP application, and 6 months after the application the results remained approximately identical.

The mean value of TLKSS before the intervention was 61.9 ± 7.4 after 3 months, and after 6 months, it was 83.4 ± 4.6 and 83.4 ± 4.3, respectively. The variance analysis showed that there were statistically significant differences concerning the TLKSS at the three points of time (Friedman ANOVA: Chi. Sqr. = 24.54, p = 0.00001). There also was a statistically significant difference between the mean values of TLKSS before the intervention and after 3 months (Wilcoxon matched-pairs test: Z = 3.407, p = 0.00062). There was also a statistically significant difference between the mean values of TLKSS before the intervention and 6 months after (Wilcoxon matched-pairs test: Z = 3.411, p = 0.00065).

Table 2: Mean Value on the TLKSS at different points in time

Tegner lysholm knee scoring scale	Mean	Std.dev.	Min	Max
Before intervention	61.9	7.4	51	74
After 3 months	83.5	4.6	77	92
After 6 months	83.4	4.3	75	90

The difference between the mean values of TLKSS after 3 and 6 months was insignificant (Wilcoxon matched-pairs test: Z = 3.411, p = 0.00065) (Table 2 and Figure 7).

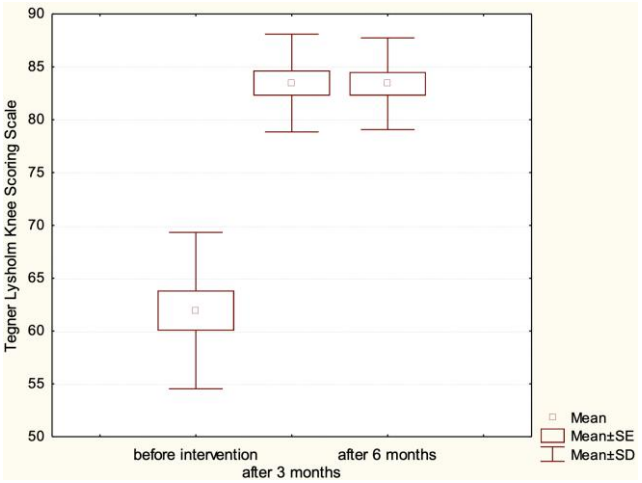

Figure 7: Mean Age Value on the TLKSS at different points in time

Discussion

The main purpose of PRP is to restore the balance between protective and aggressive factors through anti-inflammatory effects. Abrams et al., [15] state that the PRP therapy for patients that have osteoarthritis shows positive results in both clinical and pre-clinical trials. In their paper, these authors pointed out several limits in their research because of the selective selection of patients. In their research on the quality of PRP therapy in degenerative joint cartilage degeneration, Campbell at al., [16] indicated that the International Knee Documentation Committee (IKDS) achieved a six-month improvement. The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), showed significant improvement compared to hyaluronic acid (HA) when it comes to pain in 3- and 6-month follow-ups. A large number of scientific studies have shown positive short-term results in the use of PRP treatment, while a relatively small number of studies have demonstrated a long-term efficacy [17]. The demographic data of patients such as body mass index, age and sex, play a role in the effectiveness of PRP [18], [19], [20]. Several studies have failed to confirm the use of medications after the PRP treatment, while some

studies have evaluated the effect of drugs on the effectiveness of PRP [21].

In conclusion, the application of PRP in the field of medicine is widely applied, and it will continue to be because the understanding of PRP therapy is increasingly refined. This study shows the effect of PRP in 15 patients, with a significant reduction in pain, following the administration of 2-3 doses of PRP evaluated on the TLKSS. Also, no side effects were observed in any of the patients that were studied. This therapy represents a potential and latest method in short-term pain reduction, but additional studies are needed to prove its long-term effectiveness.

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References

1. Kennedy MI, Whitney K, Evans T, LaPrade RF. Platelet-Rich Plasma and Cartilage Repair. Current reviews in musculoskeletal medicine. 2018; 11(4):573-82. https://doi.org/10.1007/s12178-018-9516-x PMid:30203333 PMCid:PMC6220001

2. Goldring MB, Otero M. Inflammation in osteoarthritis. Curr Opin Rheumatol. 2011; 23:471-478. https://doi.org/10.1097/BOR.0b013e328349c2b1 PMid:21788902 PMCid:PMC3937875

3. Mueller MB, Tuan RS. Anabolic/catabolic balance in pathogenesis of osteoarthritis: identifying molecular targets. PM R. 2011; 3:S3-11. https://doi.org/10.1016/j.pmrj.2011.05.009 PMid:21703577

4. Wojdasiewicz P, Poniatowski LA, Szukiewicz D. The role of inflammatory and anti-inflammatory cytokines in the pathogenesis of osteoarthritis. Mediat Inflamm. 2014; 2014:561459. https://doi.org/10.1155/2014/561459 PMid:24876674 PMCid:PMC4021678

5. Kon E, Filardo G, Di Martino A, Marcacci M. Platelet-rich plasma (PRP) to treat sports injuries: evidence to support its use. Knee Surg Sports Traumatol Arthrosc. 2011; 19:516-527. https://doi.org/10.1007/s00167-010-1306-y PMid:21082164

6. Jang SJ, Kim JD, Cha SS. Platelet-rich plasma (PRP) injections as an effective treatment for early osteoarthritis. Eur J Orthop Surg Traumatol. 2013; 23:573-580. https://doi.org/10.1007/s00590-012-1037-5 PMid:23412170

7. Halpern B, Chaudhury S, Rodeo SA, Hayter C, Bogner E, Potter HG, Nguyen J. Clinical and MRI outcomes after platelet-rich plasma treatment for knee osteoarthritis. Clin J Sport Med. 2013; 23:238-239. https://doi.org/10.1097/JSM.0b013e31827c3846 PMid:23238250

8. Sampson S, Reed M, Silvers H, Meng M, Mandelbaum B. Injection of platelet-rich plasma in patients with primary and secondary knee osteoarthritis: a pilot study. Am J Phys Med Rehabil. 2010; 89:961-969. https://doi.org/10.1097/PHM.0b013e3181fc7edf PMid:21403592

9. Joshi Jubert N, Rodríguez L, Reverté-Vinaixa MM, Navarro A. Platelet-rich plasma injections for advanced knee osteoarthritis: a prospective, randomized, double-blinded clinical trial. Orthopaedic journal of sports medicine. 2017; 5(2):2325967116689386. https://doi.org/10.1177/2325967116689386 PMid:28255569 PMCid:PMC5315239

10. Sanchez M, Anitua E, Azofra J, Aguirre JJ, Andia I. Intra-articular injection of an autologous preparation rich in growth factors for the treatment of knee OA: a retrospective cohort study. Clin Exp Rheumatol. 2008; 26:910-913.

11. Kon E, Filardo G, Di Matteo B, Marcacci M. PRP for the treatment of cartilage pathology. Open Orthop J. 2013; 7:120-128. https://doi.org/10.2174/1874325001307010120 PMid:23730375 PMCid:PMC3664439

12. Filardo G, Kon E, Buda R, Timoncini A, di Martino A, Cenacchi A, Fornasari PM, Giannini S, Marcacci M. Platelet-rich plasma intra-articular knee injections for the treatment of degenerative cartilage lesions and osteoarthritis. Knee Surg Sports Traumatol Arthrosc. 2011; 19:528-535. https://doi.org/10.1007/s00167-010-1238-6 PMid:20740273

13. Filardo G, Kon E, Roffi A, Di Matteo B, Merli ML, Marcacci M. Platelet-rich plasma: why intra-articular? A systematic review of preclinical studies and clinical evidence on PRP for joint degeneration. Knee Surg Sports Traumatol Arthrosc. 2015; 23:2459-2474. https://doi.org/10.1007/s00167-013-2743-1 PMid:24275957 PMCid:PMC4541701

14. Kon E, Buda R, Filardo G, et al. Platelet-rich plasma: intra-articular knee injections produced favorable results on degenerative cartilage lesions. Knee Surg Sports Traumatol Arthrosc. 2010; 18:472-479. https://doi.org/10.1007/s00167-009-0940-8 PMid:19838676

15. Abrams GD, Frank RM, Fortier LA, Cole BJ. Platelet-rich plasma for articular cartilage repair Sports Med Arthrosc Rev. 2013; 21:213-219. https://doi.org/10.1097/JSA.0b013e3182999740 PMid:24212369

16. Campbell KA, Saltzman BM, Mascarenhas R, Khair MM, Verma NN, Bach BR, Jr, Cole BJ. Does intra-articular platelet-rich plasma injection provide clinically superior outcomes compared with other therapies in the treatment of knee osteoarthritis? A systematic review of overlapping meta-analyses. Arthroscopy. 2015; 31:2213-2221. https://doi.org/10.1016/j.arthro.2015.03.041 PMid:26033459

17. Huang PH, Wang CJ, Chou WY, Wang JW, Ko JY. Short-term clinical results of intra-articular PRP injections for early osteoarthritis of the knee. Int J Surg. 2017; 42:117-122. https://doi.org/10.1016/j.ijsu.2017.04.067 PMid:28476542

18. Weibrich G, Kleis WK, Hafner G, Hitzler WE. Growth factor levels in platelet-rich plasma and correlations with donor age, sex, and platelet count. J Craniomaxillofac Surg. 2002; 30:97-102. https://doi.org/10.1054/jcms.2002.0285 PMid:12069512

19. Weibrich G, Kleiss WK, Kunz-Kostomanolakis M, Loos AH, Wagner W. Correlation of platelet concentration in platelet-rich plasma to the extraction method, age, sex, and platelet count of the donor. Int J Oral Maxillofac Implants. 2001; 16:693-699.

20. Evanson JR, Guyton MK, Oliver DL, Hire JM, Topolski RL, Zumbrun SD, McPherson JC, Bojescul JA. Gender and age differences in growth factor concentrations from platelet-rich plasma in adults. Mil Med. 2014; 179:799-805. https://doi.org/10.7205/MILMED-D-13-00336 PMid:25003868

21. Schippinger G, Pruller F, Divjak M, et al. Autologous platelet-rich plasma preparations: influence of nonsteroidal anti-inflammatory drugs on platelet function. Orthop J Sports Med. 2015; 3:2325967115588896. https://doi.org/10.1177/2325967115588896 PMid:26665098 PMCid:PMC4622369

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Polymorphisms in Phase I (CYP450) Genes *CYP1A1* (rs4646421), *CYP1B1* (rs1056836), *CYP19A1* (rs749292) and *CYP2C8* (rs1058930) and Their Relation to Risk of Breast Cancer: A Case-Control Study in Mazandaran Province in North of Iran

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Abstract

BACKGROUND: The second leading cause of cancer-related death in women is breast cancer. Xenobiotic Metabolizing Enzymes (XMEs) contribute to the detoxification of numerous cancer therapy-induced products. In the metabolism of xenobiotic, cytochrome P450s or monooxygenases perform an important function by catalysing the hydroxylation reaction. In this study, the susceptibility and genetic polymorphisms of CYP450 isoenzymes was investigated that may have an etiological role in breast cancer.

AIM: The main purpose of this study was to evaluate the association of *CYP1A1* (rs4646421), *CYP1B1* (rs1056836), *CYP2C8* (rs1058930), and *CYP19A1* (rs749292) polymorphisms with the risk of breast cancer in Mazandaran province.

MATERIAL AND METHODS: This cross-sectional case-control study were recruited 72 patients and 51 healthy individuals and was performed between March 2018 to May 2018 in the Oncology Department at Imam Hospital in Sari city, Iran. Peripheral blood samples were collected in EDTA tube, and DNA extraction was performed using the salting-out method and WizPrep extraction kits. Breast cancer patients with known clinicopathological characters and healthy women as control group were genotyped for genes polymorphisms by PCR-RFLP technique, using restriction enzymes. Chi-square, Fisher exact test and Logistic regression model, were applied for statistical analysis.

RESULTS: The results of the experiments showed that there was a significant relationship between two groups and the age of the patients is significantly higher than the control group (p = 0.044). According to the chi-square and Fisher exact test, education, pregnancy, menopause status and oppose were significant between the two groups. Based on using a logistic regression model in two normalized and age-adjusted models to finding relationship between the genotypes of each gene and breast cancer risk, it was determined that in the *CYP2C8* genotype, those who have the CG allele have a 7.74 degree increased risk of breast cancer (CI = 95% 0.95-62.5) and in the *CYP19A1* gene, individuals with GA genotype, increased risk of breast cancer (CI = %95 1.52-27.21), about the *CYP1B1* gene, people with two genotypes of CG + GG had higher risk of breast cancer (CI = %95 1.19-5.71) and allele G has decreased risk of breast cancer in this gene (P = 0.0271), also allele G in *CYP2C8* gene had the protective effect (P = 0.02). In the age-adjusted model, for the *CYP2C8* gene, GG genotype increased risk of breast cancer (CI = %95 1.11-75.84) as well as, the CG + GG genotype in *CYP1B1* gene (CI = %95 1.31-6.57).

CONCLUSION: Our results confirm the association between *CYP2C8* (rs1058930), *CYP19A1* (rs749292) and *CYP1B1* (rs1056836) gene polymorphisms and increased risk of breast cancer in women in Mazandaran province.

Introduction

Breast cancer is the most frequent malignancy in women in all races and a growing number in advanced and underdevelopment countries in Asia. According to a report of Iran's Ministry of Health and Medical Education, breast cancer is the most common primary cancer in Iranian women [1]. Today, this disease accounts for about one-third of all cancers in women, and it represents the second leading cause of cancer death among women (15% of cancer deaths) after lung cancer. According to WHO statistics, one in every 8 to 10 women have breast cancer [2]. According to the latest statistics from the Iranian Cancer Research Center, breast cancer cases

in Iran are one decade younger than their western counterparts [3]. About 8500 new cases of breast cancer are reported annually in the country that 1400 cases are reported died from breast cancer; it is also estimated in 2014, about 40,000 people were living with this disease [4].

Breast cancer disease is defined as uncontrolled growth of cells in breast tissue, which is caused by abnormal growth in the glands which have role of producing milk (lobules) or in the ducts that connect lobules to the nipple duct [5] and also is a heterogeneous disease [6] that is caused by environmental and genetic factors [7]. The genetic damage caused by endogenous and exogenous metabolites could be a cause of breast cancer. Clinical and epidemiological studies have demonstrated that estrogen and progesterone play a significant role in the growth and differentiation of normal tissue in the breast [8].

The aetiology of breast cancer is complex and still poorly understood. A small proportion of breast cancer cases can be attributed purely to genetic reasons whereas risk factors such as age, reproductive events (menarche, menopause, pregnancy, breastfeeding), estrogens, exogenous hormones (hormone replacement therapy and oral contraceptives), lifestyle and exposure to environmental carcinogens (pollution, alcohol, diet, obesity), ionizing radiation, chemopreventive agents, as well as genetic factors, breast cancer susceptibility genes high penetrance genes (*BRCA1, BRCA2, PTEN*) and low penetrance genes (*CYP450, GSH, UGTA*) that encodexenobiotic metabolizing enzymes in phase I and II of metabolism [9]. As mentioned above, one of the main risk factors for breast cancer is estrogen, and there are two forms of estrogen in our body: endogenous estrogen that biosynthesises from cholesterol in the body, then undergoes metabolic processes and exogenous form (hormonal drugs, etc.). The main effect of estrogens is stimulating of the breast cells and increases the chance of errors during multiple DNA divisions and the possibility of mutations [10]. Also, it has been identified that Xeno-estrogens, which include pesticides, paints, contaminants, plastics and food preservatives, would have similar effects with estrogen, and contributed to the causes of breast cancer. They have endocrine disruption effects [11].

Many carcinogenic compounds are oxidized by phase I enzymes, represented by cytochrome P450 family, into reactive metabolites that are detoxified by phase II enzymes. GSTs are a family of Phase II detoxification enzymes that catalyse the conjugation of glutathione (GSH) to a wide variety of xenobiotic. This detoxification ability plays an important role in cellular protection from environmental and oxidative stress. Hence, the toxic effects of exposure, absorption and detoxification of carcinogens depend on a delicate balance between the phase I and phase II enzymes [12], [13]. The cytochrome P450 and *GSTs, UGTA* and etc.that phase II enzymes increase its solubility in such a way as to facilitate its excretion and detoxification. These genes involved in the metabolism of these toxins (cytochrome p450), and also contribute to the pathway of biosynthesis and estrogen metabolism, and also contribute to the progression of breast cancer Polymorphisms in both phase I and phase II enzyme genes may result in alteration of their expression, function and activity. These genes are regulated at the transcriptional level, and their expression is influenced by genetic factors, polymorphism in the structural and regulatory gene and by environmental factors [14], [15]. Polymorphisms and expression pattern of these genes are believed to be key factors in determining cancer susceptibility to toxic or environmental chemicals [16]. Polymorphisms are including single nucleotide polymorphisms (SNPs). The most common form of human genetic differences is single Nucleoid Polymorphisms (SNPs) which may play an important role in individual allergies. Single-nucleotide polymorphism is a change in only one open DNA. These single-nucleotide variations cause different phenotypes, predisposing to or susceptibility to certain diseases.

SNPs in genes involved in xenobiotic metabolism and estrogen biosynthesis and metabolism might affect circulating estrogen levels and modulate the individual susceptibility to environmental carcinogens about developing breast cancer [10]. Candidate genes for this study are low-penetrance breast carcinoma susceptibility include those encoding for Xenobiotic Metabolizing Enzymes (XMEs) involved in carcinogen metabolism and detoxification [17]. These XMEs can be divided into phase I (Cytochrome P450 family) and phase II (*GST, UGTA*, etc.) enzymes that metabolically activate potentially carcinogenic forms. In this study, polymorphisms of phase I enzymes *CYP1A1 (rs4646421C/T*), *CYP2C8* (*rs1058930C/G*), *CYP1B1* (*rs1056836 C/G*) and *CYP19A1* (*rs749292A/G*) in breast cancer patients who referred to Mazandaran province clinics, during March 2018 to May 2018 were investigated. DNA extracted from peripheral blood leukocytes of patients with breast cancer and healthy patients. Identifying the genetic polymorphisms of breast cancer patients, in order to recognizing the mechanism of the disease is effective on diagnosis and screening patients who are prone to the disease and, therefore, preventing them. So, position, structure and function of studied genes were briefly explained, then polymorphisms of them were studied.

CYP1A1 is one of the “phase I” enzymes located on chromosome 15q22-q24 and is a 5987-bp long gene. It has 7 exons and 6 introns that encodes for a 512 amino acid protein. This is one of the major components of the detoxification pathway that is highly expressed in non-hepatic cells, such as breast tissue [18]. It is a polymorphic gene involved in the

metabolism of steroids and several potentially genotoxic chemicals. In estrogen metabolism, it plays a main role in producing of2-hydroxy estrogens. Four single nucleotide polymorphisms (SNP) were identified in*CYP1A1* gene including M1, T/C transition at nucleotide 3801; M2, A/G transition at position 2455 resulting in change of Ile to Val at codon 462; M3T/C transition at nucleotide 3205; and M4 C/A transition at position 2453 resulting in change of Thr to Asn at codon 461. The M1 polymorphism at 3'-flanking region (T3801C) was verified to be associated with increased activation of carcinogens [19], [20]. *CYP1B1* is located on chromosome 2 at position 2p22.2. The gene is 42930-fold-wide and 60846 Molecular weight. It has 3 exons and 2 introns and was considered as the key enzyme of P450, which is exogenous in metabolism and endogenous substrate.

CYP1B1 plays the main role in the metabolism of androgens and estrogen substrates. *CYP1B1* can catalyse 4-hydroxyl-estrogens, which is a key reaction to hormonal carcinogenesis. *CYP1B1*, 5 different SNPs have been identified that can play a vital role in replacing amino acids A119S, R48G, L432V, A443G and N453S. Val432Leu- polymorphism has recently been identified as the most influential agent on the catalytic properties of *CYP1B1* and will also affect the function of 4-hydroxy in the Val32 allele, which indicates a 3-fold increase in activity relative to the Leu432 allele, for the specific *CYP1B1* gene rs 163077, 163086 and 162556 who are involved with invasive breast cancer [21], [22]. *CYP19A*1 is located on chromosome 15, q21.2, consisting of 18 exons and 17 introns, and has a molecular weight of 57883 Da. The enzyme is stagnant, which plays an important role in biosynthesis and the final stages of estrogen biosynthesis. The aromatase is coded by the *CYP19A1* gene, a key estrogen biosynthesis enzyme and play a vital role in the development of breast cancer. The main effect of this enzyme is in the catalysing of the final stage of estrogen biosynthesis that converts androstenedione and testosterone into estrogen and estradiol. The direct effect of aromatase on cytotoxicity in the breast is completely reported [23]. A high level of aromatase expression has been reported in breast tumours, which is also visible in the normal breast. *CYP2C8* is located on chromosome 10 at position 10q23.33 consisting of 10 exons and 9 introns. This gene has 55825 Da molecular weight. *CYP2C8* is one of the first human cytochromes and plays an important role in drug metabolism in cytochrome P450, which can also be used in response to chemotherapy and survival chances. Patients with breast cancer are more closely related to the metabolism of *CYP2C8 * 2* and *CYP2C8 * 3* [24].

Material and Methods

Subjects

A total of 123 unrelated subjects (51 controls and 72 patients), living in Mazandaran province were enrolled in this study. The cases were all new incident breast cancer patients histologically diagnosed at the Oncology Department of Cancer Research Center at Imam Hospital in Sari city in Iran, during the period of March 2018 to May 2018. Controls were randomly selected from healthy women who visited patients admitted to the same hospitals and were healthy blood donors having no evidence of any personal or family history of cancer, or other illnesses patients' age ranged from 20 to 75 years. A detailed description of the clinical-pathological characteristics of this study was summarized in (Table1). Control subjects having years ranging from 23 to 66 years. Informed consent was obtained from all participants, and a structured questionnaire was administered by trained interviewers to collect information on demographic and anthropometric data, reproductive and medical history, residential history, and occupation as well, lifestyle, exposure parameters were reported in Table 1. Tobacco smoking and alcohol consumption also were asked from the subject, but there was no case. To investigate whether certain genotypes are a susceptible marker, 5 ml peripheral blood was collected in the EDTA tube from both patients and control group and stored at -20°C.

Table 1: Comparison of cases and controls by selected demographic factors and major risk factors for breast cancer

Clinic pathological Variables	Cases	Controls	P value
Age (mean ± SD)	48.08 ± 10.3	43.69 ± 13.5	0.044*
Age at menarche (mean ± SD)	1.2 ± 13.15	1.2 ± 13.31	0.485
Age at menopause (mean ± SD)	4.4 ± 21.52	5.0 ± 22.55	0.274
Age at 1 St pregnancy (mean ± SD)	4.4 ± 49.53	8.7 ± 47.13	0.21
Age < 45, n (%)	31 (43%.1)	29 (56%.9)	0.13
Age > 45, n (%)	41 (56%.9)	22 (43%.1)	
Pregnancy, n (%)			
NO	6 (8%.3)	-	0.036*
YES	66 (91%.7)	-	
Oral contraceptive use, n (%)			
NO	38 (52%.8)	36 (70%.6)	0.047*
YES	34 (47%.2)	15 (29%.4)	
Menopause Status, n (%)			
Premenopausal	29 (40%.3)	30 (58%.8)	0.043*
Past menopausal	43 (59%.7)	21 (41%.2)	
Body mass index, n (mean±SD kg/m^2)			
BMI < 20 kg/m2	3 (4%.2)	3 (5%.9)	
20 ≤ BMI < 25	19 (26%.4)	14 (27%.5)	0.9
BMI ≥ 25	50 (69%.4)	34 (66%.6)	
Family history of breast cancer in first-degree relatives n (%)			
No	53 (73%.6)	-	< 001*
Yes	19 (26%.4)	-	
Education, n (%)			
≤ 12 years	41 (56%.9)	12 (23%.5)	< 001*
> 12 years	31 (43%.1)	39 (76%.5)	
Occupational exposure to pesticides (Agriculturist), n (%)			
NO	52 (72%.2)	-	< 001*
YES	20 (27%.8)	-	
Grade, n (%)			
I	13 (18%.1)	-	
II	47 (65%.3)	-	< 001*
III	12 (16%.7)	-	
Stage, n (%)			
I	5 (6%.9)	-	< 001*
II	42 (58%.3)	-	
III	18 (25%.0)	-	
IV	7 (9%.7)	-	

DNA extraction

Blood samples were collected in EDTA-containing tubes, and genomic DNA was isolated from buffy coats using a WizPrep DNA blood kit and salting-out method [25]. In the method by using extraction kit, in the presence of strong anionic detergents, the white blood cells are lysed, then proteins are removed with dehydration and prophylaxis. Briefly, 200 µl of blood were mixed with 20 µl protein kinase K, then 200 µl GB buffer was added then the mixture was incubated (10 min 56°C), 200 µl EtOH %100 was added. Then, washing buffer 1 and 2 was added. In the final step, 50 µl Elution Buffer was added. Precipitated proteins were removed by centrifugation. The DNA in the supernatant fluid was precipitated with ethanol. In every step, we centrifuged the mixture based on the protocol of the kit. The DNA pellet was dissolved in 400 µl of sterile distilled water. After extraction, the quality and quantity of the extracted DNA were measured by the spectrophotometer. Then, DNA samples were stored at -20°C, and its purity was checked through agarose following the protocol of the manufacturer. SNPs were genotyped.

Genotyping

Polymorphic sites of genes were genotyped by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) assay. Amplification was performed using a specific primer. Pair of Primers were designed using Gene Runner software. The sequence of primers is listed in (Table 2) reaction contain. 25 µl Polymerase Chain Reaction (PCR) containing 2 µl genomic DNA (100 ng/µl), 12.5 µl Master mix PCR, and 1 µl (10 picomols) of each primer which ultimately reached 25 µl with distilled water. The PCR reaction program was set for each gen. Then the product of the enzyme was digested. Digestion was electrophoresed on 2% agarose gel and photos were taken by Gel Doc [26].

Table 2: Primers and restriction enzymes used for polymorphism genotyping

Genes	SNP	Polymorphisms	Primers	Restriction enzymes
CYP1A1	rs 4646421	Intron/splice mutation (m1) 3'uR T→C (T56392) (+303C>T)	Forward CCATTTATTCTCTG: CTCTCTGGTA Reverse CCCACCACACTTAGGA AAATCA	*RsaI*
CYP1B1	rs1056836	CYP1B1*3 Exon3/missensc C251G (Val432 Leu)	Forward CTGTGGTTTTTGTCAA CAAGTGGTC Reverse TGAGCCAGGATGGAG ATGAAGAGA	*BsrI*
*CYP19A1*1	rs749292	Intron / Exon1	Forward CCAAGGTCCCACAGCT AATTAGTGA Reverse TAAAAGGGCAAGAGCA GAGATGAGC	*TaqI*
CYP2C8	rs1058936	CYP2C8*4 Exon5/missense C792G (ILe 264 Met)	Forward AATCAGGGCTTGGTGT AAGATA Reverse CGATGAATCACAAAAT GGACAAG	*TaqI*

Polymorphisms analysis

Previously reported primers and restricted enzymes in RFLP-PCR are listed in (Table 2). All PCR reactions were performed in an independent blinded duplicate manner, and for each polymorphism, some samples were confirmed by sequencing the PCR products.

The polymorphic site of the *CYP1A1* (rs 4646421) (C—T intron) was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). A 519 bp fragment containing C/T allele was amplified. Using forward and reverse primers that the specifications of primers and size of product and restriction enzymes of genes have been explained in the (Table 2). *CYPP1B1* (rs1056836) (C-Gval432leucin) also simultaneously revealed by Restriction Fragment Length Polymorphism (PCR-RFLP) PCR reaction was performed in a total volume of 50 µl containing 100 ng of genomic DNA, 200 µmol dNTPs, 2 mM $MgCl_2$, 1 × Taq polymerase buffer, 100 pmol of *CYP1A1* and *CYP1B1* (primers and 1 U of Taq DNA polymerase). There action conditions used with the thermal cycler were as follows for *CYP1A1*: initial denaturation at 95°C for 5 min, 32 cycles of denaturation at 95°C for 30 sec, annealing at 62°C for 30 sec and extension at 72°C for 30 sec and a final extension at 72°C for 10 min for *CYP1B1* annealing set at 66.5°C for 30 sec. To verify proper amplification conditions, 10 µl of PCR product were analysed on a 2% agarose gel and stained with ethidium bromide, the amplification of was revealed by the presence of bands. To detect *CYP1A1* (C-T) and *CYP1B1* (C-G) polymorphisms, amplified DNA was digested with 10 U of Fast Digest *RsaI* (37°C, 5 min) and *BsrI* (65°C, 16 h) restriction enzymes. In *CYP1A1* the homozygote wild-type CC genotype produces two 183 and 36 bp fragments, homozygote mutated TT genotype results in single band of 183 bp length, and heterozygote TC genotype produces three fragments of 519, 183 and 336 bp fragments and in *CYP1B1* homozygote wild-type CC genotype produce single band of 44 bp length, homozygote mutated GG genotype results from two 44 and 171 bp fragments and heterozygote GC genotype produce three fragments of 215, 171, and 44 bp fragments.

To verify proper amplification conditions, 10 µl of PCR product were analysed on a 2% agarose gel and stained with ethidium bromide, the amplification of was revealed by the presence of bands. Homozygote wild-type CC genotype produce single band of 44 bp length, homozygote mutated GG genotype results from two 44 and 171 bp fragments and heterozygote GC genotype produces three fragments of 215, 171and 44 bp fragments. Digestion conditions were performed according to the manufacturer's instructions and summarized for each gen in (Table 3).

Digestion products were separated at the appropriate concentrations on a 2, 3 or 4% Low-melting point agarose gel and stained with ethidium bromide. The splice-site mutation of *CYP19A1* (rs749292) (A-G intron) and *CYP2C8*4* (rs1058930) (C-G exon5) was also analysed by PCR-RFLP. PCR amplification also was performed the same as the above genes. The cycling conditions for both of genes including one pre-treatment cycle denaturation at 94°C for 5 min, 32 cycles of denaturation at 94°C for 30 sec, followed by annealing at 66°C for 30 sec and elongation at 72°C for 25 sec and a final elongation at 72°C for 10 min. Products were analysed by electrophoresis at 2% agarose gel and visualised by ethidium bromide staining. This amplified fragment was digested *TaqI* restriction enzyme at 65°C overnight and was analysed on 2% agarose gel. *CYP19A1* when digested with *TaqI* the homozygote wild-type GG genotype results from two 144 and 214 bp fragments, homozygote mutated AA genotype produce single band of 144 bp and heterozygote GA genotype produce three fragments of 358, 144 and 214 bp fragments whereas Digestion of CYP2C8 yielded band of 84 bp, and heterozygote GC genotype produces three fragments of 303, 84 and 219 bp fragments (Table 3).

Table 3: Restriction enzymes conditions used for Genes polymorphism genotyping

SNPS	Restriction Enzymes	Temperature and Incubation time	Fragment size (bp)
rs4646421	FastDigestRsaI (10 µ)	37ºC-5 min	519, 183 + 336
rs1056836	BSrI (10 µ)	65ºC-1h16	215, 44 + 171
rs749292	TaqI (10 µ)	65ºC-1h16	358, 144 + 214
rs1058930	TaqI (10 µ)	65ºC-1h16	303, 84 + 219

Statistical analysis

The genotype and allele frequency of the genes were tested for both patient and control group using chi-square test, Fisher exact test, quantitative (numerical) parameters analyzed by t-student test Odds ratio (OR), confidence intervals (CI) and P-values were calculated using unconditional logistic regression and adjusted estimate the association between genotypes or some other clinicopathological data and the risk of breast cancer. In this research, statistical analyses were performed using SPSS software ver. 21.

Results

Demographic and clinicopathological data

This study was performed in 72 breast cancer patients and 51 healthy controls with known demographic and clinicopathological data in Mazandaran province in the north of Iran. Characteristics of the study population were compared by case-control status, as shown in Table 1. Student's t-test showed significant relationships between the two groups and the mean age of controls (43.69 ± 13.5 years) was significantly lower (P = 0.044) than that of breast cancer patients (48.08 ± 10.3 years). Chi-square and Fisher exact test showed that Pregnancy, menopausal status, family history of breast cancer and education, stage of cancer and grade of the tumour were significantly different between cases and controls. However, no significant differences were found between them regarding BMI, Age at menarche, age at menopause and Age at 1 St pregnancy. In the case group, the frequency of level education is lower than diploma and difference is significant ($p < 0.001$).

The majority of cases have a pregnancy in the patient group, and the difference between the two groups was significant ($p = 0.036$). Regarding the menopause status, the majority of cases were not at the menopause status (P = 0.044). In the cases groups, the majority of people have used LD tablets, and the difference between the two groups was significant ($p = 0.043$). About the stage of cancer, the majority of patients were in stage 2 ($p < 0.001$), and Grades of tumours were mostly reported in Grade 2 ($p < 0.001$). Regarding the agricultural occupation, few people were in cases group ($p < 0.001$). About the family history of cancer, the majority of patients in the first-degree subjects had no history of cancer in their family ($p < 0.001$).

Regarding the distribution of genotypes, it can be said that distribution of genotypes in two groups in *CYP2C8* (P = 0.11), *CYP19A1* (P = 0.019), *CYP1B* (P = 0.026) Had a significant difference, and only in *CYP1A1 (*p = 0.416) is homogeneous (Table 4).

Table 4: Genotype frequencies of genes among cases and controls, and risk of breast cancer

Gene name	Genotype	Groups		Chi-square test		
		Cases	Controls	Test statistic	Fisher's exact test	P value
	CC	62 (%86.1)	48 (%94.1)			
CYP2C8	CG	0 (%0.0)	2 (%3.9)	-	7.4	0.011*
	GG	10 (%13.9)	1 (%2.0)			
	AA	3 (%4.2)	10 (%19.6)			
CYP19A1	GA	42 (%58.3)	27 (%52.9)	7.7	-	0.019*
	GG	27 (%37.5)	14 (%27/5)			
	CC	56 (%77.8)	38 (%74.5)			
CYP1A1	TC	16 (%22.2)	13 (%25.5)	0.17	-	0.416
	TT	0 (%0.0)	0 (%0.0)			
	CC	38 (%52.8)	38 (%74.5)			
CYP1B1	GC	20 (%27.8)	5 (%9.8)	7.26	-	0.026*
	GG	14 (%19.4)	8 (%15.7)			

Also, there was no significant correlation between age and breast cancer risk (P = 0.13). But there was a significant relationship between the risk of cancer and menopause status, and the chance of having cancer is 0.466 times lower than those who did not have menopause status ($p = 0.048$). Besides, there was a significant relationship between the risk of cancer and LD Consumption, and these people have 0.47 times higher risk of breast cancer (P = 0.043) (Table 5).

Table 5: Relation between demographic characteristic and risk of breast cancer

		Group		Result		
				OR	P-value	CI
Age	< 45	31 (25%.2)	29 (22%.6)	0.57	0.13	(0.28,1.18)
	> 45	41 (33%.3)	22 (17%.9)			
Agriculture	NO	52 (72%.2)	-	-	-	-
	YES	20 (27%.8)	-			
Menopause status	NO	36 (29%.3)	36 (29%.3)	0.466	0.048*	(0.22,0.99)
	YES	15 (12%.2)	15 (12%.2)			
Family history in the first degree	NO	-	-	-	-	-
	YES	-	-			
OCP USE	NO	30 (24%.4)	30 (24%.4)	0.47	0.043*	(0.23,0.98)
	YES	21 (17%.1)	21 (17%.1)			
Grade	I, II	-	-	-	-	-
	III	-	-			
Stage	I, II	-	-	-	-	-
	III, IV	-	-			

Regarding the relationship between the genotypes of each gene and the demographic characteristics, it can be concluded that there is no significant difference between the genotype and the demographic characteristics such as the age of the patients, education, pregnancy history, menopause status, agricultural occupation, LD consumption, stage of disease, grade of tumours and BMI. There was only a significant relationship between genotypes of *CYP1A1* gene and education (p = 0.004), and the group with under diploma education had more CC genotype, and the group with diploma education and higher had the highest TC genotype.

Frequency of Genotypes and Alleles

In this case-control study, 72 breast cancer patients and 51 healthy controls were studied. The results of the experiments showed in (Table 6). The frequency of genotypes *CYP1B1* gene among of 72 patients was CC (52.8%), CG (27.8%) and GG (19.4%), and in healthy subjects was CC (74.5%) CG (9.8%) and GG (15.7%). The frequency of alleles in this genotype, allele C (66%) and G allele (34%) in patients and control subjects was C (80%) and G allele (20%). Regarding the *CYP1A1* gene, the frequency of genotypes in patients was CC (77.8%), CT (22.2%) and TT (0%) and in healthy subjects were CC (74.5%), CT (25.5%) and TT (0%), and the frequency of allele C was determined in patients (89%) and G allele (11%) and allele C (87%) and G (13%) in controls have frequency. About. *CYP19A1* gene, the frequency of genotypes was determined in patients with GG (37.5%), GA (58.3%) and AA (4.2%), and in healthy subjects GG (27.5%), GA (52.9%) and AA (19.6%). The prevalence of alleles in patients with allele Cis (66%) and T (34%) and the healthy subjects, allele C (54%) and T (46%). Regarding the *CYP2C8* gene, the frequency of CC (86.1%), CG (0%) and GG (13.9%) genotypes in patients and healthy subjects were CC (94.1%), CG (3.9%) and GG (2%), and frequency of alleles Patients were assigned C allele (86%) and T (14%), and in the control group, allele C (94%) and T (6%) were determined (Table 6). Regarding the relationship between the genotypes of each gene and breast cancer risk using a logistic regression model (Table 6) in two normalized and age-adjusted models, it was determined that in the *CYP2C8* genotype, those who have the CG allele have a 7.74 degree increased risk of breast cancer compared to those who have the CC genotype and in the *CYP19A1* gene of individuals with GA genotype, the risk of breast cancer was 6.42 compared to those of genotype AA (CI-%95 1.52—27.21) (Table 6), about the *CYP1B1* gene, people with two genotypes of CG + GG is associated with a higher risk of breast cancer 2.61 times higher compared to the CC genotype (CI = %95 1.19-5.71) and allele G has protective effect in breast cancer and decreased risk of breast cancer (P = 0.0271) and *CYP2C8* gene allele G decreased risk of breast cancer (P = 0.02). In the age-adjusted model, for the *CYP2C8* gene, GG genotype compared to CC has an increased risk of breast cancer about 9.17 compared to other genotypes (CL = %95 1.11-75.84). Regarding *CYP1B1*, the CG + GG genotype is 2.93 times higher than CC genotype increased risk of breast cancer (CL=%95 1.31-6.57).

Table 6: Relationship between the genotypes and breast cancer risk

Genes name	Genotype and Alleles	groups		Non-Adjusted		Adjusted	
		Cases (%)	controls(%)	P	OR (95% CI)	P	OR (95% CI)
CYP2C8	CC	62 (86%.1)	48 (94%.1)	0.016	-	0.12	-
	CG	0 (0%.0)	2 (3%.9)	0.06	7.74 (0.95,62.5)	0.04	9.17 (1.11,75.84)
	GG	10 (13%.9)	1 (2%.0)	0.99	1.615E + 10	0.99	1.560E + 10
	CC vs GG + CG	72 (58%.53)	5 1(41%.47)	0.167	2.58 (0.67,9.89)	0.112	3.027 (0.77,11.88)
	C (%)	86%	%96	-	Ref	-	-
	G (%)	14%	%4	P = 0.02	0.25 (0.08,0.81)	-	-
CYP19A1	AA	3 (4%.2)	10 (19%.6)	0.038	-	0.015	-
	GA	42 (58%.3)	27 (52%.9)	0.011	6.42 (1.52,27.21)	0.61	6.21 (1.43,26.86)
	GG	27 (37%.5)	14 (27%.5)	0.601	1.24 (0.55,2.77)	0.066	1.24 (0.54,2.8)
	AA + GA vs GG	72 (58%.53)	51 (41%.47)	0.246	1.58 (0.73,3.45)	0.263	1.57 (0.71,3.46)
	G (%)	%66	%54	-	Ref	-	-
	A (%)	%34	%46	P = 0.084	1.65 (0.93,2.92)	-	-
CYP1A1	CC	56 (77%.8)	38 (74%.5)	-	-	-	-
	TC	16 (22%.2)	13 (25%.5)	0.67	0.835 (0.36,1.93)	0.63	0.81 (0.34,1.9)
	TT	0 (0%.0)	0 (0%.0)	-	-	-	-
	CC vs GG + CG	72 (58%.53)	51 (41%.47)	0.67	0.835 (0.36,1.93)	0.63	0.81 (0.34,1.9)
	C (%)	%89	%87	-	Ref	-	-
	T (%)	%11	%13	P = 0.66	1.21 (0.51,2.84)	-	-
CYP1B1	CC	38 (52%.8)	38 (74%.5)	0.034	-	0.024	-
	GC	20 (27%.8)	5 (9%.8)	0.26	1.75 (0.66,4.65)	0.165	2.045 (0.74,5.6)
	GG	14 (19%.4)	8 (15%.7)	0.22	0.44 (0.118,1.62)	0.278	0.479 (0.127,1.8)
	CC vs CG+GG	72 (58%.53)	51 (41%.47)	0.016	2.61 (1.197,5.71)	0.009	2.93 (1.31,6.57)
	C (%)	%66	%80	-	Ref	-	-
	G (%)	%34	%20	P = 0.0271	0.48 (0.25,0.92)	-	-

The results of logistic regression model regarding the frequency of genotypes of each gene and the incidence of breast cancer indicated that there was a significant relationship between the frequency of genotypes and the risk of breast cancer only in *CYP1B1* gene (P-value = 0.015, OR = 0.38, CI = 0.18 0.84), and no significant results were obtained for other genes (Table 7).

It's noteworthy that CC genotype of CYP*1B1* decreases about 0.38-time risk of breast cancer in comparison the CG + GG genotype. In addition, for correlation between CC and CG + GG genotypes with demographic and clinical variables such as age (P-value = 0.25), agriculture (P-value = 0.2), menopause (P = 0.16) (P-value = 0.29), LDL consumption (P-value = 0.86), tumor grade (P-value = 0.83), and stage of cancer (P-value = 0.16), there was no significant relationship between two groups (Table 7).

Table 7: Relationship between the frequency of *CYP1B1* genotypes and the risk of breast cancer and demographic and clinical variables

		CYP1B1		Result		
		CC	GC + GG	OR	P-value	CI
Group	Case	38 (30%.9)	34 (27.6)	0.38	0.015*	(0.18,0.84)
	Control	38 (30%.9)	13 (10%.6)			
Age	< 45	34 (27%.6)	26 (21%.1)	0.65	0.25	(0.32,1.36)
	> 45	42 (34%.1)	21 (17%.1)			
Agriculture	NO	25 (34%.7)	27 (37%.5)	0.5	0.2	(0.17,1.45)
	YES	13 (18%.1)	7 (9%.7)			
Menopause status	NO	42 (34%.1)	32 (26%.0)	0.58	0.16	(0.27,1.24)
	YES	34 (27%.6)	15 (12%.2)			
Family history in first degree	NO	26 (36%.1)	27 (37%.5)	0.56	0.29	(0.19,1.65)
	YES	12 (16%.7)	7 (9%.7)			
OCP USE	NO	36 (29%.3)	23 (18%.7)	0.94	0.86	(0.45,1.94)
	YES	40 (32%.5)	24 (19%.5)			
Grade	I, II	32 (44%.4)	28 (38%.9)	1.14	0.83	(0.33,3.94)
	III	6 (8%.3)	6 (8%.3)			
Stage	I, II	22 (30%.6)	25 (34%.7)	0.49	0.16	(0.18,1.34)
	III, IV	16 (22%.2)	9 (12%.5)			

Discussion

Breast cancer is by far the most common female cancer and comprising about 21% of all new cancers in women. The highest age-adjusted incidence rate is reported for North America, is 87 per 100 thousand women per year, while the lowest rate reported in China. Breast cancer follows a steeply increasing age gradient up to 40 years of age, after which the rate of increase slows down. Even though there are three times as many new cases diagnosed annually as in the late 1980s, breast cancer mortality has remained largely unchanged. This may at least partly be explained by earlier detection of the disease due to effective screening programs and availability of improved therapies. The highest annual mortality rates for breast cancer are reported for the UK, The Netherlands and Denmark, being over 25 per 100 thousand in these countries. So far, conflicting results have been reported from association studies [27]. The aetiology of breast cancer could not be described by allelic variability at a single locus. Instead, the main burden of breast cancer in the population probably results from complex interactions between many genetic and environmental factors over time. An improved understanding of the interplay of xenobiotic exposures, endogenous physiology, and genetic variability at multiple loci may help to identify women who are at increased risk for breast cancer. The genetic polymorphisms that may be linked to breast cancer are multiple. Cumulative lifetime exposure to estrogen, estrogen metabolites, and other physiological factors, as well as environmental exposures, could play an important role in the aetiology of breast cancer in genetically predisposed women. Carcinogenesis, determining response to drugs and cell signalling. In the metabolism of xenobiotic (foreign chemicals), cytochrome P450s or monooxygenases perform an important function by catalysing the hydroxylation reaction [28]. *CYP450* enzymes associated with the development of breast cancer which involved in biosynthesis and metabolism of estrogens and other *CYP* enzymes can involve in the development of breast cancer risks like *CYP19, CYP21, CYP17, CYP1A2, CYP11A1, CYP2D6, CYP2C19, CYP3A4/5, CYP1A1, CYP1B1, CYP2C8/9.* In this study, distribution of *CYP450* isoenzymes *CYP1A1* (*rs4646421*), *CYP1B1* (*rs1056836*), *CYP19A1* (*rs749292*) and *CYP2C8* (*rs1058930*) and gene polymorphisms in patients with breast cancer in Mazandaran province was investigated by the PCR-RFLP method using restriction enzyme activity.

In a study by Mandana Gheysar et al., they determined that through PCR-RFLP reaction, polymorphisms in genes involved in xenobiotic metabolism and estrogen biosynthesis, like *CYP1A1* (*Ile462Val; rs1048943*), *CYP1B1* (*Leu432Val; rs1056836*) and *CYP19A1* (*C> T; rs10046*) and they found an independent association of *CYP1A1* (*Val*) with BC risk. *CYP1B1* and *CYP19A1* are not associated with breast cancer risk [29]. Joanna Trubicka et al. genotyped 597 cancer patients and 597 controls for three *CYP1B1* SNPs. They found that the three SNPs rs10012, rs1056827 and rs1056836 alone did not provide any significant evidence of association with colorectal cancer risk. Haplotypes of rs1056827 and rs10012 or rs1056827 and rs1056836 revealed an association with colorectal cancer which was significantly stronger in the homozygous carriers. Genetic variants within the *CYP1B1* that are associated with altered function appear to influence susceptibility to colorectal cancer in Poland (30). In another study, Marc T Goodman et al., determined genetic variation in two *CYP19A1* single-nucleotide polymorphisms (SNPs), rs749292 and rs727479, by PCR-RFLP method and association with the risk of ovarian cancer. Results showed that the A allele of rs749292 was positively associated with ovarian cancer risk in a codominant model for all races combined (AG versus AA genotype: odds ratio (OR), 1.48 and 95% confidence interval (CI, 1.07-2.04); GG versus AA: OR, 1.87 (CI, 1.24-2.82); P trend = 0.002). Similar significant associations of the rs749292 A allele on the risk of ovarian cancer were found among Caucasian and Japanese women. No relation of the rs727479 SNP to ovarian cancer risk was observed overall, although Caucasian women carrying the variant A allele compared with women with a CC genotype had an OR of 2.91 (CI, 1.15-7.37). These data suggest *CYP19A1* variants may influence susceptibility to ovarian cancer [31].

Jernstrometal. Investigated *CYP2C8* and *CYP2C9* polymorphisms about tumour characteristics and early breast cancer. In a prospective series of 652 breast cancer patients from southern Sweden was genotyped for *CYP2C8*3, CYP2C8*4, CYP2C9*2*, and *CYP2C9*3*. Frequencies of *CYP2C8/9* polymorphisms were similar to healthy European populations. Significantly less node involvement (P¼0.002) and fewer PR tumours (P¼0.012) were associated with *CYP2C8*4*. Median follow-up was 25 months, and 52 breast cancer-related events were reported. In a multivariate model,

*CYP2C8/9*3/*1*/*2/*1* was the only factor associated with increased risk for early events in 297 tamoxifen-treated, ER-positive patients, adjusted HR 2.54 (CI = 95% 1.11–5.79). The effect appeared to be driven by *CYP2C8*3*, adjusted HR 8.56 (95%CI 1.53-51.1). They found that polymorphic variants of *CYP2C8/9* may influence breast tumour characteristics and disease-free survival in tamoxifen-treated patients [32].

In conclusion, the results of this study indicate that *CYP2C8* (*rs1058930*), *CYP19A1* (*rs749292*) and *CYP1B1 (rs1056836)* gene polymorphisms are associated with breast cancer, and screening for these genes polymorphisms can be used to prognosticate disease, prevent disease progression, and to use appropriate therapeutic.

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References

1. Mehrabani D, Almasi A, Farahmand M, Ahrari Z, Rezaianzadeh A, Mehrabani G, et al. Incidence of breast cancer in Fars province, southern Iran: A hospital-based study. World journal of plastic surgery. 2012; 1(1):16.

2. Stewart SL, King JB, Thompson TD, Friedman C, Wingo PA. Cancer mortality surveillance-United States, 1990-2000. MMWR Surveill summ. 2004; 53(3):1-108. https://doi.org/10.1037/e307142005-001

3. Khadivi R, Harrirchi I, Akbari ME. Ten year breast cancer screening and follow up in 52200 women in Shahre-Kord, Iran (1997-2006). Iranian Journal of Cancer Prevention. 2008; 1(2):73-7.

4. Nafissi N, Saghafinia M, Motamedi MHK, Akbari ME. A survey of breast cancer knowledge and attitude in Iranian women. Journal of cancer research and therapeutics. 2012; 8(1):46. https://doi.org/10.4103/0973-1482.95173 PMid:22531513

5. Allred DC. Ductal carcinoma in situ: terminology, classification, and natural history. Journal of the National Cancer Institute Monographs. 2010; 2010(41):134-8. https://doi.org/10.1093/jncimonographs/lgq035 PMid:20956817 PMCid:PMC5161057

6. Ladd AG-Z, Vásquez AA, Rivadeneira F, Siemes C, Hofman A, Stricker BC, et al. Estrogen receptor α polymorphisms and postmenopausal breast cancer risk. Breast cancer research and treatment. 2008; 107(3):415-9. https://doi.org/10.1007/s10549-007-9562-3 PMid:17453340 PMCid:PMC2217623

7. Wolff MS, Weston A. Breast cancer risk and environmental exposures. Environmental health perspectives. 1997; 105(suppl 4):891-6. https://doi.org/10.1289/ehp.97105s4891 PMid:9255576 PMCid:PMC1470027

8. Hankinson SE, Willett WC, Manson JE, Colditz GA, Hunter DJ, Spiegelman D, et al. Plasma sex steroid hormone levels and risk of breast cancer in postmenopausal women. JNCI: Journal of the National Cancer Institute. 1998; 90(17):1292-9. https://doi.org/10.1093/jnci/90.17.1292 PMid:9731736

9. Dumitrescu R, Cotarla I. Understanding breast cancer risk-where do we stand in 2005? Journal of cellular and molecular medicine. 2005; 9(1):208-21. https://doi.org/10.1111/j.1582-4934.2005.tb00350.x PMid:15784178 PMCid:PMC6741339

10. Kristensen VN, Børresen-Dale AL. Molecular epidemiology of breast cancer: genetic variation in steroid hormone metabolism. Mutation Research/Reviews in Mutation Research. 2000; 462(2-3):323-33. https://doi.org/10.1016/S1383-5742(00)00018-1

11. Gan C, Wang X, Cao Y, Ye W, Liu H, Sun Y. Association of CYP2C19* 3 gene polymorphism with breast cancer in Chinese women. Genet Mol Res. 2011; 10(4):3514-9. https://doi.org/10.4238/2011.December.5.3 PMid:22180071

12. Bartsch H, Nair U, Risch A, Rojas M, Wikman H, Alexandrov K. Genetic polymorphism of CYP genes, alone or in combination, as a risk modifier of tobacco-related cancers. Cancer Epidemiology and Prevention Biomarkers. 2000; 9(1):3-28.

13. Nebert DW, Dalton TP. The role of cytochrome P450 enzymes in endogenous signalling pathways and environmental carcinogenesis. Nature Reviews Cancer. 2006; 6(12):947. https://doi.org/10.1038/nrc2015 PMid:17128211

14. Pavek P, Dvorak Z. Xenobiotic-induced transcriptional regulation of xenobiotic metabolizing enzymes of the cytochrome P450 superfamily in human extrahepatic tissues. Current drug metabolism. 2008; 9(2):129-43. https://doi.org/10.2174/138920008783571774 PMid:18288955

15. Goth-Goldstein R, Stampfer MR, Erdmann CA, Russell M. Interindividual variation in CYP1A1 expression in breast tissue and the role of genetic polymorphism. Carcinogenesis. 2000; 21(11):2119-22. https://doi.org/10.1093/carcin/21.11.2119 PMid:11062177

16. Safarinejad MR, Dadkhah F, Asgari MA, Hosseini SY, Kolahi AA, Iran-Pour E. Glutathione Sâ€"Transferase Polymorphisms (GSTM1, GSTT1, GSTP1) and Male Factor Infertility Risk: A Pooled Analysis of Studies. Urology journal. 2012; 9(3):541-8.

17. Han W, Kang D, Park IA, Kim SW, Bae JY, Chung K-W, et al. Associations between breast cancer susceptibility gene polymorphisms and clinicopathological features. Clinical Cancer Research. 2004; 10(1):124-30. https://doi.org/10.1158/1078-0432.CCR-0834-3 PMid:14734460

18. Lu N, Wu B, Xia Y, Wang W, Gu A, Liang J, et al. Polymorphisms in CYP1A1 gene are associated with male infertility in a Chinese population. International journal of andrology. 2008; 31(5):527-33. https://doi.org/10.1111/j.1365-2605.2007.00804.x PMid:17651397

19. Srivastava DS, Mandhani A, Mittal RD. Genetic polymorphisms of cytochrome P450 CYP1A1 (* 2A) and microsomal epoxide hydrolase gene, interactions with tobacco-users, and susceptibility to bladder cancer: a study from North India. Archives of toxicology. 2008; 82(9):633-9. https://doi.org/10.1007/s00204-007-0276-4 PMid:18200441

20. Ding X, Kaminsky LS. Human extrahepatic cytochromes P450: function in xenobiotic metabolism and tissue-selective chemical toxicity in the respiratory and gastrointestinal tracts. Annual review of pharmacology and toxicology. 2003; 43(1):149-73. https://doi.org/10.1146/annurev.pharmtox.43.100901.140251 PMid:12171978

21. Harth V, Schäfer M, Abel J, Maintz L, Neuhaus T, Besuden M, et al. Head and neck squamous-cell cancer and its association with polymorphic enzymes of xenobiotic metabolism and repair. Journal of Toxicology and Environmental Health, Part A. 2008; 71(13-14):887-97. https://doi.org/10.1080/15287390801988160 PMid:18569591

22. Ickstadt K, Schäfer M, Fritsch A, Schwender H, Abel J, Bolt HM, et al. Statistical methods for detecting genetic interactions: a head and neck squamous-cell cancer study. Journal of Toxicology and Environmental Health, Part A. 2008; 71(11-12):803-15.

https://doi.org/10.1080/15287390801985745 PMid:18569579

23. Haiman CA, Dossus L, Setiawan VW, Stram DO, Dunning AM, Thomas G, et al. Genetic variation at the CYP19A1 locus predicts circulating estrogen levels but not breast cancer risk in postmenopausal women. Cancer research. 2007; 67(5):1893-7. https://doi.org/10.1158/0008-5472.CAN-06-4123 PMid:17325027

24. Daily EB, Aquilante CL. Cytochrome P450 2C8 pharmacogenetics: a review of clinical studies. Pharmacogenomics. 2009; 10(9):1489-510. https://doi.org/10.2217/pgs.09.82 PMid:19761371 PMCid:PMC2778050

25. Shokrzadeh M, Fattahi I, Mohammadpour A, Mashhadban AH. Presence of CagA gene and its antibiotic resistance pattern in Helicobacter pylori isolates. Journal of Mazandaran University of Medical Sciences. 2017; 27(154):60-72.

26. Tafrihi M, Toosi S, Minaei T, Gohari AR, Niknam V, Arab Najafi SM. Anticancer properties of Teucrium persicum in PC-3 prostate cancer cells. Asia Pac J Cancer Prevent. 2014; 15(2):785-91. https://doi.org/10.7314/APJCP.2014.15.2.785 PMid:24568496

27. Economopoulos KP, Sergentanis TN. Does race modify the association between CYP1B1 Val432Leu polymorphism and breast cancer risk? A critical appraisal of a recent meta-analysis. Breast cancer research and treatment. 2010; 124(1):293-4. https://doi.org/10.1007/s10549-010-1097-3 PMid:20686834

28. PICADO-LEONARD J, MILLER WL. Cloning and sequence of the human gene for P450cl7 (steroid 17α-hydroxylase/17, 20 lyase): similarity with the gene for P450c21. Dna. 1987; 6(5):439-48. https://doi.org/10.1089/dna.1987.6.439 PMid:3500022

29. Ghisari M, Eiberg H, Long M, Bonefeld-Jørgensen EC. Polymorphisms in Phase I and Phase II genes and breast cancer risk and relations to persistent organic pollutant exposure: a case-control study in Inuit women. Environmental Health. 2014; 13(1):19. https://doi.org/10.1186/1476-069X-13-19 PMid:24629213 PMCid:PMC4234380

30. Trubicka J, Grabowska-Kłujszo E, Suchy J, Masojć B, Serrano-Fernandez P, Kurzawski G, et al. Variant alleles of the CYP1B1 gene are associated with colorectal cancer susceptibility. BMC cancer. 2010; 10(1):420. https://doi.org/10.1186/1471-2407-10-420 PMid:20701755 PMCid:PMC2929240

31. Goodman MT, Lurie G, Thompson PJ, McDuffie KE, Carney ME. Association of two common single-nucleotide polymorphisms in the CYP19A1 locus and ovarian cancer risk. Endocrine-related cancer. 2008; 15(4):1055-60. https://doi.org/10.1677/ERC-08-0104 PMid:18667686 PMCid:PMC2663409

32. Jernström H, Bågeman E, Rose C, Jönsson P-E, Ingvar C. CYP2C8 and CYP2C9 polymorphisms in relation to tumour characteristics and early breast cancer related events among 652 breast cancer patients. British Journal of Cancer. 2009; 101(11):1817. https://doi.org/10.1038/sj.bjc.6605428 PMid:19935798 PMCid:PMC2788256

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Case Report

Surgical Treatment for Parathyroid Adenoma: A Case Report

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Abstract

BACKGROUND: Parathyroid adenoma is a rare disease. This article aimed to present the classic symptoms, diagnosis, and management of parathyroid adenoma.

CASE REPORT: We report the case of a 24-year-old Asian woman with a several-month-long history of prolonged fatigue, bone, and joint pain. The patient was admitted to our hospital with multiple fractures without significant trauma. Physical examination revealed no palpable masses in the neck. The bone survey showed multiple fractures and osteoporosis of the humerus, clavicle, femur, and lumbar vertebrae. The laboratory workup showed a significantly elevated parathyroid hormone (PTH) level of 1,276 pg/ml (reference range: 10-55 pg/ml) and hypercalcemia, at 11.3 mg/dl (reference range: 8.5-10.5 mg/dl). MRI revealed enlargement of the left inferior parathyroid gland. The patient was diagnosed with a parathyroid gland tumour. Surgical resection was performed, and histopathology revealed parathyroid adenoma. The clinical manifestations and PTH and calcium levels gradually decreased to normal after the surgery. At the two-year follow-up, there was no recurrence of the disease. The patient has resumed her daily life as a farmer.

CONCLUSIONS: Parathyroid adenoma has an excellent prognosis with surgical treatment.

Introduction

The parathyroid endocrine glands play a significant role in calcium homeostasis [1]. Cases of parathyroid adenoma are rare. Parathyroid adenoma causes excessive the autonomic formation and release of parathyroid hormone (PTH), called primary hyperparathyroidism. Primary hyperparathyroidism could be caused by solitary adenomas (80-85%), hyperplasia (10%), multiple adenomas (2%), and carcinomas (2-5%) [1], [2]. The prevalence of primary hyperparathyroidism is approximately 1:1000 in the United States. This condition occurs in women and men at a ratio of approximately 2:1. In Indonesia, there are about 1,000 cases of hyperparathyroidism every year. Women aged 50 years and over have twice as high a risk of this condition than men [2], [3].

Excess PTH secretion in hyperparathyroidism affects hypercalcemia, which directly influences receptors in the bones, intestinal tract, and kidneys. Physiologically, PTH secretion is inhibited by high serum calcium ion levels. This mechanism is inactive in adenoma, as PTH hypersecretion occurs at the same time as hypercalcemia. Calcium resorption from the bones and increased absorption from the intestines have a direct effect on the PTH level [2], [4].

Most hyperparathyroidism patients are asymptomatic. The primary manifestation of hyperparathyroidism is mainly in the bones and kidneys. Osteoporosis and bone fractures are the most common symptoms of primary hyperthyroidism. Kidney abnormalities are mostly due to calcium deposits in the renal parenchyma or recurrent nephrolithiasis. Nephrolithiasis also results in decreased kidney function and phosphate retention [2], [5].

We report a case of parathyroid adenoma diagnosed late due to the difficulties of diagnosis in rural areas and the surgical management of the case.

Case Report

A 24-year-old Asian woman complaining of prolonged fatigue and bone and joint pain for ten months prior came to the hospital. Previous treatment at the Sidrap Healthcare Centre (rural healthcare centre approximately 250 km from the capital province of Makassar) for several months yielded no improvements.

Figure 1: Appearance of the head and neck was within normal limits, no palpable neck masses

One month before admission to the hospital, the patient was walking and suddenly fell into a sitting position; after the incident, she had difficulty moving her lower limbs. Then, the patient was referred to Wahidin Sudirohusodo General Hospital, the top referral hospital for East Indonesia, due to multiple fractures. She had no history of kidney stones and no family history of endocrine neoplasia. The results of a physical examination of the head and neck were within the normal limits, and no palpable neck masses were detected (Figure 1).

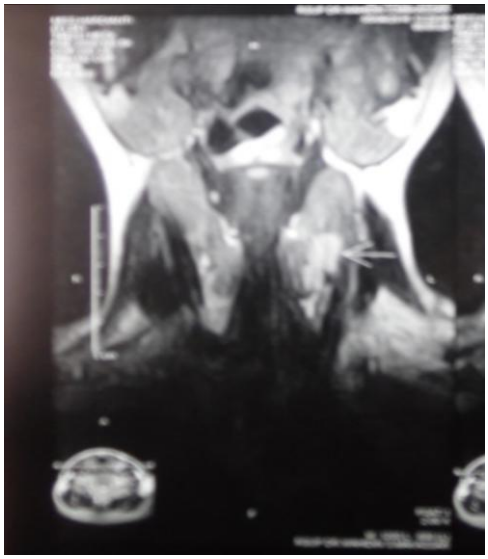

Figure 2: MRI showing left parathyroid lobe mass

Further evaluation revealed fractures of the left humerus and left femur. The bone survey showed multiple fractures and osteoporosis of the humerus (Figure 3), lumbar vertebrae (Figure 4), femur (Figure 5), and clavicle (Figure 6).

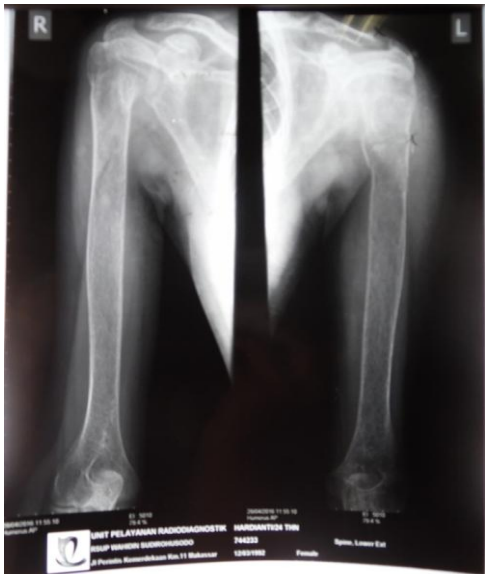

Figure 3: Humeral X-ray showing a transverse fracture of the left humeral bone, fracture of the left clavicle, and osteoporosis

The laboratory workup showed a significantly elevated parathyroid hormone (PTH) level of 1,276 pg/ml (reference range: 10-55 pg/mL) and hypercalcemia, at 11.3 mg/dl (reference range: 8.5-10.5 mg/dL). The vitamin D level was 11.2 ng/mL (30-100 ng/mL).

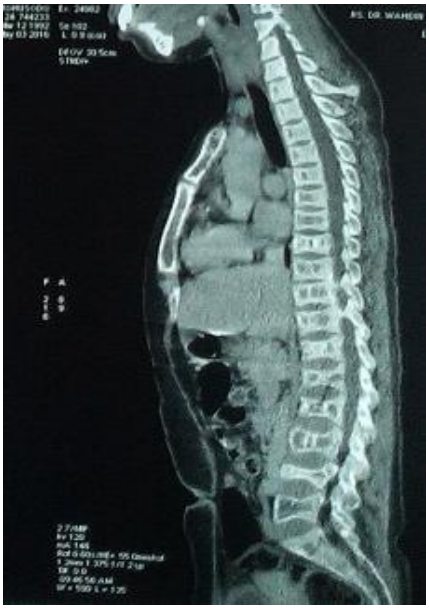

Figure 4: CT scan of lumbar vertebrae showing compression fractures of L1-L5 vertebrae

The abdominal ultrasound results were within the normal limits. Further examination by MRI identified a left parathyroid lobe mass, 1.6 x 0.8 cm, suggestive of adenoma (Figure 2). The patient was diagnosed with a parathyroid gland tumour.

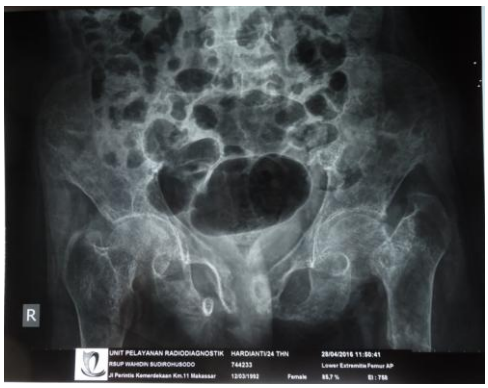

Figure 5: Pelvic X-ray showing the left femoral column fracture. Inferior ramus pubic bone fracture and osteoporosis

Surgical resection was performed using a collar incision, and the tumour was found to originate from the left lower parathyroid gland, with no infiltration into the surrounding structures (Figure 7).

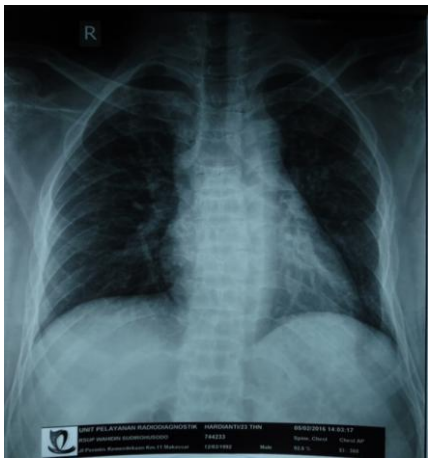

Figure 6: Left clavicle fracture and osteoporosis

Grossly, the surgical specimen was 0.9 x 0.9 x 0.8 cm (Figure 7). The tumour was excised and sent for cryosectioning, which revealed parathyroid adenoma. Histopathology confirmed parathyroid adenoma (Figure 8).

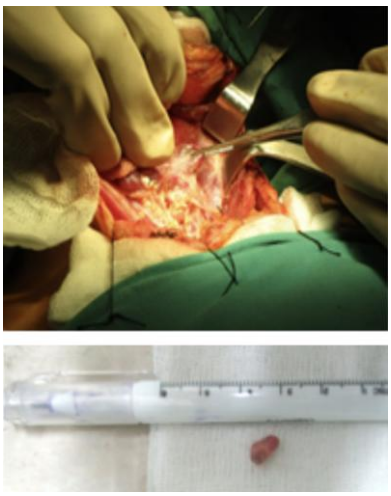

Figure 7: Intraoperative view of the parathyroid gland and specimen

The clinical manifestations and PTH and calcium levels gradually returned to normal postoperatively, as shown in Table 1. At the two-year follow-up, there were no signs or symptoms of recurrence (Figure 9). The patient has resumed her daily life as a farmer.

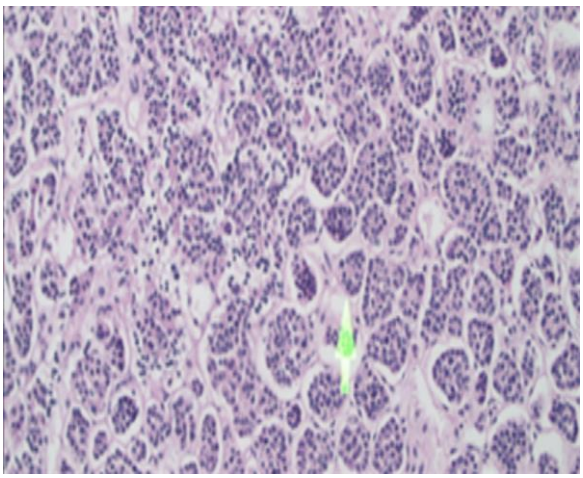

Figure 8: Histopathology found round core cells that were relatively small and monotonous and arranged in a follicular manner with little connective septal tissue. Impression: Parathyroid adenoma

Discussion

Parathyroid tumours are generally not visible or palpable clinically. The clinical symptoms of these patients are more often associated with the manifestation of hypercalcemia [1], [2]. In this case, the patient did not complain about the pain or enlargement of glands in the neck. Palpable neck masses can be found in cases of parathyroid cancer [6].

Figure 9: Follow up on 29/8/2018

Calcium affects almost all functions of the organ systems. Manifestations of hypercalcemia are very diverse [5], [6]. The classic pentad of hypercalcemia symptoms is kidney stones, painful bones, abdominal groans, psychic moans, and fatigue overtones [5], [6]. Symptoms of early hypercalcemia are often undiagnosed, especially in developing countries, in which limited laboratory facilities are available [5], [6]. Reduced bone mineral density causing osteopenia, osteoporosis, and fractures is a frequent complication in late-diagnosed hypercalcemia [6], [7].

Table 1: Laboratory analyses before and after the operation

	Baseline data	Data after the treatment	Reference value
PTH	1,276 pg/ml	95.47 pg/ml	10-55 pg/ml
Calcium	11.3 mg/dl	10.9 mg/dl	8.5-10.5 mg/dl
Vitamin D	11.2 ng/ml	15.4 ng/ml	30-100 ng/ml
Procalcitonin	0.16 ng/ml	0.15 ng/ml	< 0.05 ng/ml
FT4	1.10 ng/dl	0.96 ng/ml	0.932-1.71 ng/dl
TSH	0.57 miU/ml	0.92 miU/ml	0.270-4.20 miU/ml

Neurological disorders and multiple fractures are manifestations of primary hyperparathyroidism [2], [4]. In such cases, the patient may frequently consult with neurologists complaining of fatigue overtones, bone and joint pain, osteopenia, and osteoporosis. Numerous fractures in the patient can be diagnosed as a neurological disorder. Other symptoms can include muscle weakness. In some studies, muscle biopsies showed that the cause of muscle weakness was neuropathy, not myopathy [8], [9].

The diagnosis of parathyroid adenoma is based on clinical symptoms confirmed by laboratory findings [10]. Significant laboratory findings are increased calcium and parathyroid hormone levels. There can be an inconsistency in the laboratory results, in which there is an increase in the PTH level, an increase in the calcium level with hypophosphatemia, and an increase in urinary calcium excretion, indicating impaired calcium homeostasis in the body [11], [12]. Patients with PHPT show decreased serum phosphate (50%) and increased calcium concentrations in urine over 24 hours (60%).

Approximately 80% of cases result in mild hyperchloremic metabolic acidosis. Elevated levels of alkaline phosphatase can be found in 10% of cases, along with complications related to bone disease. A serum and urine protein electrophoresis examination can be conducted to eliminate the possibility of multiple myeloma [13], [14].

In some cases, PHPT could also manifest normocalcemic conditions due to a vitamin D deficiency, low serum albumin levels, excessive hydration, a high-phosphate diet, and low calcium levels in the normal range [13], [14]. In such cases, the laboratory results show concurrent levels of calcium and parathyroid hormone (PTH), with normal levels of FT4 and TSH, normal kidney function, and no signs of infection. Extreme increases in the parathyroid hormone level compared with the level of hypercalcemia raise suspicion of abnormalities in the parathyroid gland, which can be confirmed by radiological examination results.

Cases of hypercalcemia or PHPT with a vitamin D deficiency, determined by routine X-ray examinations of the hands and skull, could reveal osteitis fibrosa cystica, but this condition is rare. BMD examination with dual-energy absorptiometry could reveal bone conditions. Ultrasonography of the abdomen could detect stones in the kidneys or biliary tract [14], [15]. The process of locating the parathyroid glands through radiological examinations can be divided into non-invasive and invasive methods. Non-invasive methods include ultrasonography, CT, and MRI, or even radioisotope examination using Tc-99m sestamibi [16].

Parathyroid abnormalities usually cause glandular enlargement. Examination with scintigraphy has a high sensitivity of up to 80% in determining the location of a single parathyroid adenoma but of only 25% when used to locate multiple adenomas. Examination by contrast-enhanced CT and MRI can also be used primarily to determine the location of parathyroid adenomas outside of the parathyroid gland [14], [15]. The results of ultrasonography and neck CT, in this case, revealed an inferior left parathyroid lobe mass suggestive of an adenoma. Thus, the examination supported the diagnosis of parathyroid adenoma. Therefore, parathyroidectomy was performed on the patient.

In some cases, the parathyroid adenoma is diagnosed together with bone diseases, such as osteoporosis, and fractures, as reported by Mabulac and Twigt. Braverman stated that there were correlations among the PTH level, bone disease, and neuropsychiatric symptoms, in which the PTH level tended to increase the occurrence of bone disease and neuropsychiatric disorders [14], [15].

The treatment of parathyroid tumours is the surgical exploration of the neck and removal of pathological parathyroid glands followed by another parathyroid gland biopsy to determine the possibility of adenoma or multiple gland hyperplasia. If a parathyroid tumour was not found, it is necessary to consider the superior exploration of the mediastinum [16], [17].

The laboratory examination results for the patient in this case before surgery were as follows: calcium, 11.3 mg/dl; parathyroid hormone, 1,276 pg/mL; vitamin D, 11.2 ng/mL. After surgery, the calcium level was 10.9 mg/dl, and the PTH level was 95.47 pg/ml. According to these results, the levels of calcium and parathyroid hormone decreased significantly after surgery. Calo and Zawawi reported that in a case of primary hyperthyroidism, after removal of the parathyroid gland tumour, the parathyroid hormone and calcium levels would immediately decrease. In this case, during surgery, the left parathyroid gland was first removed; then, the

PTH level was measured, and the value was significantly decreased. This result indicates that parathyroid surgery decreases excessive PTH levels [15], [16].

Along with the postoperative decrease in the parathyroid hormone and calcium levels, the condition of the patient gradually improved with a reduction in the patient's perceived pain. The patient also consulted the physiotherapy unit for guidance regarding walking exercises. After surgery and physiotherapy, the patient's condition tended to improve; the patient could walk with a stick and experienced no more fractures. Regarding the osteoporosis, it was characterised by a decrease in height and multiple fractures. The patient's height before the disease was 153 cm; after surgery, her height was 150 cm, and after one year after surgery, her height was 150.2 cm, indicating a decrease in height after the illness.

In conclusion, parathyroid adenoma should be strongly suspected if a patient presents with any prolonged fatigue, bone pain, osteoporosis that is not associated with age, and multiple fractures without significant trauma — a delay in the diagnosis of parathyroid adenoma results in manifestations that can be avoided. Parathyroid adenoma has an excellent prognosis with surgical treatment.

References

1. Lofrese JJ, Lappin SL. Physiology, Parathyroid. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing, 2019. Available from: https://www.ncbi.nlm.nih.gov/books/NBK482510/

2. Bilezikian JP, Cusano NE, Khan AA, Liu JM, Marcocci C, Bandeira F. Primary hyperparathyroidism. Nature reviews Disease primers. 2016; 2:16033. https://doi.org/10.1038/nrdp.2016.33 PMid:27194212 PMCid:PMC5385896

3. Syamsu Hidayat, R., De Jong Wim. Buku Ajar Ilmu Bedah, Edisi Revisi. Jakarta: EGC, 2016.

4. Levine BS, Rodríguez M, Felsenfeld AJ. Serum calcium and bone: effect of PTH, phosphate, vitamin D, and uremia. Nefrología (English Edition). 2014; 34(5):658-69.

5. Baum M. The bone-kidney axis. Current opinion in pediatrics. 2014; 26(2):177. https://doi.org/10.1097/MOP.0000000000000071 PMid:24535498 PMCid:PMC4074396

6. McClenaghan F, Qureshi YA. Parathyroid cancer. Gland Surgery. 2015; 4(4):329.

7. Bhansali A, Gogate Y. Hyperparathyroidism. Clinical Rounds in Endocrinology. Springer, New Delhi, 2015:309-339. https://link.springer.com/chapter/10.1007/978-81-322-2398-6_14 https://doi.org/10.1007/978-81-322-2398-6_14

8. Shinjo SK, Pereira RM, Borssatto AG, Kochen JA. Musculoskeletal manifestations in primary hyperparathyroidism. Revista Brasileira de Reumatologia. 2009; 49(6):703-11. https://doi.org/10.1590/S0482-50042009000600007

9. El-Sayed, MA, El-Habashy HR, El-Taweel NS. Progressive Muscle Weakness in Hyperparathyroidism. 2010.

10. Khan M, Sharma S. Physiology, parathyroid hormone (PTH). InStatPearls [Internet]. StatPearls Publishing, 2018. https://www.ncbi.nlm.nih.gov/books/NBK499940/

11. Calò PG, Pisano G, Loi G, Medas F, Barca L, Atzeni M, Nicolosi A. Intraoperative parathyroid hormone assay during focused parathyroidectomy: the importance of 20 minutes measurement. BMC surgery. 2013; 13(1):36. https://doi.org/10.1186/1471-2482-13-36 PMid:24044556 PMCid:PMC3848580

12. Twigt BA, Scholten A, Valk GD, Rinkes IH, Vriens MR. Differences between sporadic and MEN related primary hyperparathyroidism; clinical expression, preoperative workup, operative strategy, and follow-up. Orphanet journal of rare diseases. 2013; 8(1):50. https://doi.org/10.1186/1750-1172-8-50 PMid:23547958 PMCid:PMC3623824

13. Romanidis K, Karathanos E, Nagorni EA, Giatromanolaki A, Sibridis E, Zissimopoulos A, Vogiatzaki T, Simopoulos C, Pitiakoudis M. Parathyroid adenoma detected with 99m Tc-tetrofosmin dual-phase scintigraphy: a case report. BMC research notes. 2014; 7(1):335. https://doi.org/10.1186/1756-0500-7-335 PMid:24894734 PMCid:PMC4076066

14. Zawawi F, Mlynarek AM, Cantor A, Varshney R, Black MJ, Hier MP, Rochon L, Payne RJ. Intraoperative parathyroid hormone level in parathyroidectomy: which patients benefit from it? Journal of Otolaryngology-Head & Neck Surgery. 2013; 42(1):56. https://doi.org/10.1186/1916-0216-42-56 PMid:24350891 PMCid:PMC3878236

15. Le TN, Kerr PD, Sutherland DE, Lambert P. Validation of 1-hour post-thyroidectomy parathyroid hormone level in predicting hypocalcemia. Journal of Otolaryngology-Head & Neck Surgery. 2014; 43(1):5. https://doi.org/10.1186/1916-0216-43-5 PMid:24476535 PMCid:PMC3922691

16. Williams BA, Trites JR, Taylor SM, Bullock MJ, Hart RD. Surgical management of primary hyperparathyroidism in Canada. Journal of Otolaryngology-Head & Neck Surgery. 2014; 43(1):44. https://doi.org/10.1186/s40463-014-0044-4 PMid:25367580 PMCid:PMC4221664

17. Adler JT, Sippel RS, Schaefer S, Chen H. Preserving function and quality of life after thyroid and parathyroid surgery. The lancet oncology. 2008; 9(11):1069-75. https://doi.org/10.1016/S1470-2045(08)70276-6

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Case Report

Case Series of Pre-Operative Endovascular Embolization of Nasopharyngeal Angiofibroma Using Polyvinyl Alcohol Foam Particle: A Single Centre Experience

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Abstract

BACKGROUND: Nasopharyngeal Angiofibroma is a rare neoplasm in the sphenopalatine foramen. This tumour is histologically benign, but clinically malignant because it can erode the bone and surrounding structures, such as the pterygopalatine fossa, paranasal sinuses, and nasal cavity. It is a highly vascular tumour, sometimes from multiple Feeding arteries, and tends to bleed easily.

CASE PRESENTATION: In these cases, series, we reported four cases of nasopharyngeal angiofibroma in children and one case in an elderly patient. The diagnosis was made by history taking, physical examination and Cerebral MSCT Angiography, as well as Digital Subtraction Angiography (DSA). After identification of the Feeding artery, we performed transarterial embolisation using polyvinyl alcohol (PVA) foam particles.

CONCLUSION: Preoperative embolisation in the highly vascular tumour, such as nasopharyngeal angiofibroma, is very useful to reduce peri-operative complication of surgery. This procedure can reduce blood loss during resection of the tumour and gives better outcomes.

Introduction

Nasopharyngeal Angiofibroma (NA) is a rare, benign and highly vascular tumour originating in the sphenopalatine foramen, and may extend to the pterygopalatine fossa, paranasal sinuses and nasal cavity [1]. They accounted for 0.05% of all head and neck tumours and reported to be 1 per 5,000-60,000 Ear Nose Throat (ENT) patients in the United States. NA occurs exclusively in men. NA generally occurs in the second decade of life between 7-19 years old and rarely occurs at the age of more than 25 years. Randowski et al., classified Nasopharyngeal Angiofibroma into three stages, based on the expansion of the tumour (Table1) [2], [3], [4].

Table 1: Staging systems in juvenile nasopharyngeal angiofibromas based on Randowski et al. [5]

Stage	Description of stage
IA	Limited to the nasal cavity and / or nasopharyngeal area
IB	Same as stage I with expansion to one or more paranasal sinuses
IIA	Minimal expansion to the sphenopalatine foramen includes a minimal portion of the medial part of the pterygopalatine fossa
IIB	The tumour occupies the entire sphenopalatine fossa space, forces the posterior wall of the maxilla forward, shifts laterally or anteriorly from the maxillary artery branch, superior expansion may be present, orbital bone erosion
IIC	Extension to the pterygopalatine fissure towards the cheek and infratemporal fossa or towards the posterior pterygoid plate
IIIA	Erosion of the skull base (cranial base) with minimal expansion towards the intracranial
IIIB	Erosion of the skull base (cranial base) with extensive expansion in the intracranial direction with or without involving the cavernous sinus

In this article, we reported our first five

institutional cases of nasopharyngeal angiofibroma referred from the ENT department, which is four cases in children and a rare case in an elderly patient. The diagnoses were made by history taking, physical examination and radiological imaging with Cerebral and carotid Multiple Slice Computed Tomography (MSCT) angiography. All patients underwent pre-operative Digital Subtraction Angiography (DSA) to determine the arterial Feeding of the tumour, followed by endovascular embolisation using polyvinyl alcohol (PVA) foam particles. Clinical manifestations including the symptoms and signs of each case will be discussed individually, along with the description of pre and post-procedural outcome.

Case Series

Case 1

A 13-year-old boy presented with nasal obstruction and breathing difficulties experienced for 1 year earlier, occurring slowly and then worsening in the last 3 months. He also had epistaxis in his right nasal cavity and felt pain in his right ear. Physical examination revealed a lump in the right nasal cavity. Physical examination revealed a lump in the right nasal cavity. The result of laboratory tests, Hemoglobin (Hb) at admission was 10.5 g/dl, and postoperative Hb was 10.3 g/dl. MSCT Angiography of cerebral and carotid showed an isodense mass (30.47 HU) with intense contrast uptake. The mass was well-defined, with irregular edges covering the nasopharynx particularly at the right sphenopalatine foramen extending to the right nasal cavity, right maxillary sinus, sphenoid sinus bilateral and right ethmoidal, caused bowing of right maxillary and ethmoidal bones. It also pushed the nasal septum to the left. This patient was diagnosed with stage II right nasopharyngeal angiofibroma.

Cerebral DSA procedure was performed, and the catheter tip was navigated to the right common carotid artery (RCCA). RCCA injection revealed the Feeding artery, which was originated from the C4 segment of the right internal carotid artery (RICA) and the right internal maxillary artery, a branch of the right external carotid artery (RECA). Tumour embolisation was carried out using 300-500 microns polyvinyl alcohol (PVA) foam particles, at the right internal maxillary artery, until the tumour blush decreased. Feeding artery originating from the right internal carotid artery could not be embolized for they were small and could not be reached by the available microcatheter. The post-surgical diagnosis was nasopharyngeal angiofibroma. The amount of blood loss during surgery was ± 500 cc. The patient was discharged without complication on day 6 after the operation.

Case 2

A 16-year-old boy presented with left nasal obstruction in the past 4 months before admission, accompanied by epistaxis. His physical examination revealed a tumour, covering his left nasal cavity. The result of laboratory tests was as followed: Hb at admission was 11.8 g/dl, and Hb post-surgery was 7.8 g/dl. The result of MSCT Angiography of cerebral and carotid showed a centrifuging mass in the sphenopalatine canal area with a crowded vascular pattern that expanded and widened into the left maxillary sinus. The mass encroached the pterygomaxillary fossa and filled up most of the infratemporal space area. It extended into sphenoid and ethmoid sinuses as well as to the left side of the nasal cavity which narrowed the airway. No intracranial lesions were seen. The main Feeding artery was from the branch of the left internal maxillary artery. He was diagnosed with Juvenile Nasopharyngeal Angiofibroma (JNA) with Feeding artery from an internal maxillary artery (Radkowsky Grade IIc) (Table 1).

Cerebral DSA procedure through the left external carotid artery (LECA) injection showed a tumour blush that supplied by the left internal maxillary artery. Tumour embolisation was carried out using PVA foam particles 300-500 microns in the left internal maxillary artery until the tumour blush on the left side disappeared. Tumour resection was performed afterwards. The histopathological result showed proliferation of blood vessels containing erythrocytes, between connective tissue. This finding was well-matched with angiofibroma (Figure 1). The patient was discharged 6 days after surgery without severe complications.

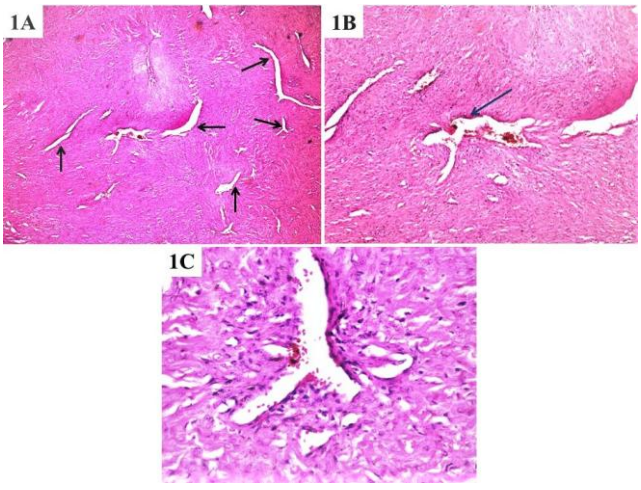

Figure 1: Histological features of angiofibroma. A) Visible erythrocyte between the proliferation of connective tissue (black arrow) 4 X magnification; B) Thin-walled vascular structure containing erythrocytes with a large layer of endothelium and fibrous component (blue arrow) 10 X magnification; C) Proliferation of connective tissue with components of fibroblast cells, with the thin layer of endothelium, 40 X enlargement (staining of hematoxylin-eosin)

Case 3

A 16-year-old boy presented with epistaxis for 4 months before admission. The epistaxis gradually worsened during the last 2 months before admission. The complaint was accompanied by nasal congestion and headache. Physical examination revealed a tumor in the right nasal cavity. The result of Hb tests was 11.2 g/dl and 10.5 g/dl for preoperative and postoperative, respectively. The result of MSCT angiography of cerebral and carotid showed a heterogeneous mass that enhances avidly upon contrast administration (90 HU). The mass showed well-definite boundaries and regular edges which originated from the right side of the nasopharynx and compressed the airway. It did not destroy the surrounding bones nor infiltrate the intracranial tissues. The Feeding artery was the right internal maxillary artery. The nasopharyngeal mass was consistent with angiofibroma. Cerebral DSA procedure was performed, and injection through RECA showed tumour blush which was supplied by the right internal maxillary artery. The tumour was embolized using PVA particles 300-500 microns in the right internal maxillary artery until the tumour blush on the right side disappeared. Subsequently, resection surgery was performed with a total of ± 500 cc blood loss during the procedure. The tissue was sent to the histopathological laboratory, and the result was consistent with angiofibroma. The patient was treated for 5 days postoperatively and eventually got discharged.

Case 4

An 11-year-old boy visited the ENT outpatient clinic with his parents. The boy came with right nasal obstruction for 3 months before admission. Physical examination showed proptosis of the right eye and a tumour in the right nasal cavity (Figure 2). Laboratory examination showed Hb results were 14.4 gr/dl and 14.5 g/dl for preoperative and postoperative, respectively.

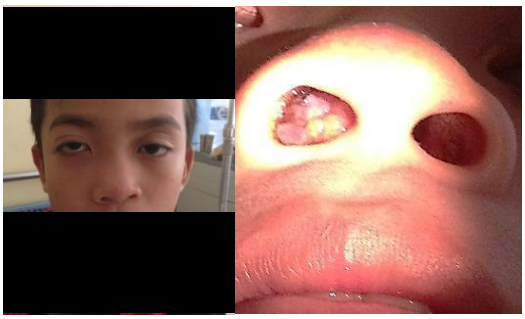

Figure 2: A) Proptosis of the right eye; B) Tumor is visible through right cavum nasi

Noncontrast CT scan on paranasal sinuses showed expansive mass with heterogeneous density, well-defined boundary, and irregular edge. The size of the mass was 4.86 x 4.34 x 4.84 cm and tended to originate from the right maxillary sinus. The tumour infiltrated the right nasal cavity, sphenoid sinus, ethmoid sinus, right frontal sinus and pressed the nasal septum to the left and caused destruction of the lateral wall of the right maxillary sinus. The diagnosis was right sinonasal mass involving more than one paranasal sinuses (Figure 3).

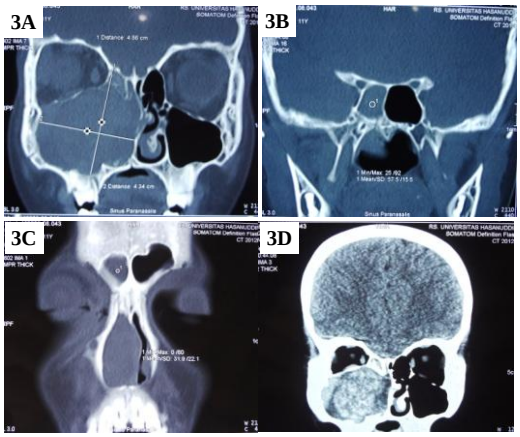

Figure 3: Paranasal Sinus CT Scan without contrast with coronal section. A) Tumours in from the right maxillary sinus compressed the right ethmoidal sinus and pushed nasal septum to the left. The tumour also destroyed the lateral wall of the right maxillary sinus; B) The tumour infiltrated the right sphenoid sinus; C) The tumour also infiltrated the right frontal sinus; D) Head CT coronal section without contrast, the nasal septum was pushed to the left, and the tumour infiltrated the ethmoidal sinus and right sphenoid sinus

Cerebral and carotid MSCT angiography revealed an isodense mass, with a regular edge, about 4.9 x 4.0 x 3.2 cm, originated from the right maxillary sinus. The tumour extended into the right nasal cavity, right ethmoidal sinus, right sphenoidal and right orbital cavity, destroyed the right maxillary and right zygomaticus bone. The right lamina papyracea of the ethmoidal bone and the nasal bone slightly pressed anteriorly (Figure 4).

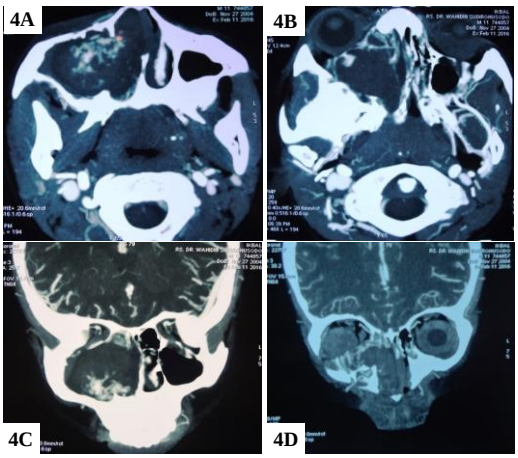

Figure 4: Cerebral and carotid MSCTA. A) There was an isodense mass with well-defined and regular edges; the size about 4.9 x 4.0 x 3.2 cm, the tumour appeared to originate from the right maxillary sinus, pressing the nasal septum to the left; B) The tumor pressed the right ethmoidal and sphenoid sinus; C) The tumour destroyed the lateral wall of right maxillary sinus and pushed nasal septum to the left; D) The tumor pressed right frontal sinus

The Feeding artery appeared to originate from the right internal maxillary artery (Figure 5). The diagnosis was a sinonasal mass corresponding to sinonasal angiofibroma. Cerebral DSA procedure via RECA showed the presence of a tumour blush that was supplied by the right internal maxillary artery (Figure 6, A and B).

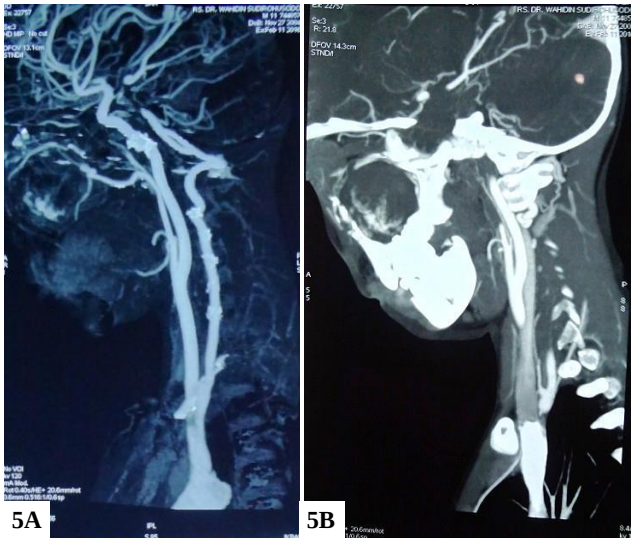

Figure 5: MSCTA Cerebral and carotid. A) Feeding artery was originating from the right internal maxillary artery; B) There was an isodense mass with well-defined regular edges; the size is 4.9 x 4.0 x 3.2 cm, the mass was from the maxillary sinus

Tumour embolisation was performed using PVA particles 300-500 microns through the right internal maxillary artery until the tumour blush on the right side disappeared (Figure 6, C). After that, a nasopharyngeal angiofibroma resection surgery was performed with a total of ± 500 cc of blood loss during surgery. The tissue was sent to the histopathological anatomy laboratory with the results was following angiofibroma. The patient was treated for 5 days without complications and got discharged.

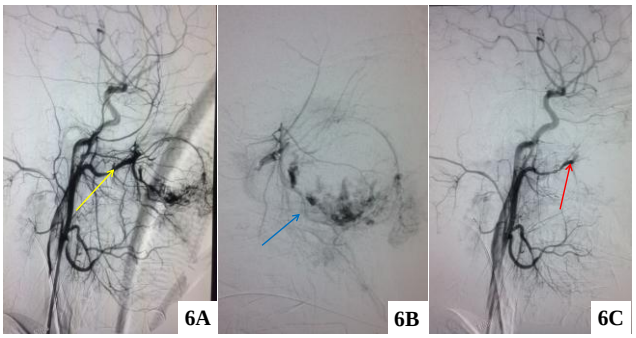

Figure 6: Digital Subtraction Angiography (DSA) cerebral, injection of the right external carotid artery (RECA). A) Pre embolization: There was a tumour blush with Feeding artery from the right internal maxillary artery (yellow arrow); B) Tumor blush was shown by blue arrow; C) Post embolization using PVA particle, in the right internal maxillary artery until the tumour blush on the right side was no longer visible (red arrow).

Case 5

A 61-years-old man came to the emergency department with unprovoked epistaxis for 5 months before admission. Laboratory examination showed the Hb results were 11.1 gr/dl and 10.5 g/dl for preoperative and postoperative, respectively. The results of Noncontrast CT scan on paranasal sinuses coronal view showed isodense mass (26 HU), with relatively firm boundaries, irregular edges, and without calcification. The tumour was from the right nasal cavity, eroded the right maxillary, extended to the right ethmoid sinus and pressed the nasal septum to contralateral. This finding was suggestive of right nasal cavity mass which extended into the right maxillary sinus and the right ethmoid sinus. Cerebral and carotid MSCT angiography showed a mass in the right nasal cavity, which was hypervascular and originated from the right maxillary artery. Cerebral DSA procedure was performed through the right internal carotid artery (RICA). Tumour blush was seen, originated from the right ophthalmic artery (Figure 7, A and B). Injection of the RECA also showed the feeding artery from the right internal maxillary artery (Figure 7, A and B). The tumour was embolized using PVA particles 300-500 microns until the tumour blush decreased (Figure 7, C and D). The feeding artery originated from the right ophthalmic artery was not embolized because of the risk of causing blindness.

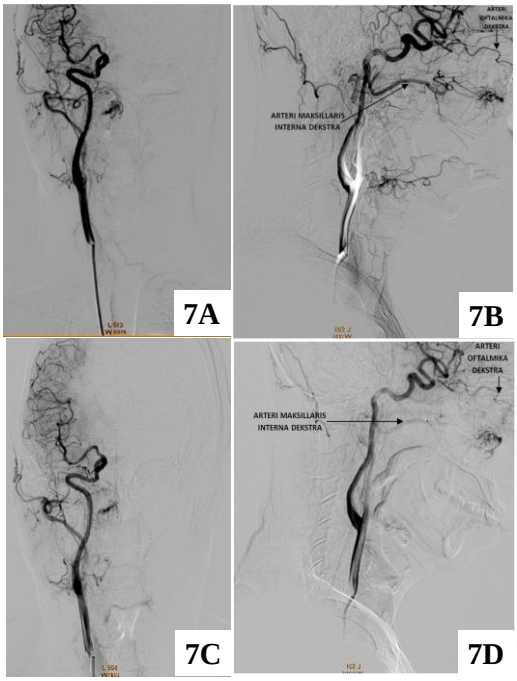

Figure 7: Digital Subtraction Angiography (DSA) Cerebral. A) Right, Common Carotid Artery (RCCA) Anterior-Posterior (AP) view pre embolisation. Tumour blush was seen (yellow arrow); B) RCCA the pre-embolized: lateral view. The Feeding arteries were from the right internal maxillary artery and the right ophthalmic artery; C) RCCA AP view post embolization, tumour blush was still visible (black arrow); D) RCCA lateral view, post embolization, blood supply from the right internal maxillary artery did not appear, while Feeding artery from the right ophthalmic artery was still visible.

The tumour blush was still visible but decreased to some extent (Figure 7D). After that, a nasopharyngeal angiofibroma resection surgery was performed with a total of ± 500 cc blood loss intraoperative. The tissue was sent to the histopathological test, and the result was angiofibroma. The patient was treated for 5 days without complications before finally discharged.

Pre-operative Endovascular Embolization and the Outcomes

All patients in this case series were treated with pre-operative embolisation before tumour resection. Tumour embolisation was performed using the endovascular trans-arterial technique, with PVA foam particles (William Cook Europe ApS, Denmark) as embolic material. The procedure was coded as Percutaneous Transcatheter Infusion Embolization (ICD-9-CM 99.29).

Cerebral DSA procedures were performed in case 1 and 4 with asepsis preparation and general anaesthesia. The right femoral artery was catheterised, using a 6F sheath (Merit Medical Systems Inc., South Jordan, USA, USA), and a 6F JR4 guide catheter (Terumo Corporation, Tokyo, Japan). The procedure was followed by tumour embolisation, using a 6F JR4 guide catheter (Terumo Corporation, Tokyo, Japan), 2.4F microcatheter (Terumo Corporation, Tokyo, Japan) and 1.4 micro guidewires (Terumo Corporation, Tokyo, Japan).

Cerebral DSA procedure was performed in case 2 with asepsis preparation and local anaesthesia. The right femoral artery was catheterised using 6F sheath (Merit Medical Systems Inc., South Jordan, Utah, USA), 5F HH1 guide catheter (Terumo Corporation, Tokyo, Japan), 6F JR3.5 catheter (Terumo Corporation, Tokyo, Japan). Subsequent tumour embolisation was done by using a 6F JR3.5 guide catheter (Terumo Corporation, Tokyo, Japan), 2.4F microcatheter (Terumo Corporation, Tokyo, Japan), 1.4F micro guidewire (Terumo Corporation, Tokyo, Japan) and particulate PVA contour 300-500 microns.

Cerebral DSA procedure in case 3 was performed with asepsis preparation and local anaesthesia, right femoral artery catheterised with 6F sheath (Merit Medical Systems Inc, South Jordan, Utah, USA), and 6F JR4 guide catheter (Terumo Corporation, Tokyo, Japan).

Cerebral DSA procedure in case 5 was performed with asepsis preparation and local anaesthesia, right femoral artery was catheterized using 6F sheath (Merit Medical Systems Inc., South Jordan, Utah, USA), 6F JR4 guide catheter (Terumo Corporation, Tokyo, Japan), and 0.038 guidewire (Terumo Corporation, Tokyo, Japan). The procedure was followed by tumour embolisation procedure; using 6F JR4 guide catheter (Merit Medical Systems Inc., South Jordan, Utah, USA), 1.8F microcatheter (Terumo Corporation, Tokyo, Japan) 1.4F micro guidewire (Terumo Corporation, Tokyo, Japan) and particulate PVA contour 300-500 microns.

The result of tumour embolisation in case 2, 3 and 4 was satisfactory, with the total disappearance of tumour blush. In case 1 and case 5, the tumour blush is still visible, although reduced to some extent, because the tumour was also received blood supply from the right internal carotid artery case 1, and the right ophthalmic artery case 5 (Table 2). Within 2 to 3 days of embolisation, the patients underwent tumour resection surgery and postoperative treatment for approximately 5-6 days. All the patients were discharged with good outcome and without any complications.

Table 2: Demographic, laboratory, and radiological characteristic of each patient

Cases	Sex	Age / yrs	Pre-Op. Hb (g/dl)	Post-Op. Hb (g/dl)	MSCT Scan of Cerebral and Carotid Angiography (Arterial Feeding)	DSA Arterial Feeding
1	M	13	10.5	10.3	Right Internal maxillary artery, a branch of the external carotid artery	C4 segment of the right internal carotid artery; Right Internal maxillary artery, a branch of the external carotid artery
2	M	11	11.8	7.8	Left internal maxillary artery, a branch of the external carotid artery	Left internal maxillary artery, a branch of the external carotid artery
3	M	16	11.2	10.5	Right Internal maxillary artery, a branch of the external carotid artery	Right internal maxillary artery, a branch of the external carotid artery
4	M	11	14.4	14.5	Right Internal maxillary artery, a branch of the external carotid artery	Right internal maxillary artery, a branch of the external carotid artery
5	M	61	11.1	10.5	Right Internal maxillary artery, a branch of the external carotid artery	Right internal maxillary artery, a branch of the external carotid artery and Right ophthalmic artery

Discussion

Nasopharyngeal angiofibroma (NA) was first described in ancient times by Hippocrates (5th century BC). NA is a rare, benign fibrovascular tumour, at the superoposterior area of the sphenopalatine foramen and is often found in young men, between 14-25 years old. It is estimated to be 0.05% of all benign Head and Neck tumours. These tumours are histopathologically benign, yet clinically destructive. The most common presenting symptoms are unilateral nasal congestion, nasopharyngeal lump and recurrent epistaxis. In the later stages, this tumour can cause facial deformity, proptosis, headache and deafness. Computed tomography (CT-Scan) and magnetic resonance imaging (MRI) is the most widely used modalities for diagnosis and evaluation of tumour growth, bone destruction and staging of angiofibroma. Also, a pre-operative angiographic procedure is performed to identify Feeding artery and to describe tumour size and location [1], [6], [7]. In our case series, 4 patients were aged between 14-25 and

1 patient was 61 years old, thus considered as a rare case. All patients are male. Almost all patients presented with the chief complaint of nasal congestion, difficulty breathing, epistaxis as well as mass in the nasal cavity. The diagnosis was confirmed by radiological examination in the form of cerebral and carotid MSCT angiography and digital subtraction angiography (DSA).

Surgical resection of nasopharyngeal angiofibroma is the mainstay of treatment. Other treatment modalities include radiation, cryotherapy, electrocoagulation, hormonal therapy, embolisation and injection of sclerosing agents [8],[9]. Although surgical excision is the definite treatment, the risk of this procedure is high, particularly due to the high risk of bleeding, since the tumour is highly vascularized. Fonseca et al. reported that there was no significant difference in bleeding risk between 15 patients who undergo surgery without preoperative embolisation and those with pre-operative embolisation [10]. Gaillard et al. reported that there could be the risk of tumour recurrence in patients who were not pre-operatively embolized. Furthermore, Gaillard confirmed that cure rates could be as high as 94% if pre-operative tumour embolisation is performed [11].

In our institution, this is the first serial cases of preoperative tumour embolisation for nasopharyngeal angiofibroma, before tumour resection was performed. Embolisation is a minimally invasive procedure, aimed at devascularization of tumour or cerebrovascular malformation. A catheter is passed through femoral, navigated until its tip is in the target vessel. In this case, the target vessel is the Feeding artery of the nasopharyngeal tumour. An embolisation agent is then ejected through the catheter tip into the blood vessel to revascularize the tumour.

In all five cases, the internal maxillary artery, a branch of the external carotid artery, is the main Feeding artery for the Nasopharyngeal Angiofibroma (Table 2), but there are other Feeding arteries, such as the C4 segment of the right internal carotid artery and the right ophthalmic artery. After the Feeding artery was identified, pre-operative tumour embolisation was performed using the PVA foam particle agent. PVA foam particle is routinely used as embolisation agent for preoperative embolisation. PVA can produce permanent and non-absorbing occlusion, with a low rate of blood vessel recanalisation. All five patients experienced anaemia both before and after tumour embolisation and tumour resection. All patients underwent tumour resection after the pre-operative embolisation. During the surgical procedure, the blood loss is only around 500 cc, and all patients were discharged, after 5-6 days of postoperative treatment, without any severe complications.

In conclusion, preoperative embolisation of the Feeding artery for Nasopharyngeal Angiofibroma is a very advisable procedure. Pre-operative embolisation can reduce blood loss during peri-operative surgical operation and improve outcome. In our institution, we routinely perform it. Before embolisation procedure, thorough evaluation with MSCT angiography and DSA must be performed to evaluate the Feeding artery.

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References

1. Ahmed Ashrafi SK, Suhail Z, Khambaty Y. Postembolization infarction in juvenile nasopharyngeal angiofibroma. J. Coll Physicians Surg Pak. 2011; 21(2):115-6.

2. Antoniades K, Antoniades DZ, Antoniades V. Juvenile angiofibroma: Report of a case with intraoral presentation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2002; 94(2):228-32. https://doi.org/10.1067/moe.2002.125582 PMid:12221391

3. Garca MF, Yuca SA, Yuca K. Juvenile Nasopharyngeal Angiofibroma. Eur J Gen Med. 2010; 7 (4):419-25. https://doi.org/10.29333/ejgm/82897

4. Baptista MAFB, Pinna FR, Voegels RL. Extra nasopharyngeal angiofibroma originating in the inferior turbinate: a distinct clinical entity at an unusual site. Int Arch Otorhinolaryngol. 2014; 18:403-405. https://doi.org/10.1055/s-0034-1387811 PMid:25992131 PMCid:PMC4297003

5. Radkowski D, McGill T, Healy GB, Ohlms L, Jones DT. Angiofibroma. Changes in staging and treatment. Arch Otolaryngol Head Neck Surg. 1996; 122(2):122-9. https://doi.org/10.1001/archotol.1996.01890140012004 PMid:8630204

6. Rahma S, Et al. Nasopharyngeal Angiofibroma in Adults. Department of Ear Nose Throat Head Neck Surgery. Faculty of Medicine, Andalas University, Padang.

7. Anggreani L, Et al. Description of Estrogen β Receptor Expression on Young Nasopharyngeal Angiofibromas Using Immunohistochemical Examination. Department of Ear Nose Throat Head Neck Surgery. Faculty of Medicine, University of Indonesia Jakarta.

8. Maniglia AJ, Mazzarella LA, Minkowitz S, et al. Maxillary sinus angiofibroma treated with cryosurgery. Arch Otolaryngol. 1969; 89:111-116. https://doi.org/10.1001/archotol.1969.00770020529015 PMid:4303494

9. Chen WL, Huang Z, Li J, Chai Q, Zhang D. Percutaneiussclerotherapy of juvenile nasopharyngeal angiofibroma using fibrin glue combined with OK-432 and bleomycin. International journal of Pediatric Otorhinolaryngology. 2010; 74:422-5. https://doi.org/10.1016/j.ijporl.2010.02.002 PMid:20189660

10. Fonseca AS, Vinhaes E, Boaventura V. et al. Surgical treatment of non-embolized patients with nasoangiofibroma. Braz J Otorhinolaryngol. 2008; 74:583-587. https://doi.org/10.1016/S1808-8694(15)30607-8

11. Gaillard AL, Anastácio VM, Piatto VB, Maniglia JV, Molina FD. A seven-year experience with patients with juvenile nasopharyngeal angiofibroma. Braz J Otorhinolaryngol. 2010; 76(2):245-250. https://doi.org/10.1590/S1808-86942010000200016 PMid:20549087

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Dental Science

Knowledge of Obstructive Sleep Apnea among Dental Fraternity in Riyadh

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Keywords: Obstructive Sleep Apnea; Oral appliance; Systemic complications; Continuous Positive Airway Pressure; Snoring; Obesity

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Abstract

AIM: To assess and compare the knowledge of obstructive sleep apnea (OSA) among final years dental students, interns, dental practitioners and faculty working in and around Riyadh.

METHODS: A questionnaire-based study comprising of 16 questions was conducted among final year dental clinical students, interns, dental practitioners and faculty working in and around Riyadh. Statistical Package for Social Sciences software (SPSS version 21) was used to analyse the statistical data. The $p < 0.05$ was considered statistically significant.

RESULTS: A total of 450 respondents took part in the study. When gender comparison was done regarding the awareness of OSA, statistically significant differences were noted for the majority of questions. The response rate for the knowledge-based questions varied statistically concerning their speciality and educational qualifications. 65% of the participants admitted that they were unaware of the diagnostic tests performed for diagnosing these patients. It was noted that 85% of the participants wanted to attend a CDE program to know more about OSA.

CONCLUSION: The study concludes that there is a significant lack of knowledge among final year students, interns and general dentists. This study emphasises the need for all dental professionals to regularly update their knowledge and equip themselves to identify and treat such patients at an early stage.

Introduction

Apnea in Greek means without breath [1]. Obstructive sleep apnea (OSA) is a chronic medical condition [2] and well-defined as a repetitive obstruction of the upper airway during sleep. It can be complete or partial. It is recognized by snoring, hypoxia, hypercapnia and insomnia [3]. OSA is broadly classified into 3 types central, obstructive and mixed and can be graded as mild, moderate and severe [1].

Untreated OSA can cause many medical problems such as hypertension, diabetes, cardiovascular diseases, cognitive dysfunction and depression [4]. It leads to tiredness, anxiety, depression, daytime sleepiness, also with increased risk of motor vehicle accidents and impairment of function in those who have it [2], [3], [4], [5]. The snoring noise can cause serious marital and social problems as well. Hence it is a serious problem and requires immediate management [5]. The main predisposing factor for OSA is obesity [1]. It is considered to be associated with older age, hereditary, smoking, alcohol, and periodontal disease, orofacial anatomical abnormalities such as mandibular micrognathia, macroglossia, and hypertrophy of palatine tonsils and enlarged uvula [3]. Most of the OSA patients are unaware of their problem due to lack of knowledge and improper guidance from their dentist or physician as well as expensive diagnostic tests which are involved in diagnosing the disease [3]. Polysomnography (PSG) still serves as the gold standard or confirmatory test in the diagnosis of OSA. It involves the overnight recording of sleep breathing patterns and oxygen saturation. A patient is diagnosed to be suffering from OSA if there is occurrence of at least five apneas or hypo apneas per hour, causing sleep fragmentation and decline in blood oxygen saturation.

Thorough clinical evaluation using a basic questionnaire helps us to diagnose the condition at an

early stage, to successfully manage the patient [4]. Dentists can play a vital role in detecting, advising, referring and treating OSA patients [5], [6]. Previous studies proved the competence of simple modern imaging techniques which are promising in identifying the disease process at an initial phase [2], [4]. Treatment of OSA is governed by the severity of symptoms, degree of clinical complications, and aetiology of upper airway obstruction. The most effective therapy considered until today is continuous positive airway pressure (CPAP), but it has suboptimal patient compliance. In 1900, Pierre Robin for the first time introduced the use of oral appliances for glossoptosis, and later the appliance was modified to treat sleep disorders [1], [2]. Of late American Academy of Sleep Medicine (AAOSM) has recommended oral appliances (OA) to treat mainly snoring and mild-to-moderate OSA and patients who cannot tolerate to CPAP or those who reject the surgery [2].

Previous studies done in the Saudi Arabian population reported 39% prevalence of OSA among females and 33.3% in males [7], [8]. So, it is important for us to recognize how well the present-day dental practitioners are equipped to identify these patients at an early stage. Many studies have evaluated the level of knowledge about OSA among medical students. However, to our knowledge, none has evaluated the same among dental students, academicians and dental practitioners in Saudi Arabia. Therefore, this study was aimed to assess the level of knowledge, awareness and attitude of Saudi dental students, interns, general dentists, specialists and academicians in and around Riyadh towards treating OSA patients.

Material and Methods

A Cross-sectional self-administered questionnaire study was distributed randomly among the final year dental students, interns, academicians, dental practitioners and specialists in around Riyadh, between February 2018 to April 2018. The questionnaire was designed comprising 16 multiple choice questions assessing knowledge and awareness of the participants regarding OSA. The questionnaire also included mentioning their gender, qualification (student or faculty or graduate or master's or PhD) and years of experience. Those who completed the questionnaire form completely and willing to participate were only included in the study. Initially 468 participants were given the questionnaire, and only 450 responded with filled forms. The questionnaire was distributed personally to the individual participants and for few other participants send through web link through e-mail and personal messages. The instructions were given on how to fill the questionnaire and stated that their participation was purely voluntary. The content authenticity was pretested on a random sample of population to ascertain practicability, strength and clarification of answers. Those who denied filling the questionnaire were excluded from our study. Statistical analysis was performed using SPSS version 21 (SPSS Inc., Chicago, IL, USA). Descriptive data were analysed using frequencies and percentages. The Wilcoxon rank-sum test and chi-square were used to identify the variation between groups. The level of significance was set at $p < 0.05$

Results

The study results demonstrated that there were 196 (44%) female respondents and 254(56%) males. Table 1 represents the response percentage for each question by males and females.

Table: Response percentage for each question by males and females

Sl. No	Question	Options	Total (100%) (n = 450)	M	F	P Value
1	Are you aware of the term, SLEEP APNEA, complete or partial occlusion of the upper airway during sleep?	YES	80%	45%	35%	0.046*
		NO	20%	9%	11%	
2	How often have you come across a patient with Obstructive Sleep Apnea (OSA)	Frequently	10%	6%	4%	0.041*
		Occasionally	50%	35%	15%	
		Never	40%	23%	17%	
3	What would you offer a patient with sleep apnea	Lifestyle modification	12.5%	7%	5.55	0.03*
		Provide an oral appliance	30%	19%	11%	
		Refer to a physician	57.5%	32%	25.5%	
4	Among the patients diagnosed with OSA, which gender has the highest prevalence	In Males	50%	24%	26%	0.21
		In Females	7.5%	3%	4.5	
		No Idea.	42.5%	26	16.5%	
5	Do you know about the investigations or tests prescribed for such patients to diagnose (OSA)?	YES	35%	20%	15%	0.026*
		NO	65%	38%	27%	
6	Have you come across the topic of management of sleep apnea and oral appliances in your Dental course/ curriculum?	YES	35%	24%	11%	0.09*
		NO	65%	33%	32%	
7	Are you aware that untreated sleep apnea can cause serious systemic diseases?	YES	62.5%	38%	24.5%	0.028*
		NO	37.5%	20%	17.5%	
8	Do you believe "OSA" patients suffer from severe snoring?	YES	62.5%	33%	29.5%	0.036*
		NO	17.5%	8%	9.5%	
		No Idea.	20%	11%	9%	
9	Can children also suffer from obstructive sleep apnea?	YES	63.5%	34%	29.5%	0.039*
		NO	18.5%	8%	10.5%	
		No Idea.	20%	9.5%	10.5%	
10	Can dentists play a major role in identifying such high-risk patients by using extra-oral -radiographs?	YES	50%	23%	27%	0.043*
		NO	35%	19%	16%	
		No Idea.	15%	8%	7%	
11	Are you aware of the Berlin Questionnaire to evaluate such patients with sleep apnea?	YES	17.5%	9%	8.5%	0.055
		NO	82.5%	45%	37.5%	
12	Is there a relation between Body mass index and Obesity with OSA?	YES	80%	43%	37%	0.034*
		NO	12.5%	7%	5.5%	
		No Idea.	7.5%	4%	3.5%	
13	Do you have the knowledge on CPAP (continuous positive airway pressure therapy), which is considered as first-line therapy for severe obstructive sleep apnea?	YES	37.5%	23%	14.5%	0.038*
		NO	63.5%	33.5	30%	
14	Are you aware of the clinical symptoms and Oro-facial characteristics to identify such patients?	YES	47.5%	27.5%	20%	0.12
		NO	52.5%	35%	17.5%	
15	Did you have the idea of Epworth sleepiness scale to assess such patient's?	YES	17.5%	10%	7.5%	0.014*
		NO	82.5%	43%	39.5%	
16	Are you interested in updating your knowledge on sleep medicine by enrolling in a CDE (continuous Dental Education) program?	YES	85%	39%	46%	0.019*
		NO	15%	7%	8%	

Statistical analysis was performed using SPSS version 21 (SPSS Inc., Chicago, IL, USA). Descriptive data were analysed using frequencies and percentages. The Wilcoxon rank-sum test and chi-square were used to identify the variation between groups. The level of significance was set at $p < 0.05$.

When gender comparison was made with knowledge responses, statistically significant

differences were noted for majority of questions with p-value < 0.05. Figure 1 demonstrates the years of experience of the participants.

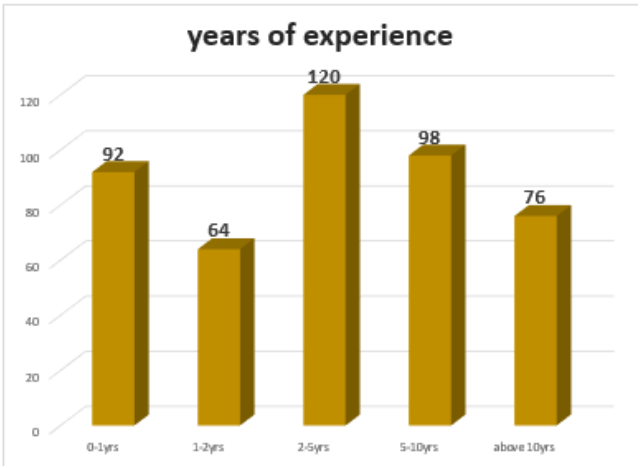

Figure 1: Years of experience in clinics for the participants

Table 2 demonstrates the details of the study population regarding their speciality and their response rate. The questions assessing the awareness of the participants were Q4, Q5, Q8, Q9, Q10, 11, Q12, Q13, 14, Q15. Participants who answered at least 6 correct responses for the above-mentioned questions were categorised to have satisfactory knowledge about OSA. It is noticed that there is significant difference regarding the knowledge on OSA based on the participant's qualification and speciality. The results showed that the specialists in surgery responded with maximum of correct responses scoring of 75% of the participants having adequate knowledge of OSA. 85% of our study population were willing to update their knowledge on sleep medicine by enrolling in a CDE (continuous Dental Education) program?

Table 2: Participants having satisfactory knowledge- who gave the correct response

Speciality	Total No of participants	No.of participants who gave the correct response	% of the correct response
Final year students	92	36	39.1%
Dental interns	154	59	38.3%
General Dentists	98	38	38.7%
Pedodontics	20	13	65%
Oral surgery	12	9	75%
Oral medicine	11	6	54.5%
Endodontics	10	5	50%
Operative	14	7	50%
Oral Pathology	7	4	57.1%
Orthodontics	13	9	69.2%
Prosthodontics	11	8	72.7%
Public health	3	2	66.6%
Periodontics	5	3	60%

Discussion

Among other medical practitioners, practising dentists are more likely to encounter first-of-contact, with such patients or detect potential OSA and other sleep disorders among their patients [9], [10]. Accordingly referring these patients to primary care physicians or sleep specialists or treat these patients with OAs is very important.

In a study done by Janhvi et al among dentists documented that 32% of the participants were not aware of the gender in which OSA was more prevalent [11], similar results were noticed in our study where 43% of our participants did not have an idea as to, in which gender it was more prevalent. In previous study 37% participants stated to provide an oral appliance whereas 29.6% of our subjects chose the option of referring the patient to a physician and 11.1% recognised Continuous Positive Airway Pressure (CPAP) as best treatment plan. Whereas in our study 58% of the participants opted to refer the OSA patients to physician, only 30% were confident to provide and treat with oral appliance, and 12.5% of the participants chose to advise lifestyle modification for these patients. 40% of our participant's declared that they never came across any patient with OSA. Only 10% of the dentists mentioned that they frequently encountered such patients. The results were almost similar to the previous study where 52% of the study population never came across these patients [6], [9]. This data enlightens us regarding the awareness of this issue among both the dentists and their patients. Most of the patients believe that sleep disorders are not in the scope of a dentist and patients might feel uncomfortable to mention about their snoring issue to a dental doctor.

The most classic orofacial features noticed in OSA patients include a maxillary and mandibular retrognathia, narrow palate, and large neck circumference, long soft palate, tonsillar hypertrophy, macroglossia, and deviation of nasal septal. In our study, 53% of the participants were unaware of these characteristic findings in OSA patients. A previous study documented that 81% of dental practitioners had no idea regarding Epworth sleep scale [9]. Similar results were identified among our dentists where 83% were unaware of Epworth sleep scale, and 86% of our study population did not know Berlin's questionnaire. We can see in Table 1 that 20% of our dentists were not having idea if OSA patients suffer from severe snoring, and 63.5% of our participants believe that OSA can be seen in children also.

Many studies discussed extensively regarding the use and implications of oral appliances in OSA. However, they overlooked the dentists' role in detecting OSA patients and making proper recommendations or referrals [12], [13]. Essentially all practitioners in dentistry, regardless of their speciality, should be prepared to detect and manage potential OSA patients.

Julia-Serda et al. stated that cephalometric radiographs combined with clinical features like physical examination, and nocturnal oximetry were valuable in the diagnosis of OSA, and recommended

that practising these methods can significantly reduce the number of polysomnography studies undergone by patients. Forty-five % of our study population admitted that they don't know if dentists can play a vital role in identifying such patient at an early stage by using extraoral radiographs [14]. Reddy et al., in their study confirmed that thorough clinical examination, risk factor analyses and standard cephalometric analyses help to identify the high-risk patients with OSA [4]. Almost 65% of our participants were unaware of the investigations that are indicated for a suspected OSA patient.

Treatment options for OSA

They range from behaviour modification to diet modification, medications, continuous positive airway pressure (CPAP), Oral appliance, as well as surgery [1]. The main aim of treating OSA is to increase the life expectancy and decrease the other systemic complications that would arise because of untreated OSA and to improve the quality of life. It is wise to select for the less invasive treatment options whenever possible. Behaviour modification is to train the patient to alter the sleep position to a lateral position instead of supine position by using a pillow, advising patients to abstain from alcohol and to control on a diet. Encourage them to do regular exercise and monitor the body weight and motivate the patients to reduce if they are overweight [16], [17], [18], [19]. Some studies suggested the use of mild sedative 3hours before sleep which are helpful to promote sound sleep, other medication advised for OSA are antibiotics, topical intranasal application of corticosteroid, leukotriene receptor antagonist and anti-inflammatory therapy to a mild to moderate case and during maintenance therapy after surgery [18], [19].

Still (CPAP) is considered as the standard treatment for patients with moderate-to-severe OSA. It acts by nonstop pumping of air under pressure through a sealed face mask into upper airway which is connected to a device with electric power. Because this is very complex to carry and use, it has less patient compliance. 64% of our contributors in our study were unaware of the use of CPAP and its function in treating sleep apnea. 83% of the participants in our study were not having knowledge about Epworth sleepiness scale to identify such patients at an early stage.

There are many other oral appliances (OAs) used to treat obstructive sleep apnea like the mandibular advancement or mandibular retaining devices with names such as snore guard, silencer (tongue retaining device) and Snor Ex (soft palate Lifter) Dentists should frequently update the guidelines and recommendations for the use of OAs in the treatment of obstructive sleep apnea (OSA) and snoring [20].

The results in our study indicate the need to enlighten the dentists in and around Riyadh with the basic knowledge about (OSA) and the clinical features to identify inpatients, the investigations helpful to diagnose such patients. Dental practitioners truly play a major role in diagnosing the suspected (OSA)patient at an early stage and to treat them. It is crucial for the dental fraternity to participate in the education programs, research and treatment of this serious and inescapable health problem.

References

1. Prabhat K, Goyal L, Bey A, Maheshwari S. Recent advances in the management of obstructive sleep apnea: The dental perspective. Journal of natural science, biology, and medicine. 2012; 3(2):113. https://doi.org/10.4103/0976-9668.101877 PMid:23225971 PMCid:PMC3510903

2. Quan SF, Schmidt-Nowara W. The Role of Dentists in the Diagnosis and Treatment of Obstructive Sleep Apnea: Consensus and Controversy. Journal of clinical sleep medicine: JCSM: official publication of the American Academy of Sleep Medicine. 2017; 13(10):1117. https://doi.org/10.5664/jcsm.6748 PMid:28942761 PMCid:PMC5612624

3. Al-Jewair TS, Nazir MA, Al-Masoud NN, Alqahtani ND. Prevalence and risks of habitual snoring and obstructive sleep apnea symptoms in adult dental patients. Saudi medical journal. 2016; 37(2):183. https://doi.org/10.15537/smj.2016.2.12852 PMid:26837402 PMCid:PMC4800918

4. Lavanya R, Gandhi Babu DB, Chavva S, Boringi M, Waghray S, Yeladandi M. The role of oral physicians in predicting the risk of obstructive sleep apnea: A case-control study. Imaging science in dentistry. 2016; 46(3):167-71. https://doi.org/10.5624/isd.2016.46.3.167 PMid:27672612 PMCid:PMC5035721

5. Jauhar S, Lyons M, Banham S, Orchardson R, Livingston E. The attitudes of general dental practitioners and medical specialists to the provision of intra-oral appliances for the management of snoring and sleep apnoea. British dental journal. 2008; 205(12):653. https://doi.org/10.1038/sj.bdj.2008.1022 PMid:19029919

6. Bian H. Knowledge, opinions, and clinical experience of general practice dentists toward obstructive sleep apnea and oral appliances. Sleep and Breathing. 2004; 8(02):85-90. https://doi.org/10.1055/s-2004-829633 PMid:15211392

7. Bahammam AS, Al-Rajeh MS, Al-Ibrahim FS, Arafah MA, Sharif MM. Prevalence of symptoms and risk of sleep apnea in middle-aged Saudi women in primary care. Saudi Med J. 2009; 30:1572-1576.

8. BaHammam AS, Alrajeh MS, Al-Jahdali HH, BinSaeed AA. Prevalence of symptoms and risk of sleep apnea in middle-aged Saudi males in primary care. Saudi Med J. 2008; 29:423-426.

9. Sri Meenakshi RB, Senthil Kumar KP, Prabhakar K. Evaluation of awareness of issues regarding obstructive sleep apnea and the orthodontist role in management: A survey among dental and medical practitioners. J Indian Acad Dent Spec Res 2016; 3:43-6. https://doi.org/10.4103/jiadsr.jiadsr_5_17

10. Quan SF, Schmidt-Nowara W. The role of dentists in the diagnosis and treatment of obstructive sleep apnea: consensus and controversy. J Clin Sleep Med. 2017; 13(10):1117-1119. https://doi.org/10.5664/jcsm.6748 PMid:28942761 PMCid:PMC5612624

11. Manohar J, Dhanraj, Rakshagan. Knowledge, awareness and practice among dental practitioners regarding oral appliances in treatment of obstructive sleep apnea. International Journal

Research. 2017; 9(02):46378-46381.

12. Mahendran R, Subramaniam M, Chan YH. Medical students' behaviour, attitudes and knowledge of sleep medicine. Singapore Med J. 2004; 45:587-9.

13. Fusetti M, Fioretti AB, Valenti M, Masedu F, Lauriello M, Pagliarella M. Cardiovascular and metabolic comorbidities in patients with obstructive sleep apnoea syndrome. Acta Otorhinolaryngol Ital. 2012; 32:320-5.

14. Julià-Serdà G, Pérez-Peñate G, Saavedra-Santana P, Ponce-González M, Valencia-Gallardo JM, Rodríguez-Delgado R, et al. Usefulness of cephalometry in sparing polysomnography of patients with suspected obstructive sleep apnea. Sleep Breath. 2006; 10:181-7. https://doi.org/10.1007/s11325-006-0073-y PMid:17053929

15. Guttal KS, Burde KN. Cephalometric evaluation of upper airway in healthy adult population: a preliminary study. J Oral and Maxillofac Radiol. 2013; 1:55-60. https://doi.org/10.4103/2321-3841.120115

16. Acar M, Turkcan İ, Ozdaş T, Bal C, Cingi C. Obstructive sleep apnoea syndrome does not negatively affect oral and dental health. J Laryngol Otol. 2015; 129:68-72. https://doi.org/10.1017/S0022215114003296 PMid:25656158

17. Knappe SW, Sonnesen L. Mandibular positioning techniques to improve sleep quality in patients with obstructive sleep apnea: current perspectives. Nat Sci Sleep. 2018; 10:65-72. https://doi.org/10.2147/NSS.S135760 PMid:29440942 PMCid:PMC5800493

18. AlRumaih HS, Baba NZ, AlShehri A, AlHelal A, Al-Humaidan A. Obstructive Sleep Apnea Management: An Overview of the Literature. J Prosthodont. 2018; 27:260-265. https://doi.org/10.1111/jopr.12530 PMid:27598517

19. Wali SO, Abalkhail B, Krayem A. Prevalence and risk factors of obstructive sleep apnea syndrome in a Saudi Arabian population. Annals of thoracic medicine. 2017; 12:88-94. https://doi.org/10.4103/1817-1737.203746 PMid:28469718 PMCid:PMC5399696

20. Ramar K, Dort LC, Katz SG, Lettieri CJ, Harrod CG, Thomas SM, et al. Clinical Practice Guideline for the Treatment of Obstructive Sleep Apnea and Snoring with Oral Appliance Therapy: An Update for 2015. J Clin Sleep Med. 2015; 11:773-827. https://doi.org/10.5664/jcsm.4858

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Aug 15; 7(15):2513-2519.
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Dental Science

Impact of Removable Partial Denture Type on Patient Satisfaction and Abutment Survival Rate-RCT

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Keywords: Removable partial dentures; Thermopress material; Patient's satisfaction; Bone loss

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Abstract

BACKGROUND: Patient's satisfaction and the preservation of abutments is the most important outcomes that the clinician seeks during fabrication of any dental treatment, especially when it is concerned with removable prosthodontic rehabilitation.

AIM: The present study evaluates three different Removable Partial Denture (RPD) types restoring mandibular class II modification I edentulous cases with regards to patient's satisfaction and abutments survival.

METHODS: Forty-two partially edentulous patients were divided into three groups (Group I rehabilitated with Vitallium RPD, Group II rehabilitated with Vitallium RPD where the modification area restored with the surveyed bridge, Group III rehabilitated with Thermopress RPD). The patients were followed up for twenty-four months. Using a questionnaire, prosthodontic maintenance required was documented at the delivery and after 3 months.

RESULTS: There was a significant difference regarding patient satisfaction for group III (P-value <0.05) while for groups I and II there was a non-significant difference (P-value >0.05). Regarding the survival rate, there was a non-significant difference between the three groups (P-value >0.05) at the end of twenty-four months of follow up.

CONCLUSION: Patient satisfaction and abutment survival were better with Thermopress RPD than conventional Vitallium RPD or Vitallium RPD with a surveyed bridge restoring the modification area. Although a non-statistically significant difference was found in the survival rate of abutments between groups, a clinically important result was revealed as no abutments failures were reported in the Thermopress group.

Introduction

Patient satisfaction is a prime concern when constructing removable partial denture (RPD). Improving phonetics, mastication and aesthetics are considered goals during partial denture (PD) designing [1]. Preservation of the remaining structures is also one of the main objectives of the RPDs. It is considered crucial for clinicians to prevent abutment loss in PD's patients.

To avoid the problems of distal extension cases, efforts should be made to preserve the posterior teeth by every means. Upon failure of these efforts, the selection of the suitable type of RPD becomes challenging especially when it comes to the relationship between the distal surface of the abutment teeth at the site of the distal extension and the framework of RPD [2].

Support is considered as the main problem in distal extension edentulous ridge as Kennedy class II partially edentulous cases. A serious problem results from the difference in compressibility between the periodontal ligament of the abutment teeth and the mucoperiosteum covering the ridge. Due to the presence of the difference in compressibility between the periodontal ligaments of the abutment teeth and the mucosa of the residual alveolar ridge, a rotational movement results causing tissue ward movement of the denture base resulting in excessive torque forces

to the abutment teeth leading to its early loss [2].

Although in Kennedy class II modification 1 mandibular cases, the anterior abutment on the tooth-supported side is secondary, serving to support and retain one end of the tooth-supported segment and adding horizontal stabilisation to the denture [2], the rotational movement is still encountered.

The problems of these cases have been solved by various concepts beginning from using the conventional RPD until introducing implants as a solution in many treatment options. Despite the problems associated with the use of the conventional partial denture; it remains the commonly used restoration [3], [4], [5].

The traditional prosthetic rehabilitation for these cases is metallic RPD or restoring the modification space with the surveyed bridge before fabricating the metallic RPD. Thermopress RPD is another option which could be considered as an alternate material overcoming the problems of the cast metal RPDs, especially with the recent improvements. This material has a superior advantage when it comes to mechanical properties as creep resistance, fatigue endurance, flexibility, dimensional stability and wear resistance. It is also light in weight and esthetically can match both the tooth and tissue colours. It also provides biocompatibility similar to that of the casted RPDs [6].

Better patient satisfaction was reported due to their flexibility and ability to engage hard and soft tissue undercuts; the RPDs made from flexible resins are more naturally felled and more comfortable in the mouth. They also improve the esthetic requirements by using invisible clear clasps on the abutment teeth [7], [8]. Flexibility allows for better distribution of the masticatory forces rather than individual support points, and they do not only engage the abutment tooth for support and retention but also engage the ridge undercuts [9], [10].

On the other hand, choosing a satisfactory path of insertion especially in the presence of soft and hard tissue undercuts aiming to maintain a superior adaptation to the tissues is considered as a challenging aim in flexible RPD. Also, the flexible RPDs are made bulkier to compensate its low impact strength making them bulky than the cast metal RPDs and makes it difficult to design occlusal rests [11], [12], [13], [14].

Designing occlusal rests are considered vital for class II mod.1cases. In metallic RPD, the framework fulcrum line “when denture base is displaced toward residual ridge” runs from the east abutment of the free end to the posterior abutment on the modification area. When forces tend to displace denture away from its basal seat, supportive element (distal occlusal rest) of direct retainer assembly on the anterior abutment of the modification area serves as an indirect retainer. If the occlusal rest on the secondary abutment lies away from the fulcrum line, it may work for indirect retention adequately (dual function) (tooth support for one end of the modification area and support for an indirect retainer).

Therefore, this study aimed to evaluate the influence of RPD type on patient satisfaction and the abutment teeth’s survivals in class II mod. 1 mandibular partially edentulous patient.

Material and Methods

Forty-two partially edentulous patients were selected from the outpatient clinic of the Removable Prosthodontics Department, Faculty of dentistry, MSA University. They were medically free ageing from 45-60 years old. Patients were Kennedy Class II modification 1 mandibular partially edentulous with existing periodontally healthy remaining teeth and opposing natural maxillary teeth (Figure 1).

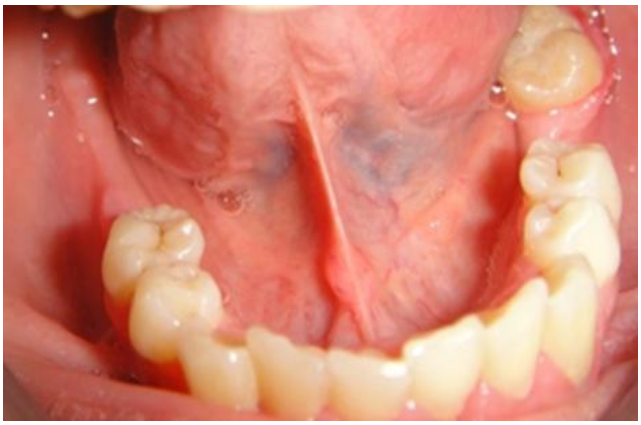

Figure 1: Intraoral photo of the mandible (occlusal view)

The patients were given a detailed explanation concerning the present state, alternative treatment plans and the proposed procedures. All patients were informed about the study protocol and objectives before they signed informed consent. The study was reviewed and approved by the Research Ethics committee of MSA University.

The selected patients were randomly divided and equally distributed fourteen patients in each group of the three groups. Each group received a different type of RPD; Group I: Vitallium RPD, Group II: Vitallium RPD with surveyed bridge restoring the modification area, Group III: Thermopress RPD. For the three groups, preoperative diagnostic panoramic radiographs and periapical radiographs for abutments were performed. Face bow records, and mounting of diagnostic casts on semi-adjustable articulators (Bioart A7 plus articulator) were implemented followed by surveying of the preliminary casts. Mouth preparations (teeth scaling and necessary teeth

fillings or another type of restoration if needed) were accomplished (Figure 2, and Figure 3 A, B, and C), (Figure 4 A, B, C, and D).

Group I: Secondary impressions were made to make the master cast. The metallic RPD framework was constructed and tried on the master cast and in the patient mouth (Figure 2D). Altered cast impressions were made and poured (Figure 2E) then the occlusion blocks were fabricated on the frameworks which were fitted on the altered cast (Figure 2F).

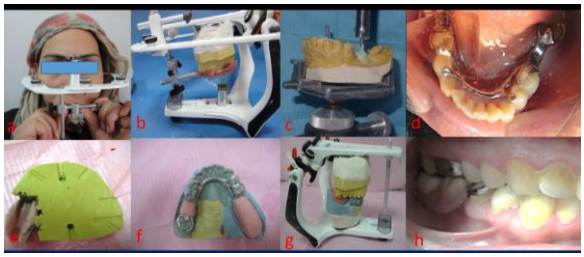

Figure 2: Vitallium RPD; A) Face bow record (facial view); B) Face bow transfer (profile view); C) Primary surveying (mandibular cast) – left side showing survey line); D) Metal try-in of the mandibular framework; E) Sawing of cast; F) Framework fitted on altered cast; G) Mounting on articulator (right side); H) Occlusion (right)

Group II: Preparation of the abutments to receive the surveyed bridge was performed. The wax pattern of the bridge was surveyed, and rest seats, as well as guiding planes, were prepared in the pattern (Figure 3D). Metal try-in of the bridge was done (Figure 3E) then cementation of the final surveyed bridge (Figure 3F). The metallic RPD framework was cast and tried first on the cast (Figure 3G) then in the patient mouth. Altered cast impression was made (Figure 3H) and poured then the occlusion blocks were fabricated on the frameworks which were fitted on the altered cast (Figure 3I).

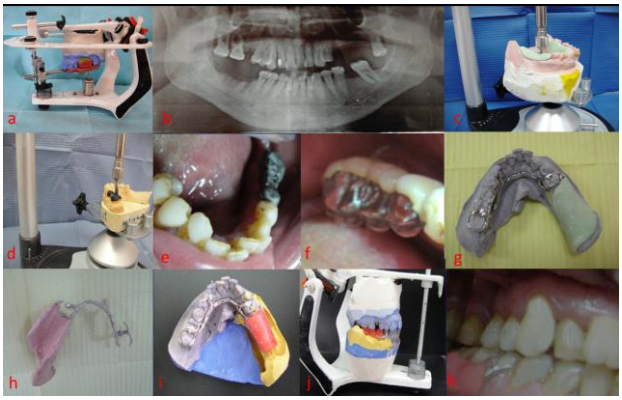

Figure 3: Vitallium RPD with surveyed bridge restoring the modification area; A) Face bow transfer (profile view); B) Panoramic radiograph; C) Surveying the cast. (path of insertion); D) Surveying the wax pattern of the bridge; E) Metal try in of surveyed bridge; F) Cementation of surveyed bridge; G) Metal try-in of the RPD framework on the cast and preparation of the tray for altered cast; H) Altered cast impression; I) The poured altered cast with the framework fully seated and wax rim prepared for jaw relation record; J) Mounted master casts (right side); K) Denture insertion (profile)

Group III: Secondary impression was made to pour the master cast, and occlusion blocks were fabricated on the master cast.

For the three groups, Jaw relation was registered then mounting of the occlusion blocks, and setting of artificial teeth was done (Figure 2G, Figure 3J, and Figure 4E) followed by the try in step. For groups, I and II heat-cured acrylic resin were used to process the PD base, while the flexible resin was used for processing the PD base of group III.

Thermopress 400 injecting unit was used for the fabrication of thermoplastic PD. The selected cartridge of the injecting material (quantity and colour) was selected and the preheating temperature (220°C), time (20 minutes) and the injecting pressure (5 bars) were adjusted according to the manufacturer's instructions. A vaseline based lubricant was applied before introducing the selected cartridge into one of the two heating cylinders where the cartridge membrane was pointed to the flask chamber. The excess of the lubricant was wiped out from the margin of the heating cylinder with a highly absorbent paper.

After processing of the dentures, denture insertion (Figure 2H, Figure 3K, Figure 4G and H) was performed, and selective grinding for intra-oral adjustments of occlusion was carried on whenever indicated.

Instructions for proper denture hygiene were stressed upon; not to wear dentures during sleeping hours and to keep it in tap water, clean the dentures after each meal under tap water only, not to use any mouthwashes or denture cleansers during the study period. They were also instructed not to use any denture adhesives.

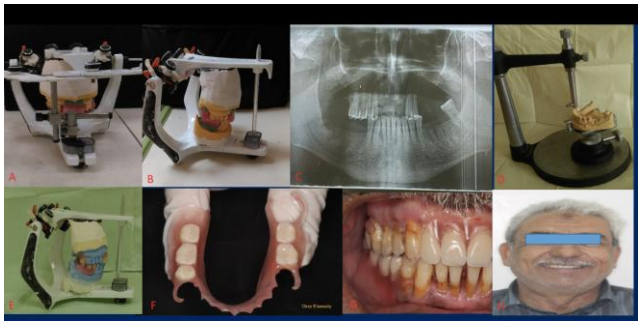

Figure 4: Thermopress RPD; A) Face bow transfer (facial view); B) Mounted diagnostic casts (left side); C) Panoramic radiograph; D) Primary surveying (mandibular cast) – right side showing survey line; E) Artificial setup on articulator (left side); F) Flexible lower partial denture; G) Denture Insertion (right); H) Extra-oral photo facial (smiling)

Patient satisfaction

Patients of the three groups were subjected to Oral Health-Related Quality of Life Measures (OHRQoL), and Chewing Function Quality (CFQ) questionnaires were taken for each group at delivering the partial denture as a baseline and after three months of function.

Patient satisfaction questionnaire based on a visual analogue scale (VAS) consisting of 12-Item short-form Oral Health-Related Quality of Life Measures (OHRQoL) with a scale from 0 to 4 (Never, Hardly ever, Occasionally, Fairly Often and Very Often) were taken from each group. The hypothesised framework included four primary dimensions: physical function, psychosocial function (with three subdimensions of role function, distress, and worry), impairment, and perceptions.

The Chewing Function Quality questionnaire consisting of 10 items with a scale from 0 to 4 (Never, Hardly ever, Occasionally, Fairly Often and Very Often) were taken from each group to prevent the mixing between psychosocial impact of a disturbed chewing function and its influence to a patient's quality of life and chewing function Disorders.

The collected data were tabulated and statistically analysed.

Survival Rate

The survival rate of abutments was evaluated. The abutments were considered surviving if they were clinically stable, functioning without any mobility. Survival analysis was done using Kaplan Maier statistics calculating the mean survival time for each group with their 95%CI (Cumulative incidence) and the corresponding survival graphs. The comparison was made between the different factors by Log-rank method using Cox-Mantel equation. P-values less than 0.05 were considered statistically significant.

Statistical analysis performed with computer program IBM SPSS 20 (Statistical Package for the Social Science; IBM Corp, Armonk, NY, USA), Graph Pad Prism (Graph Pad Technologies, USA) and Microsoft Excel 2016 (Microsoft Co-operation, USA) with the significant level set at $P \leq 0.05$. Data were presented as means and standard deviation (SD).

Results

Patient satisfaction

Oral Health Outcome Measures

Oral health outcome measures were evaluated through a written questionnaire delivered by the patient or relatives who answered twelve closed-ended questions through Likert Scale (out of 4), as listed in Table 1.

Two surveys were evaluated through this study, one considered as a baseline at the time of denture insertion and other after three months.

Table 1: Oral Health-Related Quality of Life questionnaire

Questions	Never	Hardly Ever	Occasionally	Fairly Often	Very Often
1. Have you had to avoid eating some foods? (Physical function; OHIP 28)	0	1	2	3	4
2. Have you found it difficult to relax? (Distress; OHIP 35)	0	1	2	3	4
3. Have you felt depressed? (Distress; OHIP 36)	0	1	2	3	4
4. Have you been upset? (Distress; OHIP 34)	0	1	2	3	4
5. Have you felt uncomfortable about the appearance of your teeth, mouth, or dentures? (Worry; OHIP22)	0	1	2	3	4
6. Have you been worried about dental problems? (Worry; OHIP19)	0	1	2	3	4
7. Have you had trouble getting along with other people? (Social function; OHIP 41)	0	1	2	3	4
8. Have you avoided going out? (Social function; OHIP 39)	0	1	2	3	4
9. Have you been unable to function? (Social function; OHIP 48)	0	1	2	3	4
10. How often did you feel nervous or self-conscious because of problems with your teeth, gums, or dentures? (Worry; GOHAI 10)	0	1	2	3	4
11. How much pain or distress has your teeth or gums caused you? (Pain; OHQOL 0B31)	0	1	2	3	4
12. Have you had uncomfortable dentures? (Denture; OHIP 18)	0	1	2	3	4

Regarding baseline, one-way analysis of variance (One Way ANOVA) was performed followed by Tukey`s post hoc test for multiple comparisons which revealed slight insignificant lower of group III as P-value > 0.05, as showed in Figure 5.

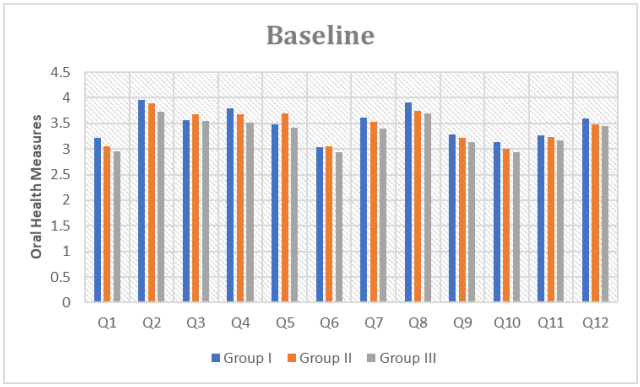

Figure 5: Means of Scale at Baseline between Studied Groups

Regarding three months, one-way analysis of variance (One Way ANOVA) was performed followed by Tukey`s post hoc test for multiple comparisons which revealed significant lower of group III as P-value < 0.05, as showed in Figure 6.

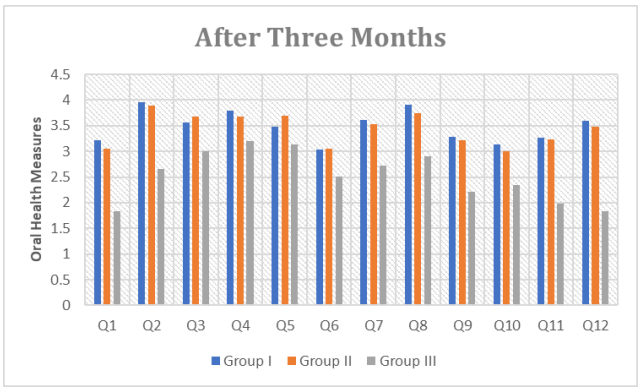

Figure 6: Means of Scale after Three Months between Studied Groups

Chewing Function Assessment:

Statistical analysis performed with SPSS 20®, Graph Pad Prism® and Microsoft Excel 2016 with a significant level set at P ≤ 0.05. Data were presented as means and standard deviation (SD).

Chewing function assessment was evaluated through a written questionnaire delivered by the patient or relatives who answered ten closed-ended questions through Likert Scale (out of 4), as listed in Table 2.

Table 2: Chewing Function Quality questionnaire

Questions	Never	Hardly Ever	Occasionally	Fairly Often	Very Often
1. Have you had any difficulty chewing apples / raw carrots, or foods of similar consistency?	0	1	2	3	4
2. Have you had any difficulty baked or fried firm meat, or foods of similar consistency?	0	1	2	3	4
3. Have you had any difficulties chewing biscuits, crackers, tea biscuits, or foods of Similar consistency?	0	1	2	3	4
4. Have you had any difficulty chewing fresh bread, doughnut or foods of similar consistency?	0	1	2	3	4
5. Have you had any difficulty chewing nuts /walnuts /almonds /macadamia/peanuts, or similar food?	0	1	2	3	4
6. Have you had any difficulty chewing lettuce, raw cabbage, or similar food?	0	1	2	3	4
7. Have you felt insecure when you are Chewing?	0	1	2	3	4
8. Have you had any difficulty when biting Different foods (food incision)?	0	1	2	3	4
9. Have you noticed food catching or food remaining stacked between or on your teeth or dentures during or after meals?	0	1	2	3	4
10. Have you had any difficulty chewing Chewing gum?	0	1	2	3	4

Two surveys were evaluated through this study, one considered as a baseline at the time of denture insertion and other after three months.

Regarding baseline, one-way analysis of variance (One Way ANOVA) was performed followed by Tukey`s post hoc test for multiple comparisons which revealed slight insignificant lower of group III as P-value > 0.05, as showed in Figure 7.

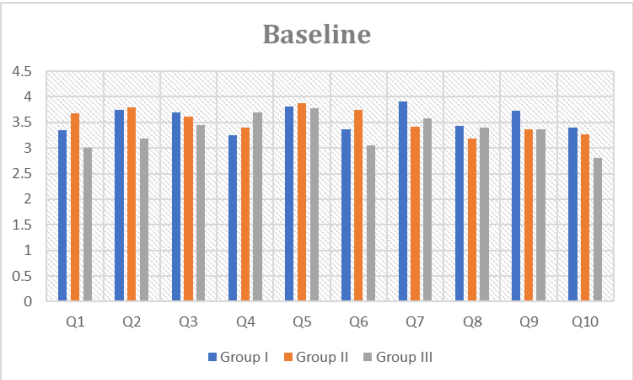

Figure 7: Means of Scale at Baseline between Studied Groups

Regarding three months, one-way analysis of variance (One Way ANOVA) was performed followed by Tukey`s post hoc test for multiple comparisons which revealed significant lower of group III as P-value < 0.05, as showed in Figure 8.

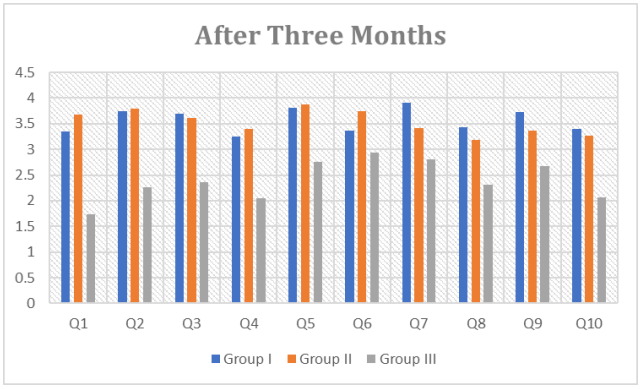

Figure 8: Means of Scale after Three Months between Studied Groups

Survival Rate

The survival rate of abutment teeth after twenty-four months of follow up in the Vitallium RPD group was 71.4%, while for RPD with surveyed bridge group was 85.7% and for Thermopress RPD group was 100% with overall survival 85.7%.

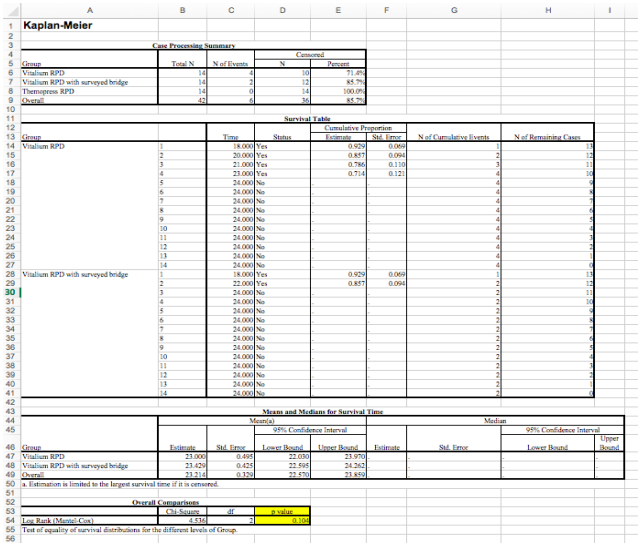

Figure 9: Survival analysis using Kaplan Meier statistics

A statistically non-significant difference in survival rate (P-value = 0.104) was revealed between the three groups.

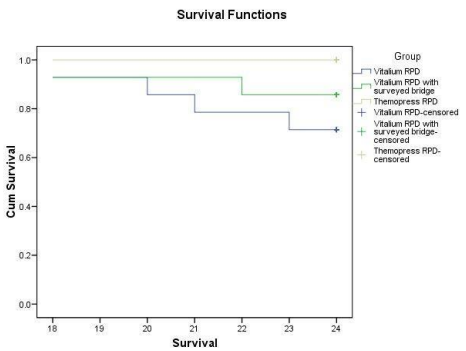

Figure 10: Kaplan Meier survival analysis

Discussion

This study was conducted to compare the patient satisfaction and survival rate of abutments outcomes of three different designs for cases with Kennedy Class II modification 1 for removable partial denture construction. Group I: Vitallium RPD; Group II: Vitallium RPD and surveyed bridge restoring the modification area; Group III: Thermopress RPD.

Patient selection was very critical to prevent the influence of some factors on the partial denture rehabilitation [15], [16], which can affect patient satisfaction results. This was crucial for the reliability and validity of the results. To exclude the effect of mechanical factors, patients having ridges with undercut areas were not included. To eliminate the effect of salivary factors, patients with Xerostomia or excessive salivation [17] and patients undertaking medications that affect salivary flow (e.g. diuretics) [18] were excluded. Similarly, patients with systemic diseases that may affect the amount or consistency of saliva (e.g. uncontrolled diabetes mellitus… etc.) were excluded.

For most of the previously mentioned reasons, patients older than 65 years were not included, to eliminate the effect of senility. Senile patients usually suffer from muscle atrophy, decreased neuromuscular coordination, stomatitis, as well as age-related limited manual dexterity [19].

In this study, Thermopress showed a significant satisfaction in all the points of satisfaction evaluated and these results may be due to the increased retention due to the decreased gap formed by the Thermopress and the underlying tissue as revealed and investigated in research work which stated that the Thermopress acryl showed an increased adaptation to the underlying tissues [20].

The results of the present study coincide with the clinical studies/observations reached by researchers [21], [22], [23]. They all concluded that the usage of flexible acryl technique plays a great role in attaining partial denture retention, providing a comfortable base and an increase in the functional denture performance.

Abutment survival prediction in RPD wearers is a major challenge for evidence-based dentistry. The factors affecting the preservation of abutments have been investigated in many studies [24], [25], [26], [27], [28], [29]. Occlusal support, pocket depth and crown root ratio are prognostic factors suggested by a study to assess the multifactorial risk factors [30]. However, no randomised controlled trials were conducted to compare the influence of the RPD type.

The influence of various types of RPDs on gingival inflammation was investigated in a study, and it was revealed that the response of the gingiva to the metallic RPD was less than the resin dentures, but these results must be related to the rate of porosity and the trapped plaque done by various types of acryl used [31]. However, the abutment loss in the present study was greater in metallic RPD groups (due to the torquing forces resulted from the difference in compressibility) compared to flexible acrylic group which may be attributed to the decreased porosity and increased plaque control of flexible acrylic resin with its flexibility nature which decreases the harmful effects of the torquing forces.

Although a statistically non-significant difference in survival rate (P-value = 0.104) was detected in this study between the three groups, a clinically important result was revealed as no abutment loss was found in the Thermopress RPD group.

Moreover, abutment loss in our study was mostly due to periodontal disease rather than due to caries which agrees with a study done in 1982 [32] which found that no marked increase in caries is caused by wearing RPDs.

The results could be used to aid the dentist to carefully select the type of the RPD aiming to restore the function, the comfort of the patient and preserve the longevity of the abutments; facilitating an evidence-based clinical decision making.

From the results of the present study, it can be concluded that the treatment for Kennedy class II modification 1 cases with Thermopress removable partial denture is satisfactory for the majority of cases. Although a non-statistically significant difference was revealed in the survival rate, a clinically important result favoured the Thermopress material as no abutments failures were reported in the patients using this type of partial denture.

References

1. McGivney GP, Carr AB and Brown TD: McCracken's removable partial prosthodontics. 11thed.The C.V. Mosby Co. St. Louis, Missouri, 2005:287-91.

2. Holmes JB: Influence of impression procedures and occlusal loading on partial denture movement. J Prosthet Dent. 2001; 86:335. https://doi.org/10.1067/mpr.2001.119826 PMid:11677525

3. D'Souza DS, Dua P. Rehabilitation strategies for partially edentulous-prosthodontic principles and current trends. Medical Journal Armed Forces India. 2011; 67(3):296-8. https://doi.org/10.1016/S0377-1237(11)60068-3

4. Janus CE, Hunt RJ, Unger JW. Survey of prosthodontic service provided by general dentists in virginia. J Prosthet Dent. 2007; 97:287-91. https://doi.org/10.1016/j.prosdent.2007.02.011 PMid:17547947

5. Dolan TA, Gilbert GH, Duncan RP, Foerster U. Risk indicators of edentulism, partial tooth loss and prosthetic status among black and white middle-aged and older adults. Community Dent Oral Epidemiol. 2001; 29:329-40. https://doi.org/10.1034/j.1600-0528.2001.290502.x PMid:11553105

6. Hundal M, Madan R. Comparative clinical evaluation of removable partial dentures made of two different materials in Kennedy Applegate class II partially edentulous situation. Medical journal armed forces india. 2015; 71:S306-12. https://doi.org/10.1016/j.mjafi.2012.08.020 PMid:26843744 PMCid:PMC4705100

7. Thakral GK, Aeran H, Yadav B, Thakral R. Flexible partial dentures - A hope for the challenged mouth. Peoples J Sci Res. 2012; 5:17-23.

8. Singh L, Kaira SL, Dayakara HR, Singh R. Flexible denture for partially edentulous arches - A case report. J Dentofacial Sci. 2012; 1:39-42.

9. Phoenix RD, Mansueto MA, Ackerman NA, Jones RE. Evaluation of mechanical and thermal properties of commonly used denture base resins. J Prosthodont. 2004; 13:17-27. https://doi.org/10.1111/j.1532-849X.2004.04002.x PMid:15032892

10. Hundal M, Madan R. Comparative clinical evaluation of removable partial dentures made of two different materials in kennedy applegate class II partially edentulous situation. Med J Armed Forces India. 2012; 71:S306-12. https://doi.org/10.1016/j.mjafi.2012.08.020 PMid:26843744 PMCid:PMC4705100

11. Abuzar MA, Bellur S, Duong N, Kim BB, Lu P, Palfreyman N, et al. Evaluating surface roughness of a polyamide denture base material in comparison with poly (methyl methacrylate). J Oral Sci. 2010; 52:577-81. https://doi.org/10.2334/josnusd.52.577 PMid:21206160

12. Dyer SR, Lassila LV, Jokinen M, Vallittu PK. Effect of cross-sectional design on the modulus of elasticity and toughness of fiber-reinforced composite materials. J Prosthet Dent. 2005; 94:219-26. https://doi.org/10.1016/j.prosdent.2005.06.008 PMid:16126074

13. Abuzar MA, Bellur S, Duong N, Kim BB, Lu P, Palfreyman N, et al.: Evaluating surface roughness of a polyamide denture base material in comparison with poly (methyl methacrylate). J Oral Sci. 2010; 52:577-81. https://doi.org/10.2334/josnusd.52.577 PMid:21206160

14. Larnier A. Partial Dentures. Dental Procedures, Dental Articles, 2012.

15. Dubravka KnezoviÊ-ZlatariÊ1et al: Patients' Satisfaction with Partial Denture Therapy. Acta Stomat Croat. 2000; 34:373-378.

16. Kamber-Cesir A, Dzonlagic A, Ajanovic M, Delalic A. Assessment of patient's satisfaction with the partial removable denture therapy. Pesquisa Brasileira em Odontopediatria e Clínica Integrada. 2011; 11(2):171-5. https://doi.org/10.4034/PBOCI.2011.112.04

17. Turner M. Hyposalivation, xerostomia and the complete denture - A systematic review. JADA. 2008; 139(2):146-50. https://doi.org/10.14219/jada.archive.2008.0129 PMid:18245681

18. Green AJ. Influence of diuretics on complete denture retention. J Prosth Dent. 1980; 43(5):506. https://doi.org/10.1016/0022-3913(80)90320-0

19. John R, Ivanhoe N, Roman M. Treating the modern complete denture patient. J Prosth Dent. 2002; 88:631. https://doi.org/10.1067/mpr.2002.130147 PMid:12488857

20. Chung-Jae LEE, Sung-Bem BOK, Ji-Young BAE, Hae-Hyoung LEE. Comparative adaptation accuracy of acrylic denture bases evaluated by two different methods. Dent Mater J. 2010; 29(4):411-417412. https://doi.org/10.4012/dmj.2009-105 PMid:20675954

21. Akinyamoju CA, Ogunrinde TJ, Taiwo JO, Dosumu OO. Comparison of Patient Satisfaction with Acrylic and Flexible Partial Dentures. Niger Postgrad Med J. 2017; 24(3):144-149. https://doi.org/10.4103/npmj.npmj_54_17 PMid:29082902

22. Mohamed El-KhodarySaad El-Din et al: Thermoplastic Distal Extension Removable Partial Dentures versus Vitallium ones Radiographic Evaluation. Mansoura Journal of Dentistry. 2014; 1(3):20-23.

23. Campbell SD, et al. Removable partial dentures: The clinical need for innovation. J Prosthet Dent. 2017; 118(3):273-80. https://doi.org/10.1016/j.prosdent.2017.01.008 PMid:28343666

24. Zlataric´ DK, Celebic´ A, Valentic´-Peruzonic´ M. The effect of removable partial dentures on periodontal health of abutment and non-abutment teeth. J Periodontol. 2002; 73:137-44. https://doi.org/10.1902/jop.2002.73.2.137 PMid:11895277

25. Sato F, Koyama S, Chiba T, Kadowaki K, Kawata T, Sasaki K. Changes in periodontal conditions of remaining teeth five years after RPD placement. Annals of Japan Prosthodontic Society. 2009; 1:130-8. https://doi.org/10.2186/ajps.1.130

26. Bergman B, Hugoson A, Olsson CO. Caries, periodontal and prosthetic findings in patients with removable partial dentures: a ten-year longitudinal study. J Prosthet Dent. 1982; 48:506-14. https://doi.org/10.1016/0022-3913(82)90352-3

27. Jepson NJA, Moynihan PJ, Kelly PJ, Waston GW, Thomason JM. Caries incidence following restoration of shortened lower dental arches in a randomized controlled trial. Br Dent J. 2001; 191:140-4. https://doi.org/10.1038/sj.bdj.4801122 PMid:11523885

28. Matsuda K, Ikebe K, Enoki K, Tada S, Fujiwara K, Maeda Y. Incidence and association of root fractures after prosthetic treatment. J Prosthodont Res. 2011; 55:137-40. https://doi.org/10.1016/j.jpor.2010.10.003 PMid:21134786

29. Miyamoto T, Morgano SM, Kumagai T, Jones JA, Nunn ME. Treatment history of teeth in relation to the longevity of the teeth and their restorations: outcomes of teeth treated and maintained for 15 years. J Prosthet Dent. 2007; 97:150-6. https://doi.org/10.1016/j.prosdent.2007.01.007 PMid:17394913

30. Tada S, Ikebe K, Matsuda K, Maeda Y. Multifactorial risk assessment for survival of abutments of removable partial dentures based on practice-based longitudinal study. J Dent. 2013; 41(12):1175-80. https://doi.org/10.1016/j.jdent.2013.07.018 PMid:23911599

31. Bissada N, Ibrahim S, Barsoum W. Gingival responses to various types of removable partial dentures. J Perio. 1974; 45:651. https://doi.org/10.1902/jop.1974.45.9.651 PMid:4529260

32. Bergman B, Hugoson A, Olsson C. Caries, periodontal and prosthetic findings in patients with removable partial dentures: a ten-year longitudinal study. J Prosthet Dent. 1982; 48:506-14. https://doi.org/10.1016/0022-3913(82)90352-3

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Dental Science

Effect of Combined Application of Growth Factors and Diode Laser Bio-Stimulation on the Osseo Integration of Dental Implants

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Keywords: Dental Implant; Diode Laser; Growth Factors; LLLT; PRF; Cone Beam Computed Tomography

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Abstract

BACKGROUND: The success of implants is associated first with their osseointegration, and later on with their survival rate. In recent years, many efforts have been exerted to develop implant design, geometry, materials and techniques to enhance the osseointegration process and also to increase the success rate of implant procedures. New techniques, like leukocyte and platelet-rich fibrin (L-PRF) and low-level laser treatment (LLLT), have been developed to enhance the osseointegration around dental implants.

AIM: This study aims at accelerating bone osseointegration process around dental implant using new techniques to increase the success rate, to allow immediate or early loading of a dental implant, and to make a comparison between the various new techniques in dental implant procedures to figure out which technique will achieve the best results.

METHODS: The study was conducted on a random sample of 40 male patients. Dental implants were placed in the posterior areas of the lower jaw. Patients were divided randomly into 4 groups; control group, LLLT group, L-PRF group and L-PRF plus LLLT group. They were assessed using cone-beam computed tomography (CBCT).

RESULTS: The results showed significant differences between all groups over different measured times. All the groups showed improvement in comparison with Normal group, where L-PRF group showed the best result followed by (L-PRF+LLLT) group, while the LLLT group showed the least improvement in comparison with bothL-PRF group and (L-PRF+LLLT) group.

CONCLUSION: The study demonstrates that L-PRF gives a better performance in the osseointegration around dental implants than LLLT.

Introduction

The use of dental implants to compensate the loss of teeth has increased through the last 30 years [1], [2]. Dental implants have become a very popular solution due to their high success rate and predictability of the procedure, as well as their relatively few complications [1], [3].

The phenomenon of osseointegration of titanium implants was discovered in 1952 by a Swedish orthopaedic surgeon, P I Brånemark, who defined osseointegration as “a direct structural and functional connection between ordered living bone and the surface of a load-bearing implant” [4]. In recent years, there has been a vast amount of scientific research and development in implant geometry, design, materials and techniques with the objective of further enhancing the success of implant treatment. Most of these developments have focused on how to improve the process of osseointegration through improvements in implant surface and design modifications [5]. New techniques have been developed to enhance the osseointegration around dental implants like L-PRF and LLLT. L-PRF and LLLT have become more and more applicable nowadays in dentistry [5].

Growth factors, which are generally considered a subset of cytokines, refer to the diffusible signaling proteins that stimulate cell growth, differentiation, survival, inflammation, and tissue repair. There are many types of growth factors, one of them is L-PRF which was first described by Choukroun *et al*., [6] as a new second generation of platelet concentrate. PRF is a simplified processing

technique without any complex handling. PRF can be used to promote wound healing, bone regeneration, graft stabilization, wound sealing, and hemostasis. Because the fibrin matrix is better organized, it is able to more efficiently direct stem cell migration and the healing program. Release of growth factors from PRF through in vitro studies and good results from in vivo studies led to optimizing the clinical application of PRF. It was shown that there were better results of PRF over PRP (Platelet-Rich Plasma). Dohan *et al*., [7] proved a slower release of growth factors from PRF than PRP and observed better healing properties with PRF.

LLLT has been used clinically in the management of several conditions based on its ability to promote stimulatory effects on the biochemical and molecular processes that occur during tissue repair, leading to increased fibroblast and epithelial proliferation, and increased collagen synthesis, which can accelerate the healing process. In addition, LLLT increases the potential for bone repair and remodeling, reduces the inflammation and edema, regulates the immune system, modulates and attenuates the pain, and manages postoperative pain [8], [9], [10], [11], [12], [13], [14]. In dentistry, preclinical findings indicate a positive effect of LLLT on bone repair and osseointegration [15], [16], [17], [18], [19], and this treatment modality has become a well-accepted adjuvant tool to enhance the osseointegration process in cases of rehabilitation involving implant-supported prostheses [16], [17], [18], [19].

Most of the techniques currently available for assessing the osseointegration process, such as histology [20], histomorphometry [21], and X-ray diffraction [22], are invasive and require sample destruction and animal euthanasia. Reproducing them in humans is often difficult [23], [24]. Other technologies for noninvasive assessment of osseointegration include X-ray imaging, cone beam computed tomography (CBCT), multislice computed tomography (CT), micro-computed tomography (μCT) [25], and digital panoramas [26].

Material and Methods

Population and Study Design

The study included 40 randomly selected male patients with missed teeth. Forty dental implants were performed, with one implant per patient. Patients were divided randomly into 4 groups, with 10 patients in each group:

Group A: (Control Group) Implant procedure without addition of growth factors or LLLT.

Group B: Implant procedure with LLLT (Diode laser 808nm).

Group C: Implant procedure with the addition of L-PRF.

Group D: Implant procedure with the combined application of both L-PRF and LLLT.

The study was approved by the NILES' ethics committee with a registration No. 018006.

Inclusion Criteria: *1) Patients were males with missed lower posterior teeth; 2) The working areas were edentulous for at least 6 months; 3) The patients' ages ranges from 30 to 40 years old; and 4) The study was performed on the lower jaw.*

Exclusion Criteria: 1) Patients with excessive bone loss; 2) Diseases affecting healing process (e.g. DM, Thyroid disease); 3) Females were excluded to avoid any hormonal changes which may affect the result of the study; and 4) Patients who receive radiotherapy or chemotherapy.

Methods

Group A: (Control Group) Implant procedure without addition of growth factors or LLLT.

Group B: Implant procedure with LLLT (Diode laser 808 nm). LLLT was applied after the implant insertion using diode laser 808 nm. There were six sessions in 2 weeks. The first session started on the same day of the implant insertion**.** The total energy dose delivered in each session is about 20 J/cm3 in one minute (10 J/cm^3 is delivered in 30 seconds buccally and 10 J/cm^3 is delivered in 30 seconds lingually) (Figure 1). The power was calculated according to the area to be stimulated, and this depends on the size of the inserted implant. The power was calculated according to this equation: Energy dose (J/cm3) = W.T/cm^3.

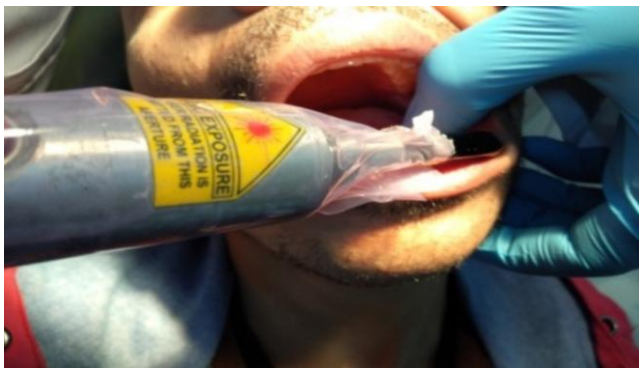

Figure 1: LLLT after implant insertion

Group C: Implant procedure with the addition of L-PRF. The osteotomy site was drilled according to the size of the previously selected implant. Then, whole venous blood (around 5 ml) in each of the two sterile vacutainer tubes (6 ml) was collected without any additives or any anticoagulants. After that the

vacutainer tubes were placed in a centrifuge machine.

Adjust the parameters of the centrifuge machine at 3,000 revolutions per minute (rpm) for 10 minutes. After the centrifugation, the following three layers were obtained: Upper part containing straw-colored acellular plasma also called platelet poor plasma (PPP), the bottom of the tube containing red blood cells (RBCs), and the middle part containing the fibrin clot (Figure 2).

Figure 2: The venous blood was collected and centrifuged

The upper straw-coloured (PPP) layer was then removed and discarded; then the middle fraction was collected, 2 mm below to the lower dividing line, which is the PRF. The formed L-PRF was cut into small pieces using scissors (Figure 3).

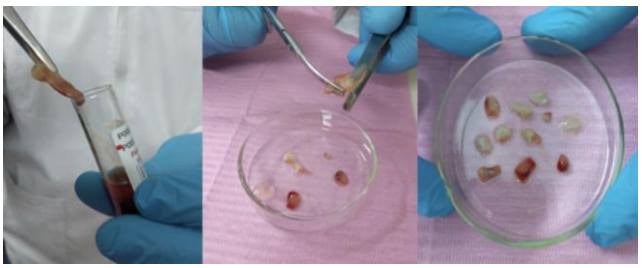

Figure 3: The formed L-PRF was cut into small pieces using scissors

Then, these small pieces of L-PRF were placed inside the osteotomy site before the insertion of the fixture of the prepared implant (Figure 4). Finally, the implant fixture was inserted inside the osteotomy site, which filled with growth factors (Figure 5).

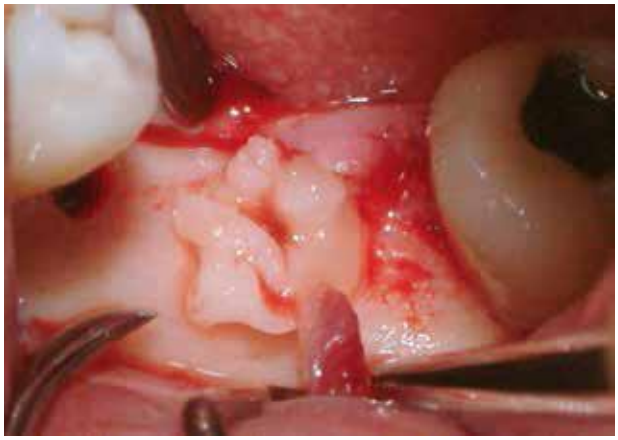

Figure 4: These small pieces of L-PRF were then placed inside the osteotomy site before the insertion of the fixture of the prepared implant

Group D: Implant procedure with the combined application of both L-PRF and LLLT (Diode laser 808 nm) with the same used protocol applied in the second and third groups.

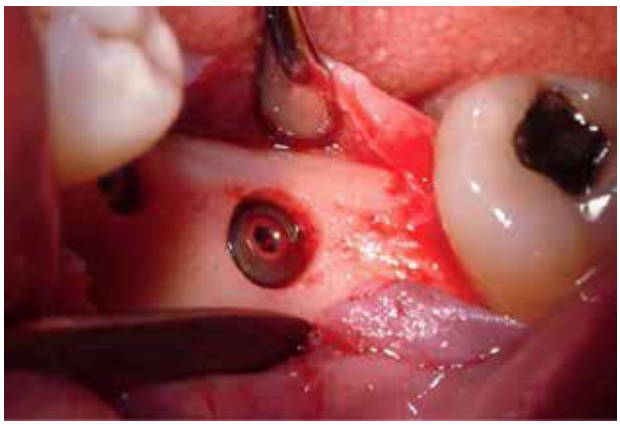

Figure 5: The implant was then inserted into the osteotomy site

All patients in the 4 groups were clinically and radiographically assessed using CBCT at baseline one week after the implant insertion (the day of suture removal), followed by another one after 6 weeks from implant insertion, and by another one after 12 weeks from the implant insertion, to evaluate the relative bone density around each implant. Relative bone density (RBD) around the implants was measured using OnDemand software, by inserting a simulated implant at the inserted implant and adjusted to the same dimensions and position, then measured the relative bone density using the verification tool in the software as shown in (Figure 6).

Diode Laser

Diode laser device class IIIB (Gallium-Aluminum-Arsenide) (808 nm). This device was made in NILES (National Institute of Laser Enhanced Science). The device was calibrated after every 10 cases in NILES.

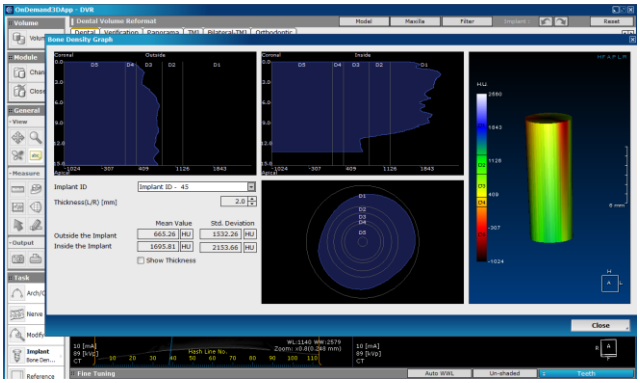

Figure 6: Measuring the relative bone density around the simulated implants using the verification tool in OnDemand software

Growth Factors

Leukocyte and Platelet-Rich Fibrin (L-PRF) products are preparations with leukocytes and with a high-density fibrin network. The preparation is completely natural, which is prepared from the patient's blood.

Centrifuge

The used centrifuge in this study is NeuationiFuge D06. The maximum speed of this device is 6500 RBM.

Dental Implant

The study used 40 dental implants of Leader system (TiXos dental implant). The dimensions of the used implants varied according to each case.

Cone Beam Machine for Radiographic Evaluation

The patients were examined using the SOREDEX (CRANEX 3Dx). All patients in this study were assessed by the same parameters (10.0 mA) (The time of exposure is 6.1 s) (90 Kv) (Field of view is 6 x 8 cm) (Resolution is high 200 µm).

Results

Descriptive Data

RBD of each implant was measured over one week, 6 weeks and 12 weeks using CBCT. The collected data of the 4 groups were summarised as average and standard deviation values for each group (Figure 7). It is well seen that the 12-week measurements in all groups are the best. The 6-week measurements in for group (A), the measured RBD decreases. On the other hand, the other 3 groups of the different treatments have clear increasing measures; except the laser group with a little increase

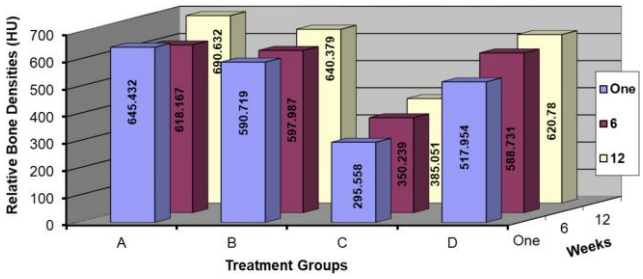

Figure 7: Relative Bone Densities at different groups and times

An illustration of efficiencies overall groups at 6 weeks and also 12 weeks can be seen at figures 8&9; one can see the highest value of the effect of L-PRF and the respective ranking as:

L-PRF group > (L-PRF+LLLT) group > LLLT group > Normal group

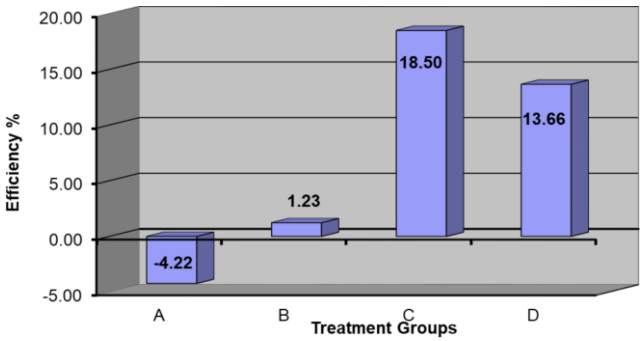

Figure 8: Efficiency of different treatment at 6 weeks

To assure the observations, we have to make statistical analysis for these collected data. An ANOVA test, t-test and correlation factor were made over the average values of treatments and the measurement times.

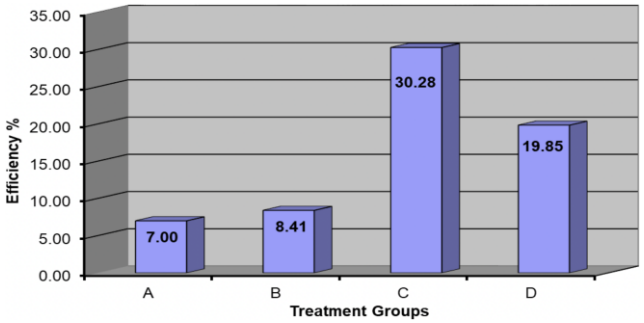

Figure 9: Efficiency of different treatment at 12 weeks

Statistical Analysis

The collected data were statistically analysed using ANOVA Test. ANOVA test was made over the average values of treatments and the measurements times, as shown in Table 1.

Table 1: The resultant data of the ANOVA test overall groups and measurement times

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Rows	10551.27	2	5275.63	9.975	0.012	5.143
Columns	171042.06	3	57014.02	107.795	0.000	4.757
Error	3173.47	6	528.91			
Total	184766.79	11				

The resultant value of P-value is 0.012 (< 0.05) this means that there are significant differences between all groups over different measured times; and to point out the internal variations over the measured times, we can use the (t-test: Paired two samples for means) and the results were as follows: T-test: Paired Two Samples for Means.

Taking into account the previous observation of these tests, they illustrate that there is a significant difference between each time of measurements (one week, 6 weeks and 12 weeks). This proves that both decreasing at 6 weeks (in the normal group) and increasing from one week up to 12 weeks (in all groups) are real, as shown in Table 2.

Table 2: P-Values of t-test

Group A			
	1 Week	6 Weeks	12 Weeks
1 Week			
6 Weeks	1.48787E-05		
12 Weeks	1.26072E-08	1.90506E-07	
Group B			
	1 Week	6 Weeks	12 Weeks
1 Week			
6 Weeks	1.98034E-06		
12 Weeks	8.0504E-09	2.75342E-08	
Group C			
	1 Week	6 Weeks	12 Weeks
1 Week			
6 Weeks	1.13269E-06		
12 Weeks	1.41573E-06	3.56459E-06	
Group D			
	1 Week	6 Weeks	12 Weeks
1 Week			
6 Weeks	1.8469E-08		
12 Weeks	2.49878E-10	5.706E-07	

On the other hand, the difference between the groups over measurement times (6 and 12 weeks) is shown in Table 3 as follows:

Table 3-a: P-Values of 6 weeks over different Groups

6 Weeks	A	B	C	D
A				
B	0.81217			
C	0.00413	0.0019		
D	0.71282	0.9034	0.0166	
12 Weeks	A	B	C	D
A				
B	0.5688			
C	0.0022	0.0017		
D	0.3938	0.8024	0.0211	

These tests show the following:

- In 6 & 12-week groups, it is well observed that the L-PRF group has a significant difference (< 0.05) with any other groups because of the higher effect of L-PRF.

- The tests between any two groups didn't show any significant difference except group C due to the positive LLLT effect on osteoclast cells was very clear on RBD during laser bio-stimulation period.

By applying the correlation factor measure, we can note the strong relation between the L-PRF group and (L-PRF+LLLT) group which means that the laser has an inhibitory role on the L-PRF effect (Table 4).

Table 4: Correlation Factor between the L-PRF group and (L-PRF+LLLT) Group at 6 Weeks and 12 Weeks

	6 Weeks			12 Weeks	
	C	D		C	D
C	1		C	1	
D	-0.736209	1	D	-0.778954	1

Discussion

Osseointegration is described as an effective interaction between bone tissue and the implant surface [27]. Osseointegration of titanium implants in rats was conducted by Haga *et al.,* This process is characterised by bone resorption in osteoclasts, followed by bone formation by osteoblasts [29]. Few days after the implant placement, osteoclast cells become more prominent in number and inaction than osteoblast cells to start bone resorption. One month after implant placement, the osteoblast cells become more active to start the osseointegration process. From 1.5 to 2.5 months after the placement of implants, the formation of bone tissue by osteoblast cells proceeds in the direction of the damaged bone containing empty osteocytic lacunae, resulting in a reduction in it. The portion of neoformed bone exhibits characteristics of spongy bone. Also, both cell types present reduced volumes, suggesting less cell activity. Three months after implant placement, there was an absence of empty osteocytic lacunae. The area of pre-existent bone has been replaced by neoformed bone containing intact osteocytes. The neoformed bone presents the morphological characteristics of compact bone [28], [30].

Previously, PDGF and PRP were applied around implants to provide bone regeneration and to increase osseointegration [31]. Anil Kumar et al., [32] reported that PRF targeting apoptosis of the osteoclasts, which may have favourable effects on combating bone resorption. Based on these findings, we propose that there is a major role of PRF to limit osteoclastogenesis.

The most challenging issue about LLLT is to define the effective dose. Several studies in the literature promote low-level laser therapy as a useful procedure to improve osseointegration with doses used between 2 and 54 J [33], [34]. Accordingly, we use 20 J/cm^3. However, it's not sensible to decide whether the amount of energy's being appropriate for biostimulation depending on the dose (Joules) only. Laser spot area should be known to have an opinion about the density of the energy given to the tissue.

When the spot area is doubled, the energy density decreases by four times, or if the spot area is halved, the energy density quadruples [33], [35]. So, one should measure the delivered energy regarding the volume of the region and not just the area of the region. In the current study, low-intensity Gallium-Aluminum-Arsenide laser with wavelength 808 nm was used as a regenerative approach to enhance osseointegration and increase the density of bone surrounding the implants. The frequently used lasers in the previous studies range from 670 to 1,064 nm [36]. The energy density delivered to the bone was calculated according to the volume of the implant using the following equation: Energy dose (J/cm3) = W.T/cm3 to ensure the delivery of sufficient energy to the bone surrounding the implant. In this study, laser light was applied from both the buccal and lingual sides. The LLLT group shows improvements in the RBD especially after 6 weeks in comparison with the control group by the previous studies [37], [38], [39].

This group, however, showed lower results of RBD in comparison with L-PRF group. This can be explained by a study by Burcu et al., 2012 [35]. This study used 38 male albino Wistar rats. It aimed at evaluating the effects of 820 nm diode laser on osteoclastic and osteoblastic cell proliferation-activity and RANKL/OPG release during orthodontic tooth movement. The study concluded a very important fact about LLLT, which is Low-level laser therapy is known to be a stimulator of the current biological process in tissue. This fact used to explain why osteoclastic activity varied and increased in laser groups that caused increasing the bone resorption process while osteoblastic activity was similar between groups on the third day of the experiment. At the end of the experiment, because bone formation had already started, the number of osteoblasts was found to have increased in the laser groups.

Moreover, Glinkowski and Pokora indicated that LLLT to bone increased phagocytosis and cytokine (IL-1, TGF-β) synthesis via accelerating macrophage migration [35]. According to Karu *et al.*, the mitochondrial cytochromes absorb the photon energy, and this absorption improves the potential activity of the cells via increasing ATP synthesis [40]. Because osteoclasts are multinuclear cells with mitochondria of high activity [41], they are readily affected by low-level laser radiation. This also explains the higher resorption levels in the irradiated animals. From the previous studies, we can conclude that the decrease in the RBD at 6 weeks in the control group is due to the pronounced activity of the osteoclast cells that are active in the first month after the implant placement. In LLLT group, there was a slight increase in the RBD after 6 weeks because laser biostimulation affects both osteoclast cells positively in the first days after the implant placement and osteoblast cells which become prominent at the end of the first month after the implant placement to compensate the bone loss in the first month and increases the relative bone densities slightly at the end of the 6th week.

Regarding the L-PRF group, the improvement efficiency percentage was the best between the 4 groups due to the great stimulating effect of PRF on healing, stimulation of the osteoblast, and limiting the osteoclastogenesis [32]. In his study, Anil Kumar *et al., [32]* reported that PRF displayed an inhibitory role in the formation and differentiation of osteoclast cells, and its molecular mechanism of action was related to the apoptosis induction through intrinsic mitochondrial pathway by activating caspase-9, -3 and -7 which are the most prevalent caspases and they are responsible for the majority of apoptotic effects.

Despite the improved RBD in both groups treated with LLLT and L-PRF, when we combine them, the density was improved but to less extent than the group used the L-PRF alone; and this means that the LLLT stimulated the osteoclasts [39], [40] which negatively affected the role of L-PRF slightly. This means that the LLLT has an inhibitory role in the PRF effect.

RBD increases after 12 weeks in the 4 groups because, within the period of 1.5 to 2.5 months after the placement of implants, the activity of the osteoclast cells decreases and the activity of osteoblast cells increases to start the process of bone formation [28], [30].

In conclusion, applying the findings of the current study, we can conclude that the osseointegration of dental implants can be clearly enhanced using new techniques like LLLT and L-PRF. These new techniques could increase the success rate of dental implants. L-PRF has a more positive effect on the osseointegration of dental implant than LLLT. The combined application of L-PRF and LLLT could enhance the osseointegration of dental implant but to a lower extent than applying L-PRF alone. Moreover, the combined application of L-PRF and LLLT could enhance the osseointegration of dental implant more than applying LLLT alone.

References

1. Zohrabian VM, Sonick M, Hwang D, Abrahams JJ. Dental implants. Semin Ultrasound CT MR. 2015; 36:415-426. https://doi.org/10.1053/j.sult.2015.09.002 PMid:26589695

2. Jenny G, Jauernik J, Bierbaum S, Bigler M, Gratz KW, Rucker M, Stadlinger B. A systematic review and meta-analysis on the influence of biological implant surface coatings on peri-implant bone formation. J Biomed Mater Res A. 2016; 104:2898-2910. https://doi.org/10.1002/jbm.a.35805 PMid:27301790

3. Shemtov-Yona K, Rittel D. An overview of the mechanical integrity of dental implants. Biomed Res Int. 2015; 2015:547384. https://doi.org/10.1155/2015/547384 PMid:26583117 PMCid:PMC4637045

4. Pal TK. Fundamentals and history of implant dentistry. Journal of the International Clinical Dental Research Organization. 2015;

7(3):6. https://doi.org/10.4103/2231-0754.172933

5. Gaviria L, Salcido JP, Guda T, Ong JL. Current trends in dental implants. J Korean Assoc Oral MaxillofacSurg. 2014; 40(2):50-60. https://doi.org/10.5125/jkaoms.2014.40.2.50 PMid:24868501 PMCid:PMC4028797

6. Choukroun J, Adda F, Schoeffler C, Vervelle A. Uneopportunité en paro-implantologie: Le PRF. Implantodontie. 2001; 42:55-62.

7. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, et al. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part II: Platelet-related biologic features. Oral Surg Oral Med Oral Pathol Oral RadiolEndod. 2006; 101:e45-50. https://doi.org/10.1016/j.tripleo.2005.07.009 PMid:16504850

8. Coelho RC, Zerbinati LP, de Oliveira MG, Weber JB. Systemic effects of LLLT on bone repair around PLLA-PGA screws in the rabbit tibia. Lasers Med Sci. 2014; 29:703-708. https://doi.org/10.1007/s10103-013-1384-4 PMid:23832178

9. De Vasconcellos LM, Barbara MA, Deco CP, Junqueira JC, do Prado RF, Anbinder AL, de Vasconcellos LG, Cairo CA, Carvalho YR. Healing of normal and osteopenic bone with titanium implant and low-level laser therapy (GaAlAs): a histomorphometric study in rats. Lasers Med Sci. 2014; 29:575-580. https://doi.org/10.1007/s10103-013-1326-1 PMid:23624654

10. Demirkol N, Sari F, Bulbul M, Demirkol M, Simsek I, Usumez A () Effectiveness of occlusal splints and low-level laser therapy on myofascial pain. Lasers Med Sci. 2015; 30:1007-1012. https://doi.org/10.1007/s10103-014-1522-7 PMid:24504660

11. Fronza B, Somacal T, Mayer L, de Moraes JF, de Oliveira MG, Weber JB () Assessment of the systemic effects of low-level laser therapy (LLLT) on thyroid hormone function in a rabbit model. Int J Oral MaxillofacSurg. 2013; 42:26-30. https://doi.org/10.1016/j.ijom.2012.06.017 PMid:22819694

12. Gasperini G, Rodrigues de Siqueira IC, Rezende Costa L. Does low-level laser therapy decrease swelling and pain resulting from orthognathic surgery? Int J Oral MaxillofacSurg. 2014; 43:868-873. https://doi.org/10.1016/j.ijom.2014.02.015 PMid:24679851

13. Park JB, Ahn SJ, Kang YG, Kim EC, Heo JS, Kang KL. Effects of increased low-level diode laser irradiation time on extraction socket healing in rats. Lasers Med Sci. 2015; 30:719-726. https://doi.org/10.1007/s10103-013-1402-6 PMid:23929563

14. Tang E, Arany P. Photobiomodulation and implants: implications for dentistry. J Periodontal Implant Sci. 2013; 43:262-268. https://doi.org/10.5051/jpis.2013.43.6.262 PMid:24455438 PMCid:PMC3891857

15. Mayer L, Gomes FV, Carlsson L, Gerhardt-Oliveira M. Histologic and resonance frequency analysis of peri-implant bone healing after low-level laser therapy: an in vivo study. Int J Oral Maxillofac Implants. 2015; 30:1028-1035. https://doi.org/10.11607/jomi.3882 PMid:26394337

16. Khadra M, Ronold HJ, Lyngstadaas SP, Ellingsen JE, Haanaes HR. Low-level laser therapy stimulates bone-implant interaction: an experimental study in rabbits. Clin Oral Implants Res. 2004; 15:325-332. https://doi.org/10.1111/j.1600-0501.2004.00994.x PMid:15142095

17. Maluf AP, Maluf RP, BritoCda R, Franca FM, De Brito RB Jr. Mechanical evaluation of the influence of low-level laser therapy in secondary stability of implants in mice shinbones. Lasers Med Sci. 2010; 25:693-698. https://doi.org/10.1007/s10103-010-0778-9 PMid:20393769

18. Primo BT, da Silva RC, Grossmann E, Miguens SA Jr, Hernandez PA, Silva AN Jr. Effect of surface roughness and low-level laser therapy on removal torque of implants placed in rat femurs. J Oral Implantol. 2013; 39:533-538. https://doi.org/10.1563/AAID-JOI-D-10-00141 PMid:21534821

19. Campanha BP, Gallina C, Geremia T, Loro RC, Valiati R, Hubler R, de Oliveira MG. Low-level laser therapy for implants without initial stability. Photomed Laser Surg. 2010; 28:365-369. https://doi.org/10.1089/pho.2008.2429 PMid:19860572

20. Rea M, Lang NP, Ricci S, Mintrone F, Gonzalez Gonzalez G, Botticelli D () Healing of implants installed in over- or under-prepared sites-an experimental study in dogs. Clin Oral Implants Res. 2015; 26:442-446. https://doi.org/10.1111/clr.12390 PMid:24684411

21. Friedmann A, Friedmann A, Grize L, Obrecht M, Dard M. Convergent methods assessing bone growth in an experimental model at dental implants in the minipig. Ann Anat. 2014; 196:100-107. https://doi.org/10.1016/j.aanat.2014.02.001 PMid:24656913

22. Lee SW, Hahn BD, Kang TY, Lee MJ, Choi JY, Kim MK, Kim SG. Hydroxyapatite and collagen combination-coated dental implants display better bone formation in the peri-implant area than the same combination plus bone morphogenetic protein-2-coated implants, hydroxyapatite only coated implants, and uncoated implants. J Oral MaxillofacSurg. 2014; 72:53-60. https://doi.org/10.1016/j.joms.2013.08.031 PMid:24331565

23. Ostman PO, Hellman M, Wendelhag I, Sennerby L. Resonance frequency analysis measurements of implants at placement surgery. Int J Prosthodont. 2006; 19:77-83.

24. Pagliani L, Sennerby L, Petersson A, Verrocchi D, Volpe S, Andersson P. The relationship between resonance frequency analysis (RFA) and lateral displacement of dental implants: an in vitro study. J Oral Rehabil. 2013; 40:221-227. https://doi.org/10.1111/joor.12024 PMid:23278128

25. Parsa A, Ibrahim N, Hassan B, van der Stelt P, Wismeijer D. Bone quality evaluation at dental implant site using multislice CT, micro-CT, and cone beam CT. Clin Oral Implants Res 2015; 26:e1-7. https://doi.org/10.1111/clr.12315 PMid:24325572

26. Barone A, Covani U, Cornelini R, Gherlone E. Radiographic bone density around immediately loaded oral implants: A case series. Clin. Oral Implants Res. 2003; 14:610-15. https://doi.org/10.1034/j.1600-0501.2003.00878.x

27. Zarb GA, Albrektsson T. Osseointegration - a réquiem for the periodontal ligament? Int J Periodontics Restorative Dent. 1991;m11:88-91.

28. Haga M, Fujii N, Nozawa-Inoue K, Nomura S, Oda K, UoshimaK, et al. Detailed process of bone remodeling after achieve-ment of osseointegration in a rat implantation model. Anat Rec. 2009; 292:38-47. https://doi.org/10.1002/ar.20748 PMid:18727113

29. Raisz LG, Rodan GA. Embryology and cellular biology of bone. In: Metabolic bone disease and clinically related disorders. Academic Press, 1998:1-22. https://doi.org/10.1016/B978-012068700-8/50002-5

30. Fujii N, Kusakari H, Maeda T. A histological study on tissue responses to titanium implantation in rat maxilla: the process of epithelial regeneration and bone reaction. J Periodontol. 1998; 69:485-95. https://doi.org/10.1902/jop.1998.69.4.485 PMid:9609380

31. Anitua E, Orive G, Aguirre JJ, Ardanza B, Andia I. 5-year clinical experience with BTI dental implants: Risk factors for implant failure. J ClinPeriodontol. 2008; 35:724-732. https://doi.org/10.1111/j.1600-051X.2008.01248.x PMid:18616758

32. Kumar A, Mahendra J, Samuel S, Govindraj J, Loganathan T, Vashum Y, Mahendra L, Krishnamoorthy T. Platelet-rich fibrin/biphasic calcium phosphate impairs osteoclasts differentiation and promotes apoptosis by the intrinsic mitochondrial pathway in chronic periodontitis. J Periodontol. 2018; 0:1-11. https://doi.org/10.1002/JPER.17-0306 PMid:29958327

33. Kawasaki K, Shimizu NV. Effects of low-energy irradiation on bone remodelling during experimental tooth movement in rats. Lasers Surg Med. 2000; 26:282-291. https://doi.org/10.1002/(SICI)1096-9101(2000)26:3<282::AID-LSM6>3.0.CO;2-X

34. Youssef M, Ashkar S, Hamade E, Gutknecht N, Lampert F, Mir M. The effect of low-level laser therapy during orthodontic movement: a preliminary study. Lasers Med Sci. 2008; 23(1):27-33. https://doi.org/10.1007/s10103-007-0449-7 PMid:17361391

35. BurcuAyseAltan, Oral Sokucu, Mahmud M. Ozkut, SevincInan. Metrical and histological investigation of the effects of low-level

laser therapy on orthodontic tooth movement. Lasers Med Sci. 2012; 27:131-140. https://doi.org/10.1007/s10103-010-0853-2 PMid:21038101

36. Diniz JS. Effect of low-power gallium-aluminum arsenium laser therapy (830 nm) in combination with bisphosph bisphosphonate treatment on osteopenic bone structure: an experimental animal study. Lasers Med Sci. 2009; 24(3): 347-52. https://doi.org/10.1007/s10103-008-0568-9 PMid:18648870

37. Lopes CB, Pinheiro AL, Sathaiah S, Duarte J, Cristinamartins M. Infrared laser light reduces loading time of dental implants: a Raman spectroscopic study. Photomed Laser Surg. 2005; 23:27-31. https://doi.org/10.1089/pho.2005.23.27 PMid:15782028

38. Shimizu N, Mayahara K, Kiyosaki T, Yamaguchi A, Ozawa Y, Abiko Y. Low-intensity laser irradiation stimulates bone nodule formation via insulin-like growth factor-I expression in rat calvarial cells. Lasers Surg Med. 2007; 39(6):551-9. https://doi.org/10.1002/lsm.20521 PMid:17659585

39. Mikhail FF, El-Din M, Ibrahim T, Zekry K, Nemat A, Nasry S. Effect of Laser Therapy on the Osseointegration of Immediately Loaded Dental Implants in Patients under Vitamin C, Omega-3 and Calcium Therapy. Open Access Maced J Med Sci. 2018; 6(8):1468-1474. https://doi.org/10.3889/oamjms.2018.291 PMid:30159079 PMCid:PMC6108810

40. Karu T. Primary and secondary mechanisms of action of visible to near-IR radiation on cells. J PhotochemPhotobiol. 1999; 49:1-17. https://doi.org/10.1016/S1011-1344(98)00219-X

41. Boyle WJ, et al. Osteoclast differentiation and activation. Nature. 2003; 423:337-342. https://doi.org/10.1038/nature01658 PMid:12748652

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Dental Science

Effect of Surface Treatments on the Shear Bond Strength of Indirect Esthetic Restorative Materials to Dentin

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Abstract

AIM: The purpose of this research was to evaluate the influence of air abrasion, hydrofluoric acid, and combination of air abrasion and hydrofluoric acid on the shear bond strength between dentin and CEREC, VITA VM7, and E-max.

MATERIAL AND METHODS: Ninety extracted human lower molars were used. The teeth were divided into three groups (n = 30) according to the surface treatment (air abrasion, hydrofluoric acid, and air abrasion + hydrofluoric acid). Each group was then subdivided into three subgroups (n = 10) according to the ceramic material (CEREC, E-max, and VITA VM7). Shear bond strength was determined by the compressive mode of force applied at the ceramic-tooth interface. The collected data were analysed using two-way analysis of variance (ANOVA), and Tukey's post-hoc test statistical significance was set at $P < 0.05$.

RESULTS: The highest mean shear bond strength value was recorded with the CEREC group treated by hydrofluoric acid (8.01MPa). While the least mean shear bond strength was recorded with the Cerec group but when treated by air abrasion alone, it was 4.33MPa.

CONCLUSION: Hydrofluoric acid etching for various types of ceramic restoration improved the bond strength to dentin.

Introduction

The prime concern of nowadays practice is to restore teeth and recovering esthetic with maximum preservation of the remaining tooth structure as much as possible. In this field, indirect ceramic restorations accomplish this concept [1]. The superior esthetic of all-ceramic restorations has resulted in the increased demand for these restorations [2]. Computer-aided design/computer-aided manufacturing (CAD / CAM) techniques are used frequently nowadays, not only for simple veneer but also for more complicated fixed prostheses [3], [4]. The pressed ceramic IPS Empress has emerged strongly in the field of all-ceramic restorations due to its high resistance to fracture and wear [5]. Even though the introduction of modern systems for indirect ceramic restoration conventional layering ceramic is still in service [6]. Long-lasting esthetic restoration is the main goal of both dentists and patients. To achieve a strong bond of the adhesive to the ceramic surface, micromechanical interlocking to the ceramic surface is essential. This requires surface activation for the ceramics [7]. Many surface treatments are used nowadays to create a surface alteration of the esthetic restorations to enhance bonding to the tooth structure. To create such alteration the surface of the restoration may be etched, silanated, or sandblasted. [8]. Etching the ceramic surface with hydrofluoric acid produces a porous surface with a larger surface area available for bonding. These pores facilitate the penetration of the adhesive to create micro retention [7]. Also, sandblasting is used to produce the same effect with different techniques [9]. Application of silane coupling agent has resulted in better wetting of the ceramic surface allowing for better bond strength [10]. All the techniques mentioned above are used solely or in combination with each other to increase the bond

strength of indirect ceramic restoration to the prepared tooth structure. Obtaining good bonding between the restoration and the prepared tooth structure has its positive reflectance to decrease the marginal discolouration. Also, microleakage will be decreased with its associated dilemma. In case of good bonding, tooth and restoration will act as one unit (tooth-restoration complex) so; the fracture resistance will be higher.

The purpose of this paper was to assess the shear bond power of triple ceramic materials bonded with the prepared teeth with three different techniques. The null hypothesizes tested are: 1) there is no effect of the ceramic type on the bond strength to the prepared tooth; 2) there is no effect of bonding technique on the bond strength to the prepared tooth, and 3) the interaction between ceramic type and bonding technique has no effect on the bond strength to the prepared tooth.

Material and Methods

Ninety freshly extracted human lower molar teeth were selected. The inclusion criteria were extracted molars free of caries or restorations and free of any developmental defects. The exclusion criteria were any carious molars or molars that have the previous restoration or developmentally affected. The teeth were manually scaled to remove any calculus or soft tissue remnants and stored in normal saline solution at room temperature during the study (not more than 3 months). All teeth were embedded into auto polymerising resin limited to the cervical line. The occlusal third of the teeth was grounded using a diamond stone under water coolant to make a flat dentin surface ready for cementation (Figure 1).

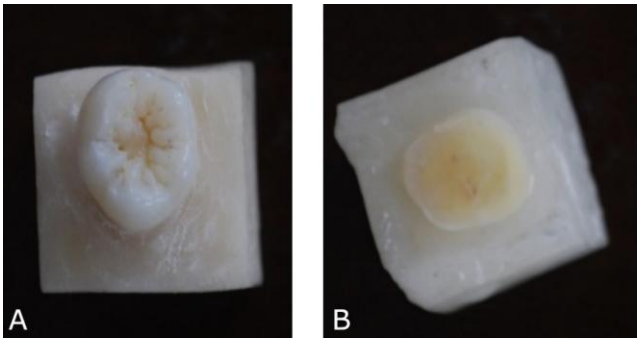

Figure 1: A photograph showing the tooth A) before, and B) after removal of the occlusal third to make a flat dentin surface ready for cementation

The teeth were then randomly divided into three groups according to the type of surface treatment (n = 30). The first group was subjected to air abrasion, the second group was subjected to hydrofluoric acid, while the third group was subjected to both air abrasion and hydrofluoric acid. Successively, each group was further subdivided into three subgroups (n = 10) according to the type of ceramic. The first subgroup was restored with Cerec, the second subgroup was restored with I.P.S. Empress, while the third subgroup was restored with VM7. For all tested materials, a standardised 30 discs were prepared with 5 mm diameter and 3mm height. Al materials used in this study are listed in the Table 1.

Table 1: The materials used in the study

No	Material	Specifications	Manufacturer	Batch No.
1	CEREC Blocs Ceramics for CEREC	CAD CAM CEREC system	Sirona the dental company Germany https://www1.dentsplysirona.com	11810
2	VITA VM7	The VITADURVEST powder	Bad Sackingen, Germany https://www.vita-zahnfabrik.com	10200801
3	E-max press medium opacity	Empress 2	(Ivoclar Vivadent. Schaan, Liechtenstein www.ivoclarvivadent.com	0346
4	Ultradent Porcelain Etch	Hydrofluoric acid	Ultradent Products, South Jordan, UT, USA. https://www.ultradent.com	10050
5	Ultradent Silane	Silane coupling agent	Ultradent Products, South Jordan, UT, USA. https://www.ultradent.com	110403
6	Dyract Cem plus	Adhesive resin cement (chemically cured)	Dentsply Germany http://www.dentsply.eu/	050103

Preparation of ceramic samples

For Cerec samples, the discs were prepared by direct grinding of the ready-made blocks. While for I.P.S. samples, the wax pattern was constructed then invested in phosphate bonded investment. While for VM7 a brass split counter die was constructed to provide a 5 x 3 mm mould space, and compensation for the shrinkage was done by adding another coat of porcelain, resulting in a full-thickness of 3 mm verified with a digital calliper.

Procedures of cementation

For each group, the samples have been separated randomly into three subgroups, according to the surface treatment. For the first subgroup, the bonding surface of the ceramic block has been treated with 0.9 HF for 4 minutes. The bonding surface of the second subgroup was air abraded with 50 μm grain-sized aluminium oxide particles at 200 kPa pressure for 14 sec. The third subgroup has undergone air abrasion with 50 μm grain-sized aluminium oxide particles at 200 kPa pressure for 14 sec and then etched with 9% hydrofluoric acid for 4 min.

All the treated samples were at that point flushed with tapping water for 10 sec silanated with a silane coupling agent and air thinned for 5 seconds. The prepared tooth surfaces were then etched for 20 seconds with 35% phosphoric acid gel, rinsed for 10 seconds, and slightly dehydrated with minimum air to guarantee that the dentine surface remains wet. The prepared dentin surfaces of the teeth were then

primed.

The silanated ceramic discs were then bonded to the dentin surface using auto polymerising resin cement (Dyract cem plus). The ceramic was placed on the centre of the dentin surface, and a fixed vertical load (5 kg) was applied to the ceramic surface to create a steady cement layer. The excess cement was removed with a sharp hand instrument, after the initial setting of the cement. The shear bond test was done after 24 hours.

Shear Bond Strength Test procedure

A circular interface shear test was planned to assess the bond quality. All tests were mounted on computer-controlled materials testing machine (Model LRX-*P*lus; Lloyd Instruments Ltd., Fareham, UK) with a load cell of 5 kN and data were recorded using computer software (Nexygen-MT; Lloyd Instruments) (Figure 2). Shear bond quality was decided by the compressive mode of force applied at the ceramic-tooth interface using a monobevelled chisel-shaped metallic rod connected to the upper movable compartment of the testing machine travelling at a cross-head speed of 0.5 mm/min.

Figure 2: Universal Testing Machine

Statistical analysis

The collected data were analysed using a two-way analysis of variance (ANOVA). Tukey's post-hoc test was used for comparison between the means when the ANOVA test is significant. For all groups, the significance level was set at $P \leq 0.05$. Statistical analysis was performed with SPSS 20.0 for windows.

Results

The results of mean shear bond strength values and standard deviations of all groups are listed in Table 2. The highest mean shear bond strength value was recorded for the Cerec group treated by hydrofluoric acid (8.01 MPa) while the least shear bond strength was also recorded for the Cerec group but when treated by air abrasion alone 4.33 MPa (Figure 3).

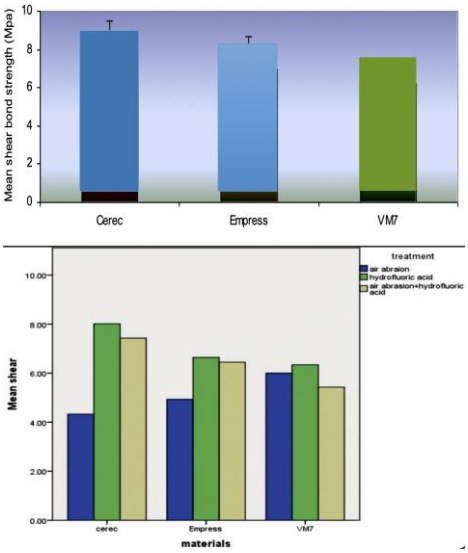

Figure 3: The effect of different surface treatment on the shear bond strength of the tested material to dentin

Regarding the tested materials, two-way ANOVA revealed no significant difference among the material groups ($P > 0.05$). However, the Cerec group yielded the highest mean shear bond strength value, while VM7 group showed the least mean shear bond strength value. For surface treatment subgroups, two-way ANOVA revealed a significant difference among different surface treatments ($P < 0.001$). Post hoc Tukey test showed a significant difference between subgroups treated with air abrasion and subgroups treated with hydrofluoric acid ($p < 0.001$), as well as subgroups treated with air abrasion followed by hydrofluoric acid ($p < 0.05$), while there was no statistically significant difference between subgroups treated with hydrofluoric acid and subgroups treated with air abrasion followed by hydrofluoric acid ($p > 0.05$). Regardless of the tested materials, surfaces treated with hydrofluoric acid showed the highest mean shear bond strength value, while those treated with air abrasion alone gave the lowest mean shear bond strength value. Two-way ANOVA revealed a significant effect of the interaction between the materials and surface treatments on the mean shear bond strength values ($p < 0.05$).

Table 2: Descriptive statistics for shear bond strength values

Ceramic	Surface treatment	Mean	SD
Cerec	Air abrasion	4.33	0.61
	Hydrofluoric acid	8.01	1.62
	Air abrasion and Hydrofluoric acid	7.43	0.98
Empress	Air abrasion	4.93	0.76
	Hydrofluoric acid	6.64	1.00
	Air abrasion and Hydrofluoric acid	6.45	0.54
VM7	Air abrasion	6.00	0.42
	Hydrofluoric acid	6.34	0.92
	Air abrasion and Hydrofluoric acid	5.43	0.77

Discussion

Nowadays, there are increased demands for esthetic restorations. Despite the increased use of CAD / CAM system, some limitations face the dentist due to its high cost and limited materials. On the other hand, it offers an easy and time-saving technique to fabricate indirect esthetic restoration [11]. IPS Empress also has been used successfully for single unit restoration or even three units fixed bridge [12]. To improve the bond strength between indirect ceramic restoration and tooth structure, the silane coupling agent is advocated. Application of a silane coupling agent to the pretreated ceramic surface gives a chemical covalent and hydrogen bond.

Moreover; it is a major factor for a sufficient resin bond to silica-based ceramic. Silanes are bifunctional molecules that bond silicone dioxide with the OH groups on the ceramic surface. They have a degradable functional group that copolymerizes with the organic matrix of the resin [13]. The ceramic bonding systems are mainly mechano-chemical bonding between the luting cement and the ceramic surface [12]. Many studies had reported high bond strength of ceramics to dentin when the ceramics were treated by hydrofluoric acid [12], [14], [15]. This was in agreement with our study. They explained this result by attacking the residual glass in the ceramics by the hydrofluoric acid leaving behind a surface of rod-shaped crystals, which enhances the mechanical interlocking possible. Other studies [14] correlates this result to the preferential dissolution of the glassy phase from the ceramic matrix that generates a micro mechanically retentive surface texture and promotes the formation of the hydroxyl group on the ceramic surface. Another study used atomic force microscopy to investigate the surface of ceramics after treatment with hydrofluoric acid. They found a very distinct surface texture enhances the bond strength [15]. The air abrasion technique showed the lowest mean bond strength value. This result was in disagreement with another study [16] who inferred that air abrasion technique could produce good bond strength. This disagreement may be due to their study was performed to repair fractured porcelain with flowable composite while this study investigated the bond between the ceramics and tooth structure. The explanation of our result may be due to the high hardness of the ceramic surface to be efficiently etched with air abrasion technique. The resulted abraded surface was smoother than those obtained after etching with hydrofluoric acid (HF) with subsequent lower bond strength values. Though HF acid was reported to provide good bond strength, it is one of the foremost hurtful compounds to handle for clinical as well as for laboratory use [12].

Regarding the ceramic material the highest mean bond strength values were obtained for the Cerec system. This was in disagreement with other studies that found no difference between the dentin bond strength of the Cerec and IPS Empress [17]. The main difference between our study and the afford mentioned one was that they performed their samples on standardised mesio-occlusal cavities, while we performed this study on a flat dentin surface. The geometry of the bonded area may affect the bond strength strongly.

In conclusion, hydrofluoric acid etching for various types of ceramic restoration results in the highest shear bond strength to dentin. The shear bond strength of the ceramic materials to dentin depends to a great extent on the surface treatment.

Data Availability

Data will be available upon request.

References

1. Girary F, Duzdar L, Oksuz M, Tanboga I. Elevation of the bond strength of resin cement used to lute ceramics on la-ser-etched dentin. Photomed Laser Surg. 2014; 32:413-21. https://doi.org/10.1089/pho.2013.3701 PMid:24992276 PMCid:PMC4082358

2. Anchieta R, Rocha E, de Almeida E, Junior A, Martini A. Bonding all-ceramic restorations with two resins cement technique: a clinical report of three-year follows up. Eur J Dent. 2011; 5:478-85. https://doi.org/10.1055/s-0039-1698922 PMid:21912505 PMCid:PMC3170033

3. Ab-Ghani Z, Jaafar W, Foo S, Ariffin Z, Mohamad D.Shear bond strength of computer-aided design and computer-aided manufacturing feldspathic and nano resin ceramics blocks cemented with three different generations of resin cement. J Conserv Dent. 2015; 18:355-9. https://doi.org/10.4103/0972-0707.164028 PMid:26430296 PMCid:PMC4578177

4. Poggio C, Pigozzo M, Ceci M, Scribante A, Beltrami R, Chiesa M. Influence of different luting protocols on shear bond strength of computer aided design/computer aided manufacturing resin nanoceramic material to dentin. Dent Res J. 2016; 13: 91-7. https://doi.org/10.4103/1735-3327.178193

5. Boaventura J, Nishida R, Elossais A, Lima D, Reis J, Cam-pos E, et al. Effect finishing and polishing procedures on the surface roughness of IPS Empress 2 ceramic. Acta Odontol Scand. 2013; 71:438-43. https://doi.org/10.3109/00016357.2012.690570

PMid:22724660 PMCid:PMC3665313

6. Borba M, de Araujo M, de Lima E, Yoshimura H, Cesar P, Griggs J, et al. Flexural strength and failure modes of layered ceramic structures. Dent Mater. 2011; 27:1259-66. https://doi.org/10.1016/j.dental.2011.09.008 PMid:21982199 PMCid:PMC3205330

7. Jetti R, Balasubramaniam M, Chidambaranathan A, Sir-Srinivasan S. Evaluation of shear bond strength of feldspathic CAD/CAM ceramic with dentin using 2 bonding agents and 2 surface treatments-An invitro study. J Clin Diagn Res. 2015; 9:36-9. https://doi.org/10.7860/JCDR/2015/15732.6779 PMid:26674522 PMCid:PMC4668520

8. Moezizadeh M, Ansari Z, Fard M. Effect of surface treatment on micro shear bond strength of two indirect composites. J Conserv Dent. 2012; 15:228-32. https://doi.org/10.4103/0972-0707.97943 PMid:22876007 PMCid:PMC3410330

9. Peumans M, Valjkova E, De Munck J, Mishevska C, Van Meerbeek B. Bonding effectiveness of luting composites to different CAD/CAM materials. J Adhes Dent. 2016:23.

10. Sattabansuk V, chanchairerk P, Punsukumtana L, Burrow M. Effect of mechanical and chemical surface treatment on the resin-glass ceramic adhesion properties. J Investig Clin Dent. 2016; 9. https://doi.org/10.1111/jicd.12220 PMid:27282642

11. Sannino G, Germano F, Arcuri E, Arcuri C, Barlattani A. CEREC CAD/CAM Chairside System. Oral & Implantology. 2014; 7(3):57-70.

12. Pattanaik S, Wadkar A. PEffect of etchant variability on shear bond strength of all-ceramic restorations - an in vitro study. J Indian Prosthodont Soc. 2011; 11:55-62. https://doi.org/10.1007/s13191-011-0064-y PMid:22379307 PMCid:PMC3095734

13. Yoo JY, Yoon HI, Park EJ. Porcelain repair- Influence of different systems and surface treatments on resin bond strength. J Adv Prosthodont. 2015; 7:343-8. https://doi.org/10.4047/jap.2015.7.5.343 PMid:26576249 PMCid:PMC4644774

14. Matinlinna JP, Vallittu PK. Bonding of resin composites to etchable ceramic surfaces-an insight review of the chemical aspects on surface conditioning. J Oral Rehabil. 2007; 34:622-30. https://doi.org/10.1111/j.1365-2842.2005.01569.x PMid:17650173

15. Zaghloul H, Elkassas DW, Haridy MF. Effect of incorporation of silane in the bonding agent on the repair potential of machinable esthetic blocks. Eur J dent. 2014; 8:44-52. https://doi.org/10.4103/1305-7456.126240 PMid:24966745 PMCid:PMC4054031

16. Erdemir U, Sancakli HS, Sancakli E, et al. Shear bond strength of a new self-adhering flowable composite resin for lithium disilicate-reinforced CAD/CAM ceramic mate-rial. J Adv Prosthodont. 2014; 6:434-443. https://doi.org/10.4047/jap.2014.6.6.434 PMid:25551002 PMCid:PMC4279040

17. Öztürk AN, İnan Ö, İnan E, Öztürk B. Microtensile Bond Strength of CAD-CAM and Pressed-Ceramic Inlays to Den-tin. Eur J Dent. 2007; 1:91-6. https://doi.org/10.1055/s-0039-1698320 PMid:19212483 PMCid:PMC2609949

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Public Health

Effectiveness of a Training Course on Accuracy of Triaging of Pediatric Patients

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Keywords: Triaging system; Pediatric emergency

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Abstract

BACKGROUND: In the context of a new but busy Pediatric Emergency Department, the risk of missing patients who need more emergent care can be reduced by timely and accurate triaging. In the emergency department of King Fahad Armed Forces Hospital, the Canadian Triage and Acuity Scale had already been implemented, including the pediatric version (PaedCTAS). However, a common observation remained that critical patients did not always receive priority with subsequent delays in management. To improve this accuracy, a training course was administered to health care professionals responsible for triaging of pediatric patients.

AIM: To determine the effectiveness of a training course on accuracy of triaging of Pediatric Patients.

METHODS: A triage training course was conducted over two months, with patient encounter sheets reviewed before the course for 6 months and after the course for 12 months. Accuracy was calculated by comparing it to level as determined by two pediatric emergency physicians. Also, admission rates were used as a surrogate marker to also determine accuracy.

RESULTS: A total of 31 053 patient sheets were reviewed. There was a considerable improvement in the correct determination of all triage levels, with accuracy ranging from 56.5% to 78.3% before the course, and reaching from 79.1% to 90.8% after the course with a statistically significant difference. Triaging errors still present were mainly in the form of down-triage.

CONCLUSION: Our training course in triage has a significant impact on the accuracy of triaging of ill pediatric patients. Further improvement can be obtained by repeated courses and direct feedback with debriefing sessions on challenges to triage level determination.

Introduction

In emergency settings, a key strategy to decrease waiting times, especially for those with critical illnesses, is an effective triage protocol. Initially introduced in the United States in 1950, triaging and its success is currently an essential component of patient care in the emergency department [1], [2]. Many systems have been developed to help prioritise patients. The one we used in this research study was the PaedCTAS (The Paediatric Canadian Triage and AcuityScale), but many others have been validated including the MTS (The Manchester Triage System) and the ATS (Australian Triage Scale) [3]. The challenge in triaging of all patients, but particularly those in the pediatric age group, is the ability to make a quick decision based on a brief encounter. Not only do heart rate and respiratory rate vary according to age, but also increase significantly in response to fever, anxiety and pain [4], [5]. To avoid this pitfall, the CTAS, initially implemented in 1999 with a recent revision in 2014, takes into consideration subjective evaluation in addition to objective assessment, and includes modifiers related to the history and the physiologic status. The acuity levels are assigned from 1 (most urgent) – 5 (least urgent) [6].

Ineffective triaging has been demonstrated to have grave consequences, including prolonged wait times, higher acuity patients deteriorating and avoidable tragic outcomes [7], [8].

In King Fahad Armed Forces Hospital, a tertiary teaching hospital in Saudi Arabia, the Pediatric emergency department serves a large population with a very high rate of visits, which highlighted the importance of being able to accurately implement a triaging protocol. Triage has always been the responsibility of nurses, but in most pediatric patients, the physician on duty would be consulted. Therefore, a PaedCTAS triage course was administered over 2 months to all nurses and physicians working in the ED, with the aim of evaluating the accuracy of triaging by reviewing the ED sheets of patients seen before and after the course.

Subjects and Methods

The study protocol was approved by the Ethics Committee of King Fahad Armed Forces Hospital in compliance with the Declaration of Helsinki and the Good Clinical Practice guidelines. This was a prospective study with the aim of assessing the accuracy of triage in the Pediatric Emergency Department before and after a PaedCTAS course. Recorded triage score was obtained by reviewing the documented notes on the ER sheet of each patient. The correct score was calculated based on findings on the sheet and was determined by two different Pediatric Emergency physicians. Only scores where there was full interobserver agreement were included in the study.

The triage course was a weekly 3-hour session that encompassed two months (total of nine sessions), and each nurse and physician was expected to attend three of these sessions. Full compliance was ensured by integrating this session into the monthly schedule. The three hours consisted of a one-hour orientation lecture on the importance and technique of triaging, followed by an hour of interactive discussion of cases, and finally time allocated for a workbook to be used for each participant with a required pass score of 90%.

The staff all felt confident with their skills following the course, but there were reports of confusion after a couple of months, so feedback was provided, and a refresher course was prepared and conducted but without complete attendance due to scheduling difficulties.

The primary outcome of this study was the comparison of accuracy of triage before and after the triage course. Secondary outcomes included identification of most difficult triage levels to identify and comparison of admission rates to expected rates for each triage level as a surrogate marker for triage accuracy.

Review of patient encounter sheets started in January 2010 for 6 months, followed by 2 months of the training course then post-course sheets review for 12 months. Only patient encounter sheets where two pediatric emergency physicians agreed upon the triage level were included in the study.

Statistical analysis was performed via SPSS for Windows Version 15.0. Rates of accurate triage were calculated as percentages, and patient characteristics were expressed as mean and standard deviation. Chi-square and McNemar tests were used to compare results before and after the course, with p-value of less than 0.05 being considered significant.

Results

The study encompassed a total of 20 months, during which patient encounter sheets were reviewed. As illustrated in Figure 1, the total number of sheets initially evaluated for inclusion were 37961 of all patients registered in the PED. Patient encounters were then excluded if the sheets were found to be incomplete (511 patients, 1.3%), if patients were over 16 years of age (231 patients, 0.6%), or if both pediatric emergency physicians did not agree on the CTAS level (6,166 patients, 16.2%). The total remaining patient encounters that were included were 31053.

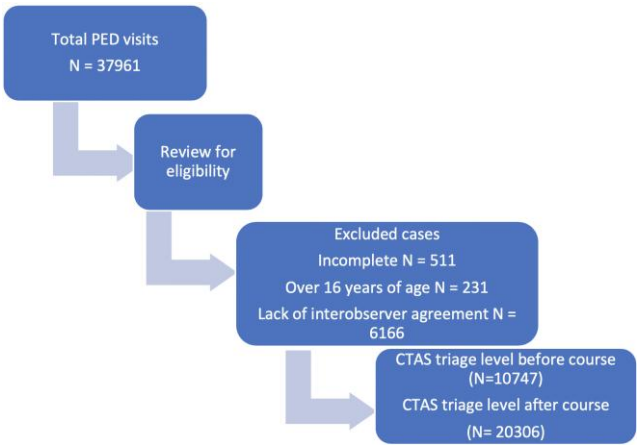

Figure 1: Selection process of sheets eligible for the research study

The patient encounter sheets reviewed showed the baseline characteristics outlined in Table 1, with a very slight female predominance. Mean age was 5.5 ± 2.9 years, showing a normal distribution curve. An important point to note is that patients who frequently presented to the PED may have more than one patient encounter sheet. The exact number falling into this category were not counted but are not expected to bias results with such a large sample size.

Regarding disposition, mortalities also

included patients who were dead on arrival, as well as those who were not aggressively resuscitated because of a Do Not Resuscitate (DNR) order.

Table 1: Baseline characteristics of the study population

Characteristic	Participants N (%) Total = 31053
Gender	
Male	14874 (47.9%)
Female	16179 (52.1%)
Age group	
0-30 days	820 (2.6%)
1-12 months	8923 (28.7%)
1-6 years	13859 (44.6%)
7-12 years	6978 (22.5%)
13-16 years	473 (1.5%)
Arrival by ambulance	1073 (3.6%)
Shift of arrival	
Day (08:01 – 16:00)	9715 (31.3%)
Evening (16:01 – 00:00)	14598 (47.0%)
Night (00:01 – 08:00)	6740 (21.7%)
Final Disposition	
Discharge	27515 (88.6%)
Admission to Pediatric department	1704 (5.5%)
Admission to Pediatric Intensive Care Unit	602 (1.9%)
Mortality	257 (0.8%)

Of the total sheets reviewed, 10747 were assessed before the triage course. Table 2 demonstrates rows of correct CTAS levels as calculated by two pediatric emergency physicians, whereas the columns demonstrate the CTAS level calculated by the triage team and show number and per cent of patients triaged in each level before the triage course.

The number of patients in Triage levels 1-5 was 115, 425, 5771, 3557 and 879 respectively. Accurate triage level assignment occurred between 56.5% and 78.3% with the least accuracy occurring in Triage level 2 and the highest in level 1.

Table 2: Comparison between calculated and correct CTAS before triage course

	CTAS level calculated by the triage team					Total
Actual CTAS	Level 1	Level 2	Level 3	Level 4	Level 5	
Level 1	90 (78.3%)	17 (14.8%)	7 (6.1%)	1 (0.9%)	0 (0.0%)	115
Level 2	55 (12.9%)	240 (56.5%)	87 (20.5%)	38 (8.9%)	5 (1.2%)	425
Level 3	172 (3.0%)	458 (7.9%)	3854 (66.8%)	846 (14.7%)	441 (7.6%)	5771
Level 4	56 (1.6%)	167 (4.7%)	125 (3.5%)	2573 (72.3%)	636 (17.9%)	3557
Level 5	0 (0.0%)	0 (0.0%)	44 (5.0%)	223 (25.4%)	612 (69.6%)	879

After completion of the triage course, a total of 20306 sheets were reviewed (Table 3), with the number of patients in each triage level being 273, 934, 11378, 6396 and 1325 respectively. Accurate triaging ranged from a minimum of 79.1%, which was in level 4 to a maximum of 90.8% in level 1.

Table 3: Comparison between calculated and correct CTAS level after triage course

	Calculated CTAS					Total
Correct CTAS	Level 1	Level 2	Level 3	Level 4	Level 5	
Level 1	248(90.8%)	15(5.5%)	10(3.7%)	0(0.0%)	0(0.0%)	273
Level 2	50(5.4%)	768(82.2%)	83(8.9%)	33(3.5%)	0(0.0%)	934
Level 3	333(2.9%)	504(4.4%)	9449(83.0%)	788(6.9%)	304(2.7%)	11378
Level 4	11(0.2%)	152(2.4%)	240(3.8%)	5057(79.1%)	936(14.6%)	6396
Level 5	0(0.0%)	0 (0.0%)	31(2.3%)	128(9.7%)	1166(88.0%)	1325

The accuracy of triaging was calculated before and after the course, with statistically significant improvements in all PaedCTAS levels, as illustrated in Figure 2.

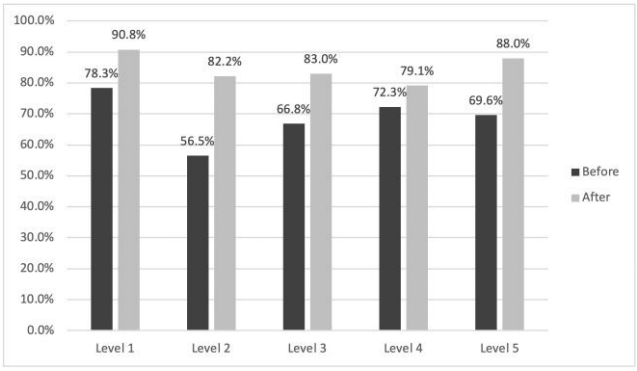

Figure 2: Percentage of accurate triaging before and after course

The greatest improvement occurred in Triage Level 2, The number and percentage of errors before and after the course decreased dramatically, the differences highly significant with a p-value of less than 0.001 for all levels (Table 4).

Table 4: Comparison of number and per cent of triage errors in each level before and after triage course

Triage level	Triage errors No (%)		P-value
	Before course	After course	
Level 1	25 (9.2%)	25 (2.2%)	0.001*
Level 2	185 (43.5%)	166 (17.8%)	< 0.001*
Level 3	1917 (33.2%)	1929 (17.0%)	< 0.001*
Level 4	984 (27.7%)	1339 (20.9%)	< 0.001*
Level 5	267 (30.4%)	159 (12.0%)	< 0.001*
Total	3378 (31.4%)	3618 (17.8%)	< 0.001*

*Statistically significant at < 0.05.

A more detailed analysis of the errors that had occurred prior to the triage course revealed that there was significantly more down-triaging than up-triaging for Triage levels 2, 3 and 4. As expected, Level 1 was only down-triaged whilst Level 5 was only up-triaged (Figure 3a and 3b).

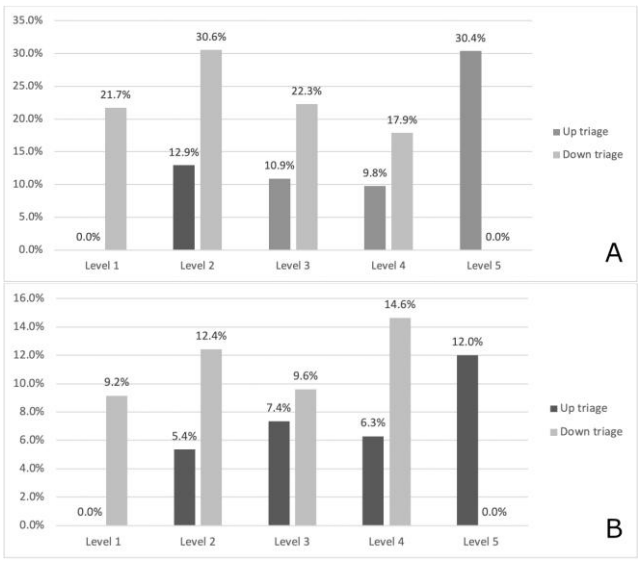

Figure 3: A) and B) Up and down triage before and after course

An alternative method to assess the accuracy of triaging is how well it correlates with admission

rates. For CTAS Level 1 patients, there was a marked decrease in admission rates into the Pediatric Inpatient department with an increase in PICU admission rates after the course. Triage level 2 patients, on the other hand, displayed higher rates of admission into the pediatric department with less admission into the PICU (Table 5).

Table 5: Patient disposition according to triage level

Triage level	Inpatient admission			ICU admission		
	Before course	After course	p value	Before course	After course N = 20306	p value
1	5/115 (4.3%)	4/273 (1.5%)	< 0.001	108/115 (93.9%)	260/273 (95.2%)	< 0.001
2	283/425 (66.6%)	850/934 (91.0%)	< 0.001	39/425 (9.2%)	72/934 (7.7%)	< 0.001
3	117/5771 (2.0%)	357/11378 (3.1%)	< 0.001	31/5771 (0.5%)	81/11378 (0.7%)	< 0.001
4	48/3557 (1.3%)	17/6396 (0.3%)	< 0.001	11/3557 (0.3%)	0/6396 (0%)	< 0.001
5	14/879 (1.6%)	9/1325 (0.7%)	< 0.001	0/879 (0.0%)	0/1325 (0.0%)	N/A

Discussion

Inspired by the importance of accurate triaging in Pediatric Emergency Department settings, we aimed in this study to enhance this using a triage course. This included a large number of patient encounter sheets, reaching 31053 after exclusion of approximately 6000 sheets for various reasons.

Our patients' characteristics were found to follow a normal distribution curve, which is expected. We found a mean age of 5.5 years, although El-Desoky et al., [9] who performed a study in a nearby hospital and found that the toddler age group had the highest number of patients. A possible explanation for this is the fact that our hospital had many sickle cell patients making up approximately 20% of our pediatric population (according to an internal census). Most of these patients were older children and adolescents, possibly pushing up the mean age in our study.

There was a significant improvement in accuracy with the training course. Of the 10747 patient sheets completed before the course, the accuracy rate was 56.5% and 78.3% jumping to 79.1% to 90.8% in the 20306 sheets after the course. The second set of sheets were included immediately after all staff were trained, and may, therefore, be falsely low as there was a learning curve with several discussions to provide feedback on incorrectly determined triage levels. Although we were hoping for higher rates, realistically, triage is a challenging task in the pediatric age group for many reasons. First of all, according to CTAS guidelines, heart and respiratory rates should be measured at rest [10]. However, the psychological stress of being in an unfamiliar environment makes this an almost impossible feat. Additionally, tachycardia and/or tachypnea may be normal physiological responses in febrile children rather than an indication of respiratory distress or hemodynamic instability [1].

In both pre-course and post-course groups, level 1 was the most easily identified, explained by the fact that patients requiring resuscitation are critically ill enough to be recognisable. Initially the least accurate was level 2 which was alarming as these may easily deteriorate if unattended to. After the course level 4 was the least recognised, which although incorrect, is expected to have less of an impact on morbidity and mortality.

The number and percentage of errors before and after the course decreased significantly, with those errors remaining being mostly down-triaging. This is contrary to findings in other studies where the main issue was over-triaging, such as with Chang et al., [12] who reported that abnormal vital signs let to over-triaging of pediatric patients, with a subsequent delay of more urgent patients. Many of the doctors and nurses at our institution realised that there was usually a tendency to up-triage and we have emphasised that excessive subjective modification of acuity level was discouraged by CTAS guidelines [6] unless it is applied with caution such as in a case of tachycardia in a crying child. We suspect that in our trials excessive down-triaging may have been due to underestimation of the patient's abnormal vital signs because of exhaustion or lack of experience.

An alternative method to assess the accuracy of triaging is how well it correlates with admission rates, which has been considered a surrogate marker for severity. Gravel et al., [13] performed a CTAS multicenter validation study and found a strong correlation between the triage level and various markers of severity, including admission rate. The admission rates reported in this study after the triage course correlate well with some of the pooled admission rates calculated after a multicenter study that included twelve Canadian Pediatric Emergency Departments [13]. The pooled rates reported by them were 61%, 30%, 10%, 2% and 0.9% for levels 1 – 5, respectively which especially agrees with our results for the lower triage levels [1], [3], [9], whilst triage levels 1 and 2 were within anticipated CTAS level admission rates of 70 – 90%, 40 – 70%, 20 – 40%, 10 – 20% and 0 – 10% respectively [10]. This contrasts with the admission rates reported according to the patient encounter sheets before the triage course, which emphasised the beneficial effect of the training the PEM staff received.

In conclusion, our assessment of the accuracy of triaging in this study was performed both via comparing assigned triage levels to that of two experienced pediatric emergency physicians, as well as by the admission rates according to assigned triage levels. There was marked improvement using both parameters. Limitations of this study were mainly that there was a high turnover of nursing staff, as well as over-crowding of the PED during the winter months, both of which may have led to falsely low accuracy. We recommend follow-up studies to assess waiting times after the training course and compare them with international standards [14].

Limitations of the study: This study was performed in an Emergency department with unique circumstances, which reduces its generalizability. We tried to maximise internal validity by having two independent physicians review the charts. Possible confounding factors were high turnover rates of nurses and a high prevalence of sickle cell disease patients, where a patient with fever would need immediate attention as per hospital policy. Repeated courses and review sessions were performed to adjust for the high turnover.

What is known: Using PaedCTAS for triaging in Emergency departments has been proven to prioritise care to critical patients and ensure optimal distribution of resources depending on the severity of illness and presentation. This is usually organised by a well-established Pediatric Emergency team with nurses well-trained in Emergency care and with expertise in pediatric care.

What this study adds: Our study assessed triaging in a newly established Pediatric Emergency Department (PED) with nurses who had little to no experience in recognising pediatric emergencies. A triage course performed by the PED team was found to be effective in that more accurate triage categorisation was performed. Our study also suggested that constant reinforcement and debriefs helps boost and maintain the learning curve.

References

1. Farrohknia N, Castrén M, Ehrenberg A, Lind L, Oredsson S, Jon-sson H, et al. Emergency department triage scales and their components: a systematic review of the scientific evidence. Scand J Trauma Resusc Emerg Med. 2011; 30:19-42. https://doi.org/10.1186/1757-7241-19-42 PMid:21718476 PMCid:PMC3150303

2. Kezirian J, Muhammad WT, Wan JY, Godambe SA, Pershad J. Cost analysis and provider satisfaction with pediatrician in triage. Pediatr Emerg Care. 2012; 28:971-6. https://doi.org/10.1097/PEC.0b013e31826c6dc4 PMid:23023460

3. Debono P, Debattista J, Attard-Montalto S, Pace D. Adequacy of pediatric triage. Disaster Med Public Health Prep. 2012; 6:151-4. https://doi.org/10.1001/dmp.2012.32a PMid:22700024

4. Nijman RG, Thompson M, van Veen M, et al. Derivation and validation of age andtemperature specific reference values and centile charts to predict lower respiratorytract infection in children with fever: prospective observational study. BMJ. 2012; 345:e4224. https://doi.org/10.1136/bmj.e4224 PMid:22761088 PMCid:PMC3388747

5. Thompson M, Harnden A, Perera R, et al. Deriving temperature and age appropriate heart rate centiles for children with acute infections. Arch Dis Child. 2009; 94:361-5. https://doi.org/10.1136/adc.2008.145011 PMid:19019883

6. Bullard MJ, Chan T, Brayman C, et al. Revisions to the Canadian Emergency Department Triage and Acuity Scale (CTAS) Guidelines. CJEM. 2014; 16:1-5. https://doi.org/10.1017/S148180350000350X

7. eiger N, van Veen M, Almeida H, Steyerberg EW, van Meurs AH, Carneiro R, et al. Improving the manchester triage system for pediatric emergency care: An international multicenter study. PLoS One. 2014; 9:e83267. https://doi.org/10.1371/journal.pone.0083267 PMid:24454699 PMCid:PMC3893080

8. Tsai VW, Sharieff GQ, Kanegaye JT, Carlson LA, Harley J. Rapid medical assessment: Improving pediatric emergency department time to provider, length of stay, and left without being seen rates. Pediatr Emerg Care. 2012; 28:354-6. https://doi.org/10.1097/PEC.0b013e31824d9d27 PMid:22453731

9. El Desoky S, Mashat S, Bana S, Alama M, Dhabab N, Malibari GM, Halwani M, Albanna AS, Kari JA. Efficiency of Using Pediatrics Emergency Services and Triage Evaluation. Pediatric emergency care. 2018; 34(6):417-21. https://doi.org/10.1097/PEC.0000000000000754

10. Warren D, Jarvis A, LeBlanc L. Canadian Paediatric Triage and Acuity Scale:implementation Guidelines for Emergency Departments. CJEM. 2001; 3(Suppl 4):S1-27.

11. Fleming S, Thompson M, Stevens R, et al. Normal ranges of heart rate and respiratory rate in children from birth to 18 years of age: a systematic review ofobservational studies. The Lancet. 2011; 377:1011-18. https://doi.org/10.1016/S0140-6736(10)62226-X

12. Chang YC, Ng CJ, Wu CT, et al. Effectiveness of a five-level Paediatric TriageSystem: an analysis of resource utilisation in the emergency department in Taiwan.Emerg Med J. 2013; 30:735-9. https://doi.org/10.1136/emermed-2012-201362 PMid:22983978 PMCid:PMC3756519

13. Gravel J, Fitzpatrick E, Gouin S, et al. Performance of the Canadian Triage andAcuity Scale for children: a multicenter database study. Ann Emerg Med. 2013; 61:27-32.e3. https://doi.org/10.1016/j.annemergmed.2012.05.024 PMid:22841173

14. Partovi SN, Nelson BK, Bryan ED, Walsh MJ. Faculty triage shortens emergency department length of stay. Acad Emerg Med. 2001; 8:990-5. https://doi.org/10.1111/j.1553-2712.2001.tb01099.x PMid:11581086

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Inter - Relationship of Awareness, Knowledge, Attitude, Some Socio-Economic Variables and Osteoporosis in Sample of Egyptian Women

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Abstract

BACKGROUND: Osteoporosis is a global health problem, and its prevalence is rapidly increasing worldwide.

AIM: The aim was to assess the awareness concerning some nutritional and socio-economic variables causes the disease in a sample of Egyptian women.

METHODS: This study was done among 116 female volunteers. They were divided into two groups, pre and post-menopausal, with a mean age of 42.05 ± 8.25 & 51.13 ± 5.82 years and mean body mass index (BMI) of 30.83 ± 8.18 & 34.24 ± 8.80 kg/m^2. A standardised questionnaire, socioeconomic and food frequency chart were used to assess osteoporosis and food intake awareness. Bone mineral density was measured by dual-energy X-ray absorptiometry (DEXA). Statistical analyses were done using simple percentage and Chi-square test.

RESULTS: Data revealed that a low percentage of pre and post-menopausal women were aware of osteoporosis and fracture (16.67% & 12.96% and 30.65% & 19.35%). They had incomplete knowledge about the sources and the beneficial effects of consumption of calcium and vitamin D rich diet. Non-osteoporotic women showed more awareness. Odds ratio predict occupations and educations levels as risk factors for osteoporosis.

CONCLUSION: Awareness about osteoporosis and consumption healthy diet were low among Egyptian women, so it is important to implement special osteoporosis prevention program.

Introduction

The International Osteoporosis Foundation reported that osteoporosis is a serious disease in the Middle East [1]. Because of the overwhelming impacts of osteoporosis, International Osteoporosis Foundation (2011) creates preventive strategies which should be executed to lessen the risk of osteoporotic fractures later on. Three stages to unbreakable bones" is prescribed by the International Osteoporosis Foundation which pieces of advice remaining active, eating regimen rich in calcium, and avoid vitamin D inadequacy to improve bone and muscle health and decrease the danger of osteoporosis [2].

Boonen and his colleagues (2004) reported that both calcium and vitamin D are essential nutrients of an integrated strategy for the prevention and treatment of osteoporosis in patients with dietary insufficiency [3]. Regardless of the increase in its significance, osteoporosis is broadly perceived as a preventable and treatable illness; along these lines, a suitable identification and the board framework that incorporates a way of life changes may minimise the burden on public health resources worldwide. Recommendation rules prescribe discontinuance of tobacco use, avoidance of excessive alcohol intake investment in standard exercise, and a satisfactory intake of calcium and vitamin D for keeping up bone health [4]. However, several studies have demonstrated that osteoporosis patients don't pursue

the suggested clinical rules after the diagnosis of illness [5].

Moreira et al. found that physical exercise is an essential stimulus for the prevention and treatment of osteoporosis. In any case, it is not clear yet which methodology would be better to activate bone metabolism and upgrade the physical capacity of postmenopausal women [6].

Du and his colleagues suggested a conclusive positive or negative association between bone mineral density (BMD) and socioeconomic status (SES) proved to be difficult. However, individuals who are at an extreme SES are the most vulnerable group to have relatively low BMD [7]. The importance of education for osteoporosis has been confirmed by several studies. Okumus et al. found that both pre-and postmenopausal women that obtained a higher level of education exhibited better information about osteoporosis dependent on their awareness questionnaire score [8].

The objective of this study was to identify and to evaluate the risk factors of osteoporosis in the studied sample, and to assess their knowledge level concerning some nutritional and socio-economic variables causes the disease.

Methods

The sample size was calculated based on the previous study regarding the association between osteoporosis and menopausal state, 53 subjects in each group were adequate, assuming α = 0.05, B = 0.04 and power of 95.2%.

This study was done amongst 116 female volunteers. The exclusion criteria; any critical health problems as cardiovascular, hepatic or renal diseases, diabetes mellitus, as well any diseases that could affect bone health (thyroid, parathyroid, adrenal).

They were divided into 2 groups, 54 women were pre-menopausal, and 62 women were post-menopausal regarding (menopausal state), with a mean age of 42.05 ± 8.25 & 51.13 ± 5.82 years and mean body mass index (BMI) of 30.83 ± 8.18 & 34.24 ± 8.80 kg/m^2. Women were considered to be postmenopausal if they were ≥ 55 years or reported not having had a menstrual period during the past 12 months. Institutional ethics committee approval was taken (number 16 / 127) through a project titled " Bone mass among Overweight and Obese Women: Mechanism and Intervention". Besides, informed written consent was obtained from each participant to be included in the study. Data collected in "Management of visceral obesity and growth disturbance unit" in the Medical Research Centre of Excellence (MRCE) – National Research Centre.

A standardised questionnaire regarding the perception of personal risk of osteoporosis and fractures was used. Socioeconomic data were collected, the frequency chart was used to assess their awareness about nutrients intake especially food rich in calcium and vitamin D in addition to physical activity evaluation. The data for this study was collected by the direct method with the help of a self-prepared and validated questionnaire under the supervision of a dietary consultant. Education level was evaluated by questionnaire commonly divided into four stages: illiteracy, primary school, secondary and university level. Occupation types also divided into four groups: not working, workers, employee and professional and owner of private business.

Each group divided into three groups: normal, osteopenia, and osteoporosis according to their bone health status. BMD (in grams per square centimetre) was measured in the total hip (trochanter, Ward's area and femoral neck) and lumbar spine (L2 – L4) by dual-energy X-ray absorptiometry (DEXA) with (NorlandXr-46, with host software version: 3.9.6 / 2.3.1., USA). The instruments were calibrated daily according to the manufacturer's instructions. Osteoporosis is established by measurement of BMD of the hip and spine using the T-score which was calculated using the following formula:

T score = (measured bone density-maximum bone density) / the maximum standard deviation

T-score ≥ -1.0 were grouped as normal, T-score < -1.0 to - < 2.5 were put in the osteopenia, and T-score < -2.5 were categorised as having osteoporosis following the diagnostic criteria established by the World Health Organization (WHO, 2003) in adults [9].

Anthropometric parameters**:** Relevant anthropometric measurements were recorded, including height and weight using standard methods following the recommendations of the International Biological Program [10].

Statistical analyses were carried out with SPSS version 22 software, using simple percentage and Chi-square test of significance and alpha level set at p = 0.05.

Results

Table 1 shows the mean ± SD of age, weight, height and BMI of pre and post-menopausal women. The post-menopausal women were heavier but shorter. However, both of them were obese as their BMI was 30.83 ± 8.18 and 34.24 ± 8.80, respectively.

Table 1: Mean ± SD of age, weight, height and BMI of pre and post-menopausal women

Parameters	Pre-menopausal No. (54)	Post-menopausal No. (62)
	Mean ± S D	Mean ± S D
Age (Year)	42.05 ± 8.25	51.13 ± 5.82
Weight (kg)	76.96 ± 2.27	80.15 ± 1.90
Height (m)	1.58 ± 5.59	1.53 ± 6.73
Body Mass Index (kg/ht^2)	30.83 ± 8.18	34.24 ± 8.80

Table 2 shows the distribution of pre and postmenopausal women according to their awareness of osteoporosis and fracture. Out of 54 pre-menopausal women, 16.67%had heard about osteoporosis while and 12.96% about the fracture, while 30.65% among the post-menopausal had heard about osteoporosis and 19.35% about the fracture.

Table 2: Distribution of pre and postmenopausal women according to their awareness of osteoporosis and fracture

Parameters	Pre-menopausal No. (54)		Post-menopausal No. (62)	
	No.	%	No.	%
Risk perception of osteoporosis				
No	45	83.33	43	69.35
Yes	9	16.67	19	30.65
Fracture				
No	47	87.04	50	80.65
Yes	7	12.96	12	19.35

Table 3 shows a significant difference between the percent of the frequent consumption of different food items for pre and post-menopausal women. The higher percent frequency consumption of some of the common carbohydrate foods was range from 36.5- 39.1% at the period twice / week for the pre and postmenopausal women respectively. For the milk and dairy products, the most common items consumed were cheese, where 60.4% of the premenopausal women consumed cheese every day.

Table 3: The percent of the frequency consumption (%) of different food items for pre and post-menopausal women

Items	Pre-menopausal No. (54)					Post-menopausal No. (62)					
	Didn't eat	Every day	Twice / week	Every / week	More	Didn't eat	Every day	Twice / week	Every / week	More	P-Value
Carbohydrate Foods											
Bread	0.0	38.1	31.1	30.8	0.0	0.0	50.2	37.4	12.5	0.0	
Bakery products	9.5	57.1	33.4	0.0	0.0	6.2	60.3	25.4	8.1	0.0	
Pasta	2.1	2.5	45.1	50.2	0.0	1.4	3.2	54.2	41.5	0.0	
Total	3.7	32.6	36.5	27.2	0.0	2.5	37.9	39.1	20.7	0.0	0.010*
Milk & Milk Products											
Milk	12.4	35.7	28.3	18.7	4.9	16.2	26.4	24.8	27.4	5.2	
Cheese	14.3	60.4	10.2	5.1	10.0	15.9	32.7	21.4	18.8	11.2	
Yoghurt	19.0	11.5	12.4	42.6	14.5	27.1	5.8	11.7	25.1	30.3	
Total	15.3	35.9	16.9	22.1	9.8	18.7	21.6	19.3	23.8	16.6	0.301
Animal protein foods											
Chicken	1.3	2.1	41.3	52.1	3.2	1.1	3.4	54.2	39.5	1.8	
Meat	4.1	0.0	21.4	43.2	31.3	6.3	0.0	49.7	38.4	5.6	
Fish	4.8	0.0	35.2	46.2	13.8	6.2	0.0	30.1	40.9	22.8	
Egg	5.6	54.8	23.8	10.3	5.5	4.1	22.9	18.8	36.4	17.8	
Total	3.9	14.3	30.4	37.9	13.5	4.4	6.5	38.2	38.8	12.1	0.092
Plant protein food											
Legumes	1.3	56.1	23.6	16.0	3.0	2.3	50.2	24.1	15.3	8.1	0.690
Fresh& Cooked Vegetables											
Fresh	8.6	14.5	32.4	28.6	15.9	10.4	9.8	30.6	38.7	10.5	
Cooked	0.0	21.9	36.2	33.6	8.3	0.0	11.3	28.4	41.1	19.2	
Total	4.3	18.3	34.1	31.2	12.1	5.1	6.3	31.5	42.4	14.7	0.004**
Fruits											
Fresh Fruits	2.2	19.3	40.6	23.8	14.1	2.1	11.5	32.4	36.7	17.3	
Fruit Juices	9.5	11.9	28.6	33.3	16.7	14.9	5.2	22.1	33.4	24.4	
Total	5.9	15.7	34.6	28.5	15.3	8.3	8.6	27.3	35.1	20.7	0.015*
Sweet											
Sweet	4.8	18.4	36.5	29.1	11.2	3.6	21.4	28.7	32.1	14.2	0.000**
Beverages											
Tea	3.7	48.4	29.2	11.6	7.1	1.2	56.1	30.7	8.6	3.4	
Carbonated drink	2.6	12.3	40.1	42.6	2.4	1.3	14.8	43.5	38.7	1.7	
Total	3.4	30.1	34.6	27.1	4.8	1.1	36.1	37.1	23.4	2.3	0.499

*Significant at P≤ 0.05; **Highly Significant at P ≤ 0.001.

However, 9.8% and 16.6% of both groups had a very low rate of consumption. Egg consumption reported a high rate of frequency consumption among the premenopausal women (54.8% every day), while chicken consumption represents animal protein reported 52.1 and 54.2% / week, legumes consumption as a plant protein food was 56.1- 50.2 / day for both groups. Vegetables and fruits showed the high percent frequency of consumption at range from 34.1 and 34.6% twice / week for the premenopausal women and 42.3 and 35.1% every week for the postmenopausal women. Beverages represent by tea and carbonate carbohydrate, about half of the women of both groups drunken tea every day (48.4, 56.1%), while carbonate beverage consumed at the rate 42.6% every week for the premenopausal and 43.5% twice/week for the postmenopausal women.

Table 4 shows significant differences between the percent distribution of pre and post-menopausal women' according to their attitude and health awareness for improving their bone health. The women awareness in both groups about the consumption of milk, milk products and leafy vegetables was very low especially the last item where the percent under definitely yes was ranged from 0 to 2.38%. Animal protein foods including meat, chicken and fish also showed a low degree of awareness that ranges from 7.14 to 27.09, while that for the egg was zero percent in both groups. Awareness about the harmful effect of the carbonate beverages between the studied two groups was ranging from 14.29 to 37.50%. The beneficial effect of the physical exercise was achieved by 56.29 & 38.50% of the two groups respectively.

Table 4: Percent distribution of pre and post-menopausal women' according to their attitude and health awareness for improving their bone health

Questions	Answers				
	No %	Probably No %	Probably yes %	Definitely yes %	P-Value
Total No: (116): Pre-menopausal: (54), Post-menopausal: (62)					
Are drinking milk and eating dairy products improve your bone health?					
Pre-menopausal	7.14	26.19	40.48	26.19	0.068
Post-menopausal	27.10	39.58	25.00	8.33	
Are milk and dairy products contain a lot of Vitamin D & Calcium?					
Pre-menopausal	9.52	28.57	42.86	19.05	0.055*
Post-menopausal	25.00	39.58	29.17	6.25	
Are eating leafy vegetables improve your bone health?					
Pre-menopausal	26.19	45.24	26.19	2.38	0.003**
Post-menopausal	52.08	31.25	16.67	0.00	
Are leafy vegetables contain a lot of Vitamin D& Calcium?					
Pre-menopausal	30.95	50.00	19.05	0.00	0.000**
Post-menopausal	72.92	18.75	8.33	0.00	
Are eating meat & liver to improve your health?					
Pre-menopausal	2.38	45.24	33.33	19.05	0.281
Post-menopausal	6.25	37.50	39.58	16.67	
Does our meats & liver contain a lot of Vitamin D?					
Pre-menopausal	21.43	38.10	33.33	7.14	0.568
Post-menopausal	10.42	20.82	41.67	27.09	
Is eating egg improve your health?					
Pre-menopausal	23.81	42.86	33.33	0.00	0.000**
Post-menopausal	39.58	45.83	14.59	0.00	
Is eating Baladi bread to improve your health?					
Pre-menopausal	4.76	28.57	42.86	23.81	0.010*
Post-menopausal	25.00	18.88	35.57	20.55	
Are drinking a lot of Beverages harm your health?					
Pre-menopausal	14.29	23.80	42.86	19.05	0.499
Post-menopausal	37.50	41.67	14.58	6.25	
Is physical exercise improve bone health?					
Pre-menopausal	3.14	19..30	21.27	56.29	0.013*
Post-menopausal	10.27	22.84	28.69	38.20	

*Significant at P ≤ 0.05; **Highly Significant at P ≤ 0.001.

Table 5 shows the attitude and awareness about osteoporotic protective foods, calcium intake and physical activity among women according to their education levels and occupations. Data proved that education was associated with higher awareness. The awareness among the illiterate premenopausal

women about the beneficial effects of the five items was ranging from 4.2 to 10.3%, while for higher education, the percent was 37.2 to 43.4%. The same results were found among the post-menopausal women, where the percent was ranging from 3.5 to 14.9% and 28.4 to 49.7% for the two educational levels respectively. The association between occupation and osteoporosis awareness was also highest among the employee and the professional jobs followed by those who had private business compared to others who did not work or work as workers in both groups.

Table 5: Attitude and awareness of osteoporosis among studied women according to their Education and occupations

Awareness items			Drinking milk & Eating Dairy Products %	Eating leafy vegetables %	Eating Fish %	Taking Calcium %	Doing physical activity %
Education Levels:Total No: (116):Pre-menopausal: (54), Post-menopausal: (62)							
Illiteracy No:8 (6.9%)	No:3 (5.6%)	Pre- menopausal	8.7%	7.9% **	5.1%	10.3%**	4.2%**
	No:5 (8.1%)	Post-menopausal	5.7%	4.6%**	4.2%	14.9%**	3.5%**
Primary No:12 (10.3%)	No:5 (9.2%)	Pre- menopausal	20. 6%	19.7%**	22.3%*	24.6%*	18.2%
	No:7 (11.3%)	Post-menopausal	18.3%	15.3%**	13.6%*	29.8%*	17.9%
Secondary No:44 (37.9%)	No:20 (37.1%)	Pre- menopausal	31.9%**	33.5%*	32.2%**	27.9%	34.2%**
	No:24 (38.7%)	Post-menopausal	29.9%**	31.2%*	32.5%**	26.9%	33.9%**
University No:52 (44.8%)	No:26 (48.1%)	Pre- menopausal	38.8%*	38.9%**	40.4%**	37.2%	43.4%**
	No:26 (41.9%)	Post-menopausal	46.1%*	48.9%**	49.7%**	28.4%	44.7%**
Type of Occupations							
Not working No:31 (26.7%)	No:9 (16.7%)	Pre- menopausal	11.5%*	4.2%**	5.7%*	18.5%*	7.5%**
	No:22 (35.5%)	Post-menopausal	9.1%*	3.4%**	4.7%*	17.3%*	7.2%**
Workers No:7 (6.0%)	No:3 (5.6%)	Pre- menopausal	20.2%	6.4%*	8.5%*	17.6%	9.7%*
	No:4 (6.5%)	Post-menopausal	18.7%	5.7%*	6.9%*	16.8%	8.9%*
Employee & Professional No:50 (43.1%)	No:24 (44.4%)	Pre- menopausal	49.4%	62.0%**	62.1%**	47.1%	54.4%**
	No:26 (41.9%)	Post-menopausal	48.1%	60.5%**	58.5%**	44.5%	55.8%**
Owner of private Business No:28 (24.1%)	No:18 (33.3%)	Pre- menopausal	18.9%**	27.4%**	23.7%**	16.7%	28.4%**
	No:10 (16.1%)	Post-menopausal	24.1%**	30.4%**	27.9%**	21.4%	28.1%**

*Significant at P ≤ 0.05; **Highly Significant at P ≤ 0.001.

Table 6 shows the association between awareness, behaviour, education and occupations with the bone density of the lumbar spine and femur;

Regarding the lumbar spine, there was a statistically significant association with an awareness of eating leafy vegetables and doing physical activity (p = 0.018 and 0.014), as the majority of participants (67.8% and 73.6%) respectively had an osteopenic spine.

Also, there was a statistically significant association between femur density and awareness of eating fish (p = 0.053), as 24.2% of participants had normal bone density while 59.7% had an osteopenic femoral neck. The behaviour of taking calcium was statistically significant (p = 0.019), as 41.2% of participants had osteoporotic and 47.1% had an osteopenic femoral neck.

Regarding education, there was statistically significant association (p = 0.018 and 0.007) with lumbar spine and femur respectively as, the incidence of osteoporotic bone density was increased (36.5% at the spine and 40.4% at the femur) with higher educational level (university), moreover most of the participated women had osteopenic bone regarding to level of education.

Table 6: Association between awareness, behaviour, education and occupations with the bone density of the spine & femur

Bone status (Anatomical sites)		Normal bone density No. (%)		Osteopenia No. (%)		Osteoporosis No. (%)		P -Value	
		Spine 16 (13.8%)	Femur 27 (23.3%)	Spine 74 (63.8%)	Femur 60 (51.7%)	Spine 26 (22.4%)	Femur 29 (25%)	Spine	Femur
Awareness and Behavior: Total No (116)									
Eating dairy products	No: 50 (43.1%)	5 (10%)	12 (24%)	33 (66%)	26 (52.0%)	12 (24%)	12 (24.0%)	0.582	0.972
Eating leafy vegetables	No:59 (50.9%)	3 (5.1%)	10 (16.9%)	40 (67.8%)	30 (50.8%)	16 (27.1%)	19 (32.2%)	0.018*	0.103
Eating Fish	No:62 (53.5%)	8 (12.9%)	15 (24.2%)	44 (71.0%)	37 (59.7%)	10 (16.1%)	10 (16.1%)	0.174	0.053*
Taking Calcium	No:39 (33.6%)	2 (5.9%)	4 (11.8%)	21 (61.8%)	16 (47.1%)	11 (32.4%)	14 (41.2%)	0.115	0.019*
Doing physical activity	No:53 (45.7%)	2 (3.8%)	9 (17.0%)	39 (73.6%)	31 (58.5%)	12 (22.6%)	13 (24.5%)	0.014*	0.282
Education									
Illiteracy	No:8 (6.9%)	0 (0%)	0 (0%)	8 (100%)	7 (87.5%)	0 (0%)	1 (12.5%)	0.018*	0.007**
Primary	No:12 (10.3%)	2 (16.7%)	5 (38.5%)	10 (83.3%)	8 (61.5%)	0 (0%)	0 (0%)		
Secondary	No:44 (37.9%)	7 (15.9%)	12 (27.9%)	30 (68.2%)	24 (55.8%)	7 (15.9%)	7 (16.3%)		
University	No:52 (44.8%)	7 (13.5%)	10 (19.2%)	26 (50.0%)	21 (40.4%)	19 (36.5%)	21 (40.4%)		
Occupation									
Not working	No:31 (26.7%)	7 (22.6%)	9 (29.0%)	23 (74.2%)	21 (67.7%)	1 (3.2%)	1 (3.2%)	0.017*	0.001**
Workers	No:7 (6.0%)	0 (0%)	0 (0%)	5 (71.4%)	4 (57.1%)	2 (28.6%)	3 (42.9%)		
Employee and Professional	No:50 (43.1%)	6 (12.0%)	11 (22.0%)	33 (66.0%)	28 (56.0%)	11 (22.0%)	11 (22.0%)		
Owner of private Business	No:28 (24.1%)	3 (10.7%)	7 (25.0%)	13 (46.4%)	7 (25.0%)	12 (42.9%)	14 (50.0%)		

*Significant at P ≤ 0.05; **Highly Significant at P ≤ 0.001.

Occupations was highly statistically significant with bone density (p = 0.017 and 0.001 for spine and femur respectively), as the highest percentage of osteoporotic bone was detecting at who had private business (42.9% at the spine and 50% for the femur), while the manual worker; (71.4% &57.1%) were osteopenic and (28.6% & 42.9%) were osteoporotic for spine and femur respectively.

Finally, the odds ratio was done to predict risk factors for osteoporosis at the lumbar spine and femur; Table 7 revealed that there was a statistically significant difference with occupations and educations which could be detected by the 95% confidence interval (CI) and p-value (≤ 0.05-0.01).

Table 7: Odds ratio to predict risk factors for osteoporosis at the lumbar spine and femur

		Odd Value	95% Confidence Interval	P-Value
Occupation	Spine	12.5	1.62-96.74	0.003**
	Femur	16.4	2.13-126.03	0.001**
Education	Spine	1.5	1.196- 1.497	0.040*
	Femur	4.93	0.61- 39.63	0.100

*Significant at P ≤ 0.05; **Highly Significant at P ≤ 0.001.

Discussion

Osteoporosis is a global medical problem, and its prevalence is quickly expanding around the world. In Egypt; osteopenia prevalence (53.9%) and osteoporosis prevalence (28.4%) [22]. Misunderstanding about osteoporosis and the absence of preventive activity among women are as yet not change, making primary avoidance at an early

age the best mediation [11]. As a result of hormonal changes or deficiency of calcium or vitamin, bones become weak and fragile due to loss of tissue. Besides, after menopause, due to a decrease in estrogen levels in the women body bone loss increases [12].

Data of this study revealed the low osteoporosis awareness of the pre and postmenopausal women about the term osteoporosis and fracture; yet data showed that the postmenopausal women were more aware of the risk of the disease, as 30.65% & 19.35% of them had heard about osteoporosis and fracture compared to 16.67% & 12.96% of the pre-menopausal women. In this context; Njeze Ngozi et al. stated that increased prevalence of osteoporosis disease is attributed to low awareness of the disease among the general population referred to as a 'silent disease' [13].

Even though the genetic factor is the important determinant of bone density, however, lifestyle and environmental factors like sedentary life, low intake of calcium and vitamin D and smoking are all other hazard variables of osteoporosis [14]. Knowledge concerning healthy foods was determined by responses to the specific questions; in this study, we had asked questions related to predicting factors of osteoporosis. Including; important components of building bones, to access the frequency of consumption and the awareness of the subjects. Data revealed that women had incomplete knowledge about the sources and the healthy effect of these protective foods. Low percent of the pre and post-menopausal women knowing the beneficial effects of milk and milk products and confirmed the information that milk and dairy products are good sources of calcium and vitamin D (definitely yes). The same results were obtained with other food items that were included. Fujii and his colleagues studied the everyday consumption of green and yellow vegetables and its impact on keeping up bone mass in young women [15]. They found that bone density is linked with lifestyle factors including good nutrition, in the light of the fact that vegetables and fruits, are rich in the nutrient vitamin C and their daily intake had a favourable effect to bone metabolism.

Our data revealed that moderate percent of premenopausal women had knowledge about the healthy effect of increasing activity and exercise on osteoporosis compared to the low percentage of postmenopausal women. It is found that sedentary life which neglects physical exercise and unloading the skeleton leading to decreased bone mass, whereas mechanical loading through exercise increases bone mineral density [16]. Muscle mass is important fact determined and measure the muscle-strengthening exercise which is a defensive factor against bone fracture. A few investigations have announced a positive connection between ‘lean body mass and BMD, which suggested that the muscle applies mechanical burden powers on the bone and is subsequently a metabolically dynamic organ influencing bone health [17], [18].

Alternatively, related awareness to bone density status showed that moderate percent including non- osteoporotic women (normal bone density and osteopenic) were more aware of the importance of consumption of healthy food items which protect against osteoporosis like leafy vegetables and fish, and also about the beneficial effect of physical activity. Also, the knowledge of taking calcium supplement was high for participants had osteopenia which could be explained that they might use calcium as a medication to elevated bone density.

To identify the effect of the socioeconomic status on the women awareness and the bone density status, the educational situation and the type of job were selected. Odds ratio that predicts risk factors for osteoporosis at the lumbar spine and femur revealed that there was a statistically significant difference with occupations and educations which could be detected by the 95% confidence interval (CI) and p-value (≤ 0.05-0.01).

Current findings indicated that education levels were significantly associated with the knowledge about the importance of consuming healthy food items influencing bone health and protect against osteoporosis. Specifically, awareness was observed among the higher percent of both pre and post-menopausal women who had attained a high education level (p-value < 0.05), compared to other levels. On the contrary, the association between education levels and bone density showed a higher incidence of osteoporotic change with a higher educational level (p = 0.018 and 0.007). In agreement with this result Kim et al., have demonstrated that education level was only significantly associated with osteoporosis in men, after adjusting for age, sex, and health behaviours, while the relationship was inverted with women [19]. Other studies demonstrated that there were complicated relationships among the levels of attitudes, knowledge, osteoporosis behaviours and educational level [20]. Since osteoporosis is multifactorial diseases, as hormonal levels, genetic and other causes which might play an important role in the onset of the disease that could explain the previous information obtained [14].

In this study, the association of awareness and different types of occupations among the pre and post-menopausal women was in favour of those holding professional jobs. Also, data concerning the association between types of occupations with bone density revealed a high statistically significant difference between the different groups. The highest percentage of osteoporotic state was detected among women who had a private business. This may attribute to the nature of their work which does not need much physical activity. Njeze Ngozi et al. showed that osteoporosis awareness was significantly

associated with age and occupation but not gender, marital status, and level of education [13].

In conclusion, data of this study revealed the low osteoporosis awareness of the pre and post-menopausal Egyptian women about the term osteoporosis and fracture. Also, a high percent of them was not aware of the healthy effect of consumption of the osteoporosis protective foods rich in calcium, and vitamin D. Moderate percent of them had awareness about the beneficial effect of physical activity. The results indicated that the socioeconomic status like education levels and the type of work were considered as risk factors for osteoporosis. This is why it is important to work in different ways on a special osteoporosis prevention program helping women to maintain good bone health.

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Author contribution

Prof. Dr Nayera E. Hassan is the Principal investigator (P.I.), designed the project and the study as well revised every step of the project and gave conceptual advice; Prof. Dr Salwa M. El Shebini is the nutritional consultant and wrote the manuscript; Dr Nihad H. Ahmed collected nutritional data and performed the statistical analysis; Dr Safenaz Y. El Sherity performed the anthropology measurements, DEXA Scan and shared at the statistical analysis; Prof. Dr Sahar A. El-Masry and Prof. Dr Enas R. Abdel Hamed gave their conceptual advice; Dr Heba T. Aboud collected the nutritional data from participates and taken anthropology measurements. All authors read and approved the final manuscript.

References

1. Fuleihan G, Adib G, Nauroy L. The Middle East and Africa regional audit, epidemiology, costs and burden of osteoporosis in 2011. International Osteoporosis Foundation. 2011; 102011-5000.

2. International Osteoporosis Foundation: Three steps to unbreakable bones Available from: http://www.iofbonehealth.org/bonehealth/three-steps-unbreakable-bones-world-osteoporosis-dayAccessed October 13, 2011.

3. Boonen S, Rizzoli R, Meunier PJ, et al. The need for clinical guidance in the use of calcium and vitamin D in the management of osteoporosis: a consensus report Osteoporos. Int. 2004; 15(7):511-519. https://doi.org/10.1007/s00198-004-1621-6 PMid:15069595

4. Greenspan SL, Bilezikian JP, Watts NB, Berry CA, Mencia WA, Stowell SA, Karcher RB.A clinician performance initiative to improve quality of care for patients with osteoporosis J Women's Health. 2013; 22(10):853-861. https://doi.org/10.1089/jwh.2013.4388 PMid:24011023 PMCid:PMC3837565

5. Patel A, Coates PS, Nelson JB, Trump DL, Resnick NM, Greenspan SL. Does bone mineral density and knowledge influence health-related behaviors of elderly men at risk for osteoporosis? J Clin Densitom. 2003; 6(4):323-330. https://doi.org/10.1385/JCD:6:4:323

6. Moreira LD, Oliveira ML, Lirani-Galvão AP, Marin-Mio RV, Santos RN, Lazaretti-Castro M. Physical exercise and osteoporosis: effects of different types of exercises on bone and physical function of postmenopausal women. Arq Bras EndocrinolMetabol. 2014; 58(5):514-22. https://doi.org/10.1590/0004-2730000003374 PMid:25166042

7. Du Y, Zhao LJ, Xu Q, Wu KH, Deng HW. Socioeconomic status and bone mineral density in adults by race/ethnicity and gender: the Louisiana osteoporosis study. Osteoporos Int. 2017; 28(5):1699-1275. https://doi.org/10.1007/s00198-017-3951-1 PMid:28236128

8. Okumus M, Ceceli E, Tasbas O, Kocaoglu S, Akdogan S, Borman P. Educational status and knowledge level of pre- and postmenopausal women about osteoporosis and risk factors: a cross-sectional study in a group of Turkish female subjects. J Back Musculoskelet Rehabil. 2013; 26(3):337-343. https://doi.org/10.3233/BMR-130389 PMid:23893150

9. World Health Organ. Prevention and Management of Osteoporosis. Tech Rep Ser. 2003; 921:1-164.

10. Tanner JM, Hiernau J, Jerman S. Growth and physical studies. In: Human Biology: A guide to field methods. Eds. Weiner J.S., Lourie S.A., IBP. London, Blackwell Scientific Publications. Oxford. U.K, 1969.

11. Al-Muraikhi H, Said H, Selim N, Chehab MA. The knowledge of osteoporosis risk factors and preventive practices among women of reproductive age in the state of Qatar: a cross-sectional survey. Home. 2017; 4(2):522-527. https://doi.org/10.18203/2394-6040.ijcmph20170284

12. Dabholkar T, Doctor A, Dabholkar A. Awareness of Osteoporosis in the Parsi Community. Journal Of Humanities And Social Science. 2017; 22(7): 27-29.

13. Njeze Ngozi R, Ikechukwu O, Miriam A, Olanike AU, AkpagbulaUlugo D, NjezeNneze C. Awareness of osteoporosis in a polytechnic in Enugu, South East Nigeria.Arch Osteoporos. 2017; 12(1):51. https://doi.org/10.1007/s11657-017-0342-3 PMid:28540650

14. Ralston SH. Genetics of osteoporosis. Ann N Y Acad Sci. 2011; 1192:181-189. https://doi.org/10.1111/j.1749-6632.2009.05317.x PMid:20392235

15. Fujii H, Noda T, Sairenchi T, Muto T. Daily Intake of Green and Yellow Vegetables Is Effective for Maintaining Bone Mass in Young Women. The Tohoku journal of experimental medicine. 2009; 218(2):149-154. https://doi.org/10.1620/tjem.218.149 PMid:19478471

16. Howe TE, Shea B, Dawson LJ, Downie F, Murray A, Ross C, Harbour RT, Caldwell LM, Creed G Exercise for preventing and treating osteoporosis in postmenopausal women. Cochrane Database Syst Rev. 2011; 6(7). https://doi.org/10.1002/14651858.CD000333.pub2

17. Visser M, Kiel DP, Langlois J, Hannan MT, Felson DT, Wilson PW, Harris TB. Muscle mass and fat mass in relation to bone mineral density in very old men and women: the Framingham Heart Study. Appl Radiat Isot. 1998; 49(5):745-747. https://doi.org/10.1016/S0969-8043(97)00101-2

18. Shin D, Kim S, Kim KH, Park SM. Importance of fat mass and lean mass on bone health in men: the Fourth Korean National

Health and Nutrition Examination Survey (KNHANES IV) Osteoporos Int. 2014; 25(2):467-474. https://doi.org/10.1007/s00198-013-2412-8 PMid:23779082

19. Kim J, Lee J, Shin J-Y, Park B-J. Socioeconomic disparities in osteoporosis prevalence: different results in the overall Korean adult population and single-person households. J Prev Med Public Health. 2015; 48 (2):84-93. https://doi.org/10.3961/jpmph.14.047 PMid:25857646 PMCid:PMC4398150

20. Puttapitakpong P, Chaikittisilpa S, Panyakhamlerd K, Nimnuan C, Jaisamrarn U, et al. Inter-correlation of knowledge, attitude, and osteoporosis preventive behaviors in women around the age of peak bone mass. BMC Women's Health. 2014; 14:35. https://doi.org/10.1186/1472-6874-14-35 PMid:24588970 PMCid:PMC3942254

21. Doventas A, Bolayirli IM, Incir S, Yavuzer H, Civelek S, Erdincler S, Konukoglu D, Beger T, Seven A. Interrelationships between obesity and bone markers in postmenopausal women with either obesity or osteoporosis. European Geriatric Medicine. 2015; 6(1):15-20. https://doi.org/10.1016/j.eurger.2014.06.033

22. El-Tawab S, Saba E, Elweshahi H, Ashry M. Knowledge of osteoporosis among women in Alexandria (Egypt): A community based survey. The Egyptian Rheumatologist. 2016; 38:225-231. https://doi.org/10.1016/j.ejr.2015.08.001

ID Design Press, Skopje, Republic of Macedonia
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Public Health

Consumption of Anti-Epileptic Drugs in Primary Health Care in Albania, 2004-2016

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Abstract

BACKGROUND: Epilepsy is a serious neurological condition requiring sometimes lifelong pharmacological treatment, and continuous specialist monitoring.

AIM: To investigate the use of Anti-Epileptic Drugs (AEDs) in epilepsy, with focus on the exposure of AEDs, differences and changes in prescription patterns over time; to evaluate the relation between the consumption data of AED and the level of epileptic morbidity for the period 2004-2016.

STUDY DESIGN: Official data regarding the consumption of AEDs within Albania were collected retrospectively. Every year of the period, 2004-2016 has been considered separately.

METHODS: The data were assembled from Health Insurance Institute (HII) in Tirana, Albania and analysed for the period 2004-2016. The consumption of drugs was expressed as several Defined Daily Dose (DDDs)/1000 inhabitants/day. Also, for all the period under study 2004-2016, we analysed the data of import and domestic production of drugs, which represent the real consumption of drugs in the country. These data were subsequently involved in a comparative analysis with the utilisation data according to the HII, as well as through performing international comparisons of the consumption of AEDs drugs.

RESULTS: Epilepsy morbidity data indicate that there exists a correlation statistically significant between this disease and the trend of consumption of AEDs.

CONCLUSION: The present study suggests that the level of consumption for AEDs in Albania is very low when compared globally; with a decrease in the consumption of classic antiepileptic drugs and a parallel increase in the consumption of new generation drugs.

Introduction

Epilepsy is a group of neurological diseases characterised by a predisposition to recurrent unprovoked seizures [1], [2]. Epileptic seizures are episodes that can vary from brief and nearly undetectable to long periods of vigorous shaking [3]. The human brain is the source of human epilepsy. Although the symptoms of a seizure may affect any part of the body, the electrical events that produce the symptoms occur in the brain. The location of that event, how it spreads and how much of the brain is affected, and how long it lasts all have profound effects. These factors determine the character of a seizure and its impact on the individual. The cause of most cases of epilepsy is unknown, although some people develop epilepsy as the result of traumatic or vascular injury, brain tumours, infections of the brain, and congenital disabilities [3]. Epileptic seizures are the result of excessive and abnormal nerve cell activity in the cortex of the brain [4]. The mainstay treatment of epilepsy is anti-epileptic medications, possibly for the person's entire life [5]. The choice of anticonvulsant is based on seizure type, epilepsy syndrome, other medications used, other health problems, and the person's age and lifestyle [6]. A single medication is recommended initially; if this is not effective, switching to a single other medication is recommended [7], [8]. Two medications at once are recommended only if a single medication does not work [8].

There are a number of medications available. Phenytoin, carbamazepine and valproate appear to be equally effective in both partial and generalised seizures [9], [10]. The least expensive anticonvulsant is phenobarbital. The World Health Organization gives it a first-line recommendation in the developing world, and it is commonly used there [11], [12].

Adverse effects from medications are reported in 10 to 90% of people, depending on how and from whom the data is collected [13]. Most adverse effects are dose-related and mild [13]. Despite this, treatment is often continued once effective because the risk of untreated epilepsy is believed to be greater than the risk of the medications [14].

The study aimed to assess the out-of-hospital AEDs use in Albania during the period 2004-2016.

Material and Methods

The data were obtained from the HII [15]. All data were collected for the period 2004-2016 and analysed. The analysis included the total number of prescriptions made and quantities of drugs used.

The data about the population were obtained from the Institute of Statistics (INSTAT) [16]. The data about the consumption of drugs were expressed as several Defined Daily Dose (DDDs)/1000 inhabitants/day. All drugs were classified by groups of Anatomic Therapeutic Chemical Classification (ATC).

Data on real consumption (import and domestic production)

For all the period under study 2004-2016, there were collected and analysed data from the import and domestic production of the drugs [17], which represent the real consumption of drugs in the country. It was noted that the increase in consumption from one year to another was small, e.g. the consumption from 2012 to 2016 (i.e. 4 years) was increased by only 2.32%. Consequently, to obtain an updated study, there were chosen the data of import and domestic consumption only for the last three years, 2014, 2015, 2016, and those were involved in a comparative analysis with the equivalent consumption data according to HII. To minimise the effect of variations between consumption and stock inventory balances from one year to another, it was calculated and put to analysis the annual average value of the three chosen years (on the one hand that of the import and domestic consumption, and on the other hand that of HII).

Presentation of the results and statistical elaboration

The database of HII was modified in Microsoft Office Excel 2007, whereas the statistical elaboration of the obtained results was conducted with the statistical package StatsDirect (version 2.7.2.). A descriptive statistic was used to report all data on drugs consumption and the results obtained were displayed in tabular form as well as through the histogram method.

Average annual values of consumption in the country level and for each district were used as a basis to generate the overviews and the graphics that illustrate the trends of consumption for each class of drugs during the 10 years 2004-2016. The linear regression model was used to evaluate the trends of consumption of drugs relative to the time. A value of $p \leq 0.05$ was considered as significant.

To asses, if there exists a correlation statistically significant between the level of consumption of drugs and the level of morbidity, it was applied the Spearman correlation (with a significance level of ≤ 0.05).

Results

The data were expressed as a number of defined daily doses per 1000 inhabitants/day (DDDs/1000 inhabitants/day).

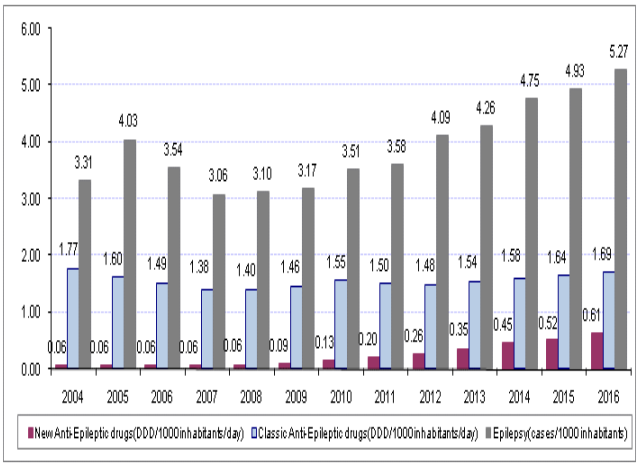

Figure 1: Consumption of different classes of AEDs at national level (DDD/1000 inhabitants/day) versus Epilepsy morbidity (cases/1000 inhabitants) (For the new AEDs: p = 0,0421; strength (with significance level ≤ 0,05) = 53.6%; correlation coefficient statistically significant; for the classic AEDs: p = 0,1618; strength (with significance level ≤ 0,05) = 27.62%; correlation coefficient is not statistically significant).

The consumption of AEDs was 1.82-2.30 DDD/1000 inhabitants/day respectively in 2004-2016. The AEDs most prescribed are the classic or the old

generation with values of 1.77-1.69 DDD/1000 inhabitants/day, 2014-2016. Meanwhile, the new generation of AEDs included in the reimbursement scheme is lamotrigine, gabapentin, levetiracetam and topiramate. Their values of consumption are 0.06-0.61 DDD/1000 inhabitants/day.

Epilepsy morbidity data indicate that there exists a correlation statistically significant between this disease and the trend of consumption of AEDs (P = 0.0034), (Figure 1, and 2).

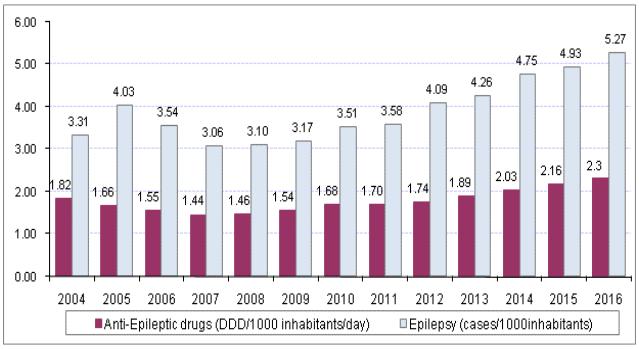

Figure 2: Total consumption of AEDs at the national level (DDD/1000 inhabitants/day) versus Epilepsy morbidity (cases/1000 inhabitants)

Discussion

Epilepsy is a frequent disorder in the western countries occurring in 4-8/1000 inhabitants [30]. The earliest effective pharmacological treatments to prevent epileptic seizures date in 1857, when Locock prescribed for the first-time potassium bromide. However, until 1970, there were in circulation, only a small number of antiepileptic drugs. Since then, the introduction of new antiepileptic molecules in the pharmaceutical market has been highly accelerated, to the point that some new antiepileptic drugs are prescribed even for pain control, psychiatric disorders or migraine prevention [31], [32], [33], [34].

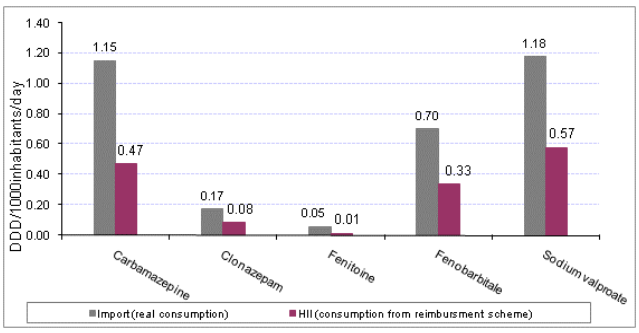

Figure 3: Annual average value of consumption of classic AEDs: consumption-based on import (real consumption) [*] versus consumption based on HII. [*] The "Import" item includes the consumption based on import data as well as the consumption-based on domestic production: this represents the factual consumption

In Figure 1 and 2 can be noted an expressed inconsistency between the level of reported morbidity and the very low consumption of antiepileptic drugs. *First* of all, in some years it results that more than half of epileptic patients have not taken medication under the scheme. *Secondly*, there is noted refraction in the morbidity trend, going through a significant increase in 2005 and a noticeable decrease in 2006-2007, which is difficult to explain from the medical perspective. The consumption reports do reflect the same trend too, but with slighter refraction and with a decrease in consumption in 2007. However, the consumption for this drugs class, although in low levels compared to the morbidity levels, seems relatively consistent and stable. A reason could be the fact that the main drugs in the treatment of this disease continue to be the classic antiepileptic drugs at low cost and low impact on the HII's budget. As old preparations, they do not attire the interest of pharmaceutical companies, and hence, their promotion and marketing remain low. Another reason could be the fact that new antiepileptic drugs were included under the scheme only in 2009.

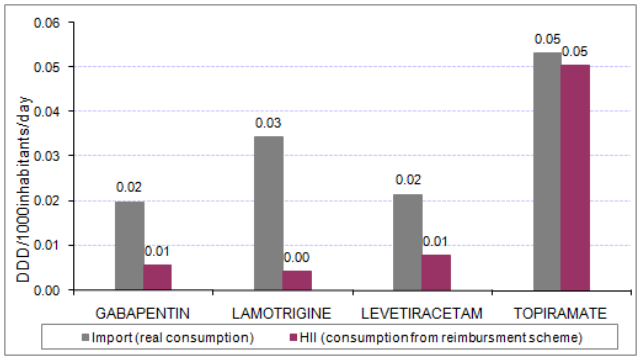

Figure 4: Annual average value of consumption of new AEDs: consumption-based on import (real consumption) [*] versus consumption based on HII. [*] The "Import" item includes the consumption based on import data as well as the consumption-based on domestic production: this represents the factual consumption

To have a better understanding, we have analysed the data of consumption based on imports, comparing them to the analogue consumption data based on HII reports.

As it can be noted in Figures 3, 4 and 5, the consumption of classical antiepileptic drugs is covered in only 57% by the scheme; the remainder of the patients take these drugs without a prescription from the family doctor. It should be considered in this case that such drugs are useable for other purposes apart from for epilepsy, whereas the HII reimburses them only for the treatment of different forms of epilepsy. For instance, sodium valproate is also reimbursed for the treatment of the manic phase of bipolar disorder and is also prescribed for the increase of pain tolerance for chronic pains.

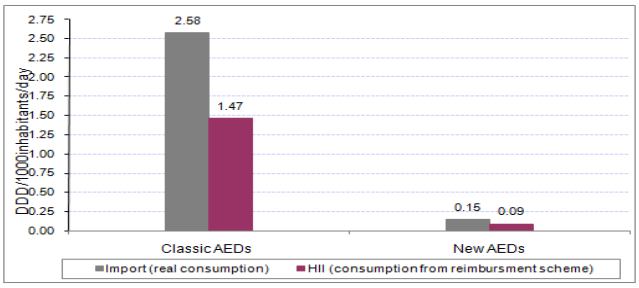

Figure 5: Annual average value of consumption of both classes of AEDs: consumption-based on import (real consumption) [*] versus consumption based on HII

On the other hand, the consumption of new antiepileptic drugs reported by HII consists in around 60% of their total consumption based on import figures. Some of them, e.g. *gabapentin*, is increasingly indicated by therapeutic guidelines for the treatment of neuropathic pain. However, it is of interest the fact that the total value of consumption of antiepileptic drugs based on import data is very much approximate to the reported values of epileptic morbidity.

Among the new antiepileptic drugs, the only one that has been part of the list since in 2004, is *topiramate*: 3.08%-5.43%, 2004-2016. Antiepileptic drugs of the new generation have been included in the list only by 2009.

The consumption of antiepileptic drugs in different regions (Figure 6) remain relatively low. The region with the minimum consumption along the years is Vlora, whereas the maximum consumption is in Berat.

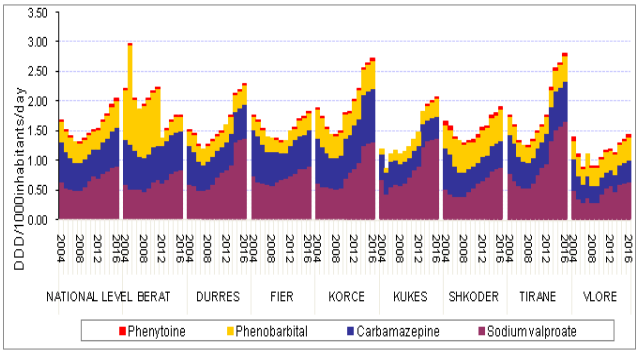

Figure 6: Consumption of AEDs in different regions and at the national level (DDD/1000 inhabitants/day)

A common feature for all regions is the gradual reduction of the consumption in 2006, and especially in 2007 (a year in which the list of reimbursable antiepileptic drugs was the poorest compared to any other year), whereas in subsequent years the list gets re-extended, a fact which is reflected also in the consumption, particularly in the last years of the study.

At the national level, the consumption of antiepileptic drugs undergoes a slight increase (from 1,82 DDD/1000 inhabitants/day in 2004 to 2.30 DDD/1000 inhabitants/day in 2016). This increase in consumption does not reflect the evident increasing morbidity trend of 5.97% (3.31-5.27 cases/1000 inhabitants during 2004-2016). In total, we note a decrease in the consumption of classic antiepileptic drugs and a simultaneous increase in the drugs of the new generation. The clinical trials performed report little evidence that supports the usage of monotherapy with antiepileptic drugs of the new generation or their usage as adjuvants in the antiepileptic treatment, compared to the classic antiepileptic drugs. Furthermore, there is no evidence indicating the priority of usage of an antiepileptic drug over another one [35], [36]. *Levetiracetam*, as well as the other similar antiepileptic drugs of the last generation such as *gabapentin*, *lamotrigine*, *oxcarbazepine*, *tiagabine* and *topiramate*, are recommended by international therapeutic guidelines to be used as drugs of first choice, in order to reinforce the “on” phenomena and the improvement phase in partial epilepsies at adults, resistant to previous drugs (with partial or generalized seizures) [36], [37], [38].

In general, the data obtained in the clinical efficacy, the security of consumption and the pharmacological tolerance to them, have not been able to demonstrate statistically significant differences between these drugs. As an exception, the comparison between new antiepileptic drugs and placebo has resulted in significant differences in favour of the new generation. However, the clinical trials are generally short-termed, limiting to a certain extent the applicability of the data obtained. New antiepileptic drugs used as monotherapy, may have a favourable efficacy-cost report in the treatment of patients that have incurred severe negative side effects from the old classic antiepileptic drugs, of those where the therapy with classic antiepileptic drugs has failed, or of those cases where the drugs of the old generation have been counter-indicated [39].

From the perspective of the efficacy-cost report, an investigation of the British Sanitary system published in 2005, has indicated that new antiepileptic drugs may be superior in patients with partial or generalised seizures refractory to traditional antiepileptic drugs [39].

Comparisons of consumption at the international level

In international comparisons, the consumption of antiepileptic drugs in Albania remains in very low values (*Figure 7*). The total consumption of antiepileptic drugs in our country in 2016 results 2,36 DDD/1000 capita/day, whereas in the Netherlands the consumption of this class appeared since in 2001 around the value 7,02 DDD/1000 capita/day [40]. A reason can be the lack of combined schemes of therapy and the usage of lower than needed doses. These may be indicators of the under-treatment of this pathology in our country.

In conclusion, the consumption values of anti-epileptic drugs in Albania are very low. We noted a decrease in the consumption of classic antiepileptic drugs and a simultaneous increase in the drugs of the new generation. Also, an important part of anti-epileptic drugs flows out of the reimbursement scheme.

References

1. Chang BS, Lowenstein DH. Epilepsy. N Engl J Med. 2003; 349(13):1257-66. https://doi.org/10.1056/NEJMra022308 PMid:14507951

2. Fisher RS, Acevedo C, Arzimanoglou A, Bogacz A, Cross JH, Elger CE, Engel J Jr, Forsgren L, French JA, Glynn M, Hesdorffer DC, Lee BI, Mathern GW, Moshé SL, Perucca E, Scheffer IE, Tomson T, Watanabe M, Wiebe S. ILAE official report: a practical clinical definition of epilepsy. Epilepsia. 2014; 55(4):475-82. https://doi.org/10.1111/epi.12550 PMid:24730690

3. WHO, Media Centre. Epilepsy, Fact Sheet, Feb 2016. Available at: https://www.who.int/news-room/fact-sheets/detail/epilepsy. [Last accessed March 3rd, 2019].

4. Fisher RS, van Emde Boas W, Blume W, Elger C, Genton P, Lee P, Engel J Jr. Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). Epilepsia. 2005; 46(4):470-2. https://doi.org/10.1111/j.0013-9580.2005.66104.x PMid:15816939

5. The Epilepsies: The Diagnosis and Management of the Epilepsies in Adults and Children in Primary and Secondary Care: Pharmacological Update of Clinical Guideline 20. National Clinical Guideline Centre (UK). London: Royal College of Physicians (UK); 2012 Jan.

6. Nunes VD, Sawyer L, Neilson J, Sarri G, Cross JH. Diagnosis and management of the epilepsies in adults and children: summary of updated NICE guidance. BMJ. 2012; 344:e281. https://doi.org/10.1136/bmj.e281 PMid:22282528

7. Wyllie's treatment of epilepsy: principles and practice. - 5th ed./editor-in-chief, Elaine Wyllie; associate editors, Gregory D. Cascino, Barry E. Gidal, Howard P. Goodkin. Lippincott Williams & Wilkins, 2011:187-188.

8. Alderson P, Kosky N, Cross H. NICE response to World Report on epilepsy guidance. Lancet. 2012; 379(9823):1300. https://doi.org/10.1016/S0140-6736(12)60557-1

9. Nolan SJ, Marson AG, Pulman J, Tudur Smith C. Phenytoin versus valproate monotherapy for partial onset seizures and generalized onset tonic-clonic seizures. Cochrane Database Syst Rev. 2013; (8):CD001769. https://doi.org/10.1002/14651858.CD001769.pub2

10. Tudur Smith C, Marson AG, Clough HE, Williamson PR. Carbamazepine versus phenytoin monotherapy for epilepsy. Cochrane Database Syst Rev. 2002; (2):CD001911. https://doi.org/10.1002/14651858.CD001911 PMid:12076427

11. Ilangaratne NB, Mannakkara NN, Bell GS, Sander JW. Phenobarbital: missing in action. Bull World Health Organ. 2012; 90(12):871-871A. https://doi.org/10.2471/BLT.12.113183 PMid:23284189 PMCid:PMC3524964

12. Shorvon S, Perucca E, Engel Jr J, editors. The treatment of epilepsy. John Wiley & Sons, 2015:587. https://doi.org/10.1002/9781118936979

13. Perucca P, Gilliam FG. Adverse effects of antiepileptic drugs. Lancet Neurol. 2012; 11(9):792-802. https://doi.org/10.1016/S1474-4422(12)70153-9

14. Kamyar M, Varner M. Epilepsy in pregnancy. Clin Obstet Gynecol. 2013; 56(2):330-41. https://doi.org/10.1097/GRF.0b013e31828f2436 PMid:23563876

15. Health Insurance Institute, Tirana, Albania, 2016. Available at: http://www.fsdksh.com.al/images/2017/Botime/Raporti_Vjetor_2016/Raporti_Vjetor_FSDKSH_Anglisht.pdf [Last accessed March 3rd, 2019].

16. Institute of Statistics; INSTAT, Tirana, Albania, 2019. http://www.instat.gov.al/en [Last accessed March 3rd, 2019].

17. General Customs Directorate, Ministry of Finance, Tirana, Albania, 2019. http://www.dogana.gov.al/english/c/171/197/199/general-directorate-of-customs [Last accessed March 3rd, 2019].

18. Sull'impiego dei Medicinali ON. L'uso dei farmaci in Italia-Rapporto Nazionale, 2007. Available at http://www. agenziafarmaco. it/allegati/rapporto_osmed_2007. pdf [Last accessed March 3rd, 2019].

19. Sull'impiego dei Medicinali ON. L'uso dei farmaci in Italia-Rapporto Nazionale, 2008. Available at http://www.aifa.gov.it/content/rapporti-osmed-luso-dei-farmaci-italia [Last accessed March 3rd, 2019].

20. OSMED, G., 2011. L'uso dei farmaci in Italia: rapporto nazionale anno 2010. Il Pensiero Scientifico Editore.

21. Estonian Statistics on Medicines 2006-2010. Ravimiamet State Agency of Medicines, 2010. Available at: http://www.ravimiamet.ee/en/statistics-medicines [Last accessed March 3rd, 2019].

22. Statistical Yearbook of the State Agency of medicines 2015. Available at: http://www.ravimiamet.ee/sites/default/files/documents/publications/statistika_aastaraamat_2015/index.html [Last accessed March 3rd, 2019].

23. Statistical Yearbook of the State Agency of medicines 2017. Available at: http://www.ravimiamet.ee/sites/default/files/ravimiamet_aastaraamat_a5_100lkkaaned_k3_final.pdf [Last accessed March 3rd, 2019].

24. Norwegian Institute of Public Health. Drug Consumption in Norway 2006-2010. Department of pharmaco-epidemiology, Norwegian Institute of Public Health; Available at: http://www.legemiddelforbruk.no [Last accessed March 3rd, 2019].

25. Drug Consumption in Norway 2011-2015. Available at: https://www.fhi.no/globalassets/dokumenterfiler/rapporter/2016/legemiddelforbruket-i-norge-2011-2015-pdf.pdf [Last accessed March 3rd, 2019].

26. Drug Consumption in Norway 2011-2015. Available at: https://www.fhi.no/en/publ/2017/drug-consumption-2012-2016/ [Last accessed March 3rd, 2019].

27. Finnish Statistics on Medicines 2007. National Agency for Medicines, Department of safety and drug information. Available at: https://www.kela.fi/web/en/statistical-publications_finnish-statistics-on-medicines [Last accessed March 3rd, 2019].

28. Finnish Statistics on Medicines 2014. National Agency for Medicines, Department of safety and drug information. Available at: https://www.kela.fi/web/en/statistical-publications_finnish-statistics-on-medicines [Last accessed March 3rd, 2019].

29. Finnish Statistics on Medicines 2016. National Agency for Medicines, Department of safety and drug information. Available at: https://www.kela.fi/web/en/statistical-publications_finnish-statistics-on-medicines [Last accessed March 3rd, 2019].

30. Banerjee PN, Hauser WA. Incidence and prevalence. Epilepsy: a comprehensive textbook. 2008; 1:45-56.

31. Beyer JL, Burchitt B, Gersing K, Krishnan KR. Patterns of pharmacotherapy and treatment response in elderly adults with bipolar disorder. Psychopharmacol Bull. 2008; 41(1):102-114.

32. Geddes JR, Calabrese JR, Goodwin GM. Lamotrigine for treatment of bipolar depression: independent meta-analysis and meta-regression of individual patient data from five randomised trials. Br J Psychiatry. 2009; 194(1):4-9.

https://doi.org/10.1192/bjp.bp.107.048504 PMid:19118318

33. Johannessen Landmark C. Antiepileptic drugs in non-epilepsy disorders: relations between mechanisms of action and clinical efficacy. CNS Drugs. 2008; 22(1):27-47. https://doi.org/10.2165/00023210-200822010-00003 PMid:18072813

34. Mulleners WM, Chronicle EP. Anticonvulsants in migraine prophylaxis: a Cochrane review. Cephalalgia. 2008; 28(6):585-597. https://doi.org/10.1111/j.1468-2982.2008.01571.x PMid:18454787

35. French JA, Kanner AM, Bautista J, et al; American Academy of Neurology Therapeutics and Technology Assessment Subcommittee; American Academy of Neurology Quality Standards Subcommittee; American Epilepsy Society Quality Standards Subcommittee; American Epilepsy Society Therapeutics and Technology Assessment Subcommittee. Efficacy and tolerability of the new antiepileptic drugs, I: Treatment of new-onset epilepsy: report of the TTA and QSS Subcommittees of the American Academy of Neurology and the American Epilepsy Society. Epilepsia. 2004; 45(5):401-9. https://doi.org/10.1111/j.0013-9580.2004.06204.x PMid:15101821

36. National Institute for Health and Clinical Excellence. CG20 Epilepsy in adults and children. Available at: http://www.nice.org.uk/nicemedia/pdf/CG020NICEguideline.pdf [Last accessed March 3rd, 2019].

37. Scottish Intercollegiate Guidelines Network. Diagnosis and management of epilepsy in adults. A national clinical guideline, 2003. Addendum released June 2004. Available at: http://www.sign.ac.uk/pdf/sign70.pdf, and at http://www.sign.ac.uk/guidelines/fulltext/70/update.html. [Last accessed March 3rd, 2019].

38. Wilby J, Kainth A, Hawkins N, Epstein D, McIntosh H, McDaid C, Mason A, Golder S, O'Meara S, Sculpher M, Drummond M, Forbes C. Clinical effectiveness, tolerability and cost-effectiveness of newer drugs for epilepsy in adults: a systematic review and economic evaluation. Health Technol Assess. 2005; 9(15):1-157. https://doi.org/10.3310/hta9150 PMid:15842952

39. Hollingworth SA, Eadie MJ. Antiepileptic drugs in Australia: 2002-2007. Pharmacoepidemiol Drug Saf. 2010; 19(1):82-9. https://doi.org/10.1002/pds.1871 PMid:19802824

40. Knoester P, Deckers C, van der Vaart R, Leufkens B, Hekster Y. Volume and market share of anti-epileptic drugs in The Netherlands: impact of new drugs. Pharm World Sci. 2005; 27(2):129-34. https://doi.org/10.1007/s11096-005-1558-7 PMid:15999925

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Public Health

The Point Prevalence Survey Research of Antibacterial Drugs' Prescription for Outpatient Treatment of Urinary System Infections

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Keywords: PPS-research; Antibiotic resistance; Urinary tract infections; Prescription; Treatment; Choice of antibiotic drugs

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Abstract

BACKGROUND: Antibiotic resistance of microorganisms is the subject of numerous discussions and initiatives, it has a well-defined tendency to increase which is largely related to a big number of errors when prescribing antibacterial drugs at the outpatient stage of treatment and disease prevention, as well as a lack of information and data on the quantity and quality of antimicrobial therapy. One of the elements aimed at reducing antibiotic resistance growth is audit and analysis of application practice.

AIM: To assess qualitative and quantitative characteristics for urinary tract infections treatment using antimicrobial drugs by general physicians at the outpatient level.

MATERIALS AND METHODS: The Point Prevalence Survey (PPS) analysis of antimicrobial drugs prescription was conducted by general physicians of No. 1, 4, 5 Hospitals of Karaganda city. There was carried out a daily prospectively data collecting on antibacterial drugs prescription by Doctor of Medical institutions, which was performed during patients' visit. For each person who was prescribed the AD, there was filled a special questionnaire developed by the European Center for Disease Prevention and Control, including passport data, data on disease and prescription of antibacterial drugs. There were considered 200 cases of antibiotic prescription. The study included patients of both sexes, all age groups, who were prescribed the antibiotic therapy for the UTI treatment.

RESULTS: When selecting antimicrobial therapy, the Protocols for diagnosis and treatment indicate the need for a microbiological study to determine sensitivity to antibacterial drugs, that was not performed in 100% of cases, and initial treatment was empirically prescribed, namely, in 34% of cases there were used drugs from the cephalosporin group (Ceftriaxone), nitrofurans (Furazidin)-42%, fluoroquinolones (Levofloxacin)-24%. In treating acute cystitis, in most cases, alternative medications were prescribed, though according to current recommendations, first-line therapy includes fosfomycin trometamol, pivmecillins and nitrofurantoin macrocrystals, which according to the results of this study were not used at all.

CONCLUSION: In most cases (71%), alternative antibacterial drugs were prescribed for initial treatment of urinary tract infections. In majority cases, the dosage regimen of antibacterial drugs, dosage frequency, treatment course did not meet current recommendations.

Introduction

The number of urinary system diseases in the Republic of Kazakhstan tends to continuous growth, mainly due to urinary tract infections (UTI). So, in 2015, the overall incidence in this nosological category was 7532.2, in 2016-8784.8, in 2017 - 8765.7 per 100,000 people. In absolute numbers, the statistics is the following: 2015-1321460 persons, 2016-1563158 persons, 2017-1581114 persons [1], [2].

Every year in the US, more than 7 million people with the UTI symptoms are seeking specialised medical assistance, and about 15% of all prescribed antibacterial drugs (AD) are used specifically by a group of childbearing age female patients. A similar trend is observed in Western European countries. Among childbearing age women, the UTI is diagnosed ten times more often than in

men. Nearly half of women in this age group have had the UTI during their life with every third woman under the age of 24, and from 20 years old before menopause women's cystitis is diagnosed 50 times more often than in men, which indicates the need to improve the diagnosis and treatment of the UTI in women, which is also important in periconceptional supplementation due to strong correlation with infectious diseases of the pelvic organs, especially when the infection is generalized [3]. Besides, savings by reducing antibiotics costs may be greater than the cost of intervention or program (from $ 200,000 to $ 900,000, depending on the study) [4], [5].

According to numerous studies, the etiological factor in approximately 70 – 85% of cases are Enterobacteriaceae. The remaining pathogens (Staphylococcus saprophyticus, Pseudomonas aeruginosa, Enterococcus faecalis) are much less common [6], [7], [8]. Today, the literature suggests the choice of therapy drugs for urinary tract infections are cephalosporins of the II-III generations, fluoroquinolones and aminoglycosides, which indicates an increase in resistance [3].

Antibiotic resistance of microorganisms is the subject of numerous discussions and initiatives [9], it has a well-defined tendency to increase which is largely related to a big number of errors when prescribing antibacterial drugs at the outpatient stage of treatment and disease prevention, as well as a lack of information and data on the quantity and quality of antimicrobial therapy. One of the elements aimed at reducing antibiotic resistance growth is audit and analysis of application practice.

The Point Prevalence Survey (PPS) is the most appropriate tool for monitoring doctors' actions regarding antibiotics. The PPS is pointed prevalence study which allows identifying the goals of doctors in charge of diseases therapy. At the same time, the points prevalence means the number of people with a certain characteristic in a selected period of the relatively interested population for researchers. The Global Prevalence Study (Global PPS) on consumption and antimicrobial resistance was developed after the Fourth Worldwide Forum on Infections and Antimicrobial Resistance. Its goal was to assess the international prevalence of antimicrobial use and resistance with a focus on countries with low resources, support and experience [10]. The project was based on the results of three-pointed prevalence studies conducted by the European antimicrobial consumption supervision in 2006 and 2009 [11], [12].

Many modern studies have brought into sharp focus that the PPS analysis is a valid method of rationality assessment for antimicrobial therapy prescribing in each medical institution [13], [14], [15], [16], [17]. Several studies [18], [19], [20], [21] on applicability and benefits of the antimicrobial use prevalence points survey have shown its value within the range of European hospitals. In addition, the European supervision of antimicrobial consumption network methods have been adapted for the European Center for Disease Prevention for the use of antimicrobial agents in emergency hospitals [22] and determination the antibiotic resistance in the European children's project which focuses on antimicrobial drugs for children and newborns worldwide [23], [24], [25]. Thus, to prevent resistance growth, it is necessary to conduct an analysis for rational antibiotics uses based on valid methods.

Purpose of the study: to assess qualitative and quantitative characteristics for urinary tract infections treatment using antimicrobial drugs by general physicians at the outpatient level.

Material and Methods

The Point Prevalence Survey (PPS) analysis of antimicrobial drugs prescription was conducted by general physicians of No. 1, 4, 5 Hospitals of Karaganda city. There was carried out a daily prospectively data collecting on antibacterial drugs prescription by Doctor of Medical institutions, which was performed during patients' visit. For each person who was prescribed the AD, there was filled a special questionnaire developed by the European Center for Disease Prevention and Control [26], including passport data, data on disease and prescription of antibacterial drugs. There were considered 200 cases of antibiotic prescription. The study included patients of both sexes, all age groups, who were prescribed the antibiotic therapy for the UTI treatment.

Results

In the research, 200 cases of the AD prescription for urinary tract infections therapy were considered. The survey included 161(80.5%) women and 39 (19.5%) men.

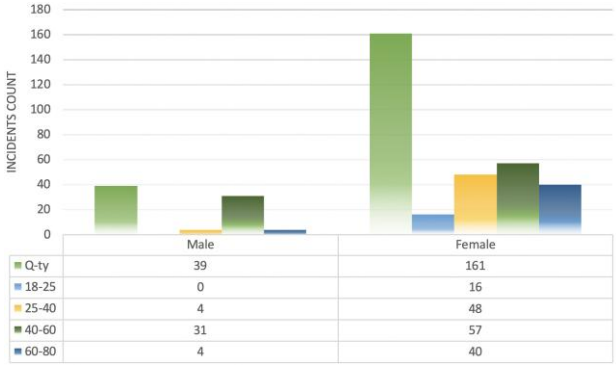


Figure 1: Sex-age structure of those under study by groups

One hundred twenty-one persons under study (75%) are related to the childbearing age women category. Their sex-age structure by groups is provided in Figure 1.

As the Figure indicates, women are 4 times more than men which corresponds to literary data [3].

When analysing the obtained data, there was data homogeneity: most often antibacterial drugs were prescribed by general physicians in the Hospitals No. 1, it is 46% of cases, in the Hospitals No. 4 and No. 5 it is 31% and 23% respectively. The structure of the nosology is shown in Figure 2.

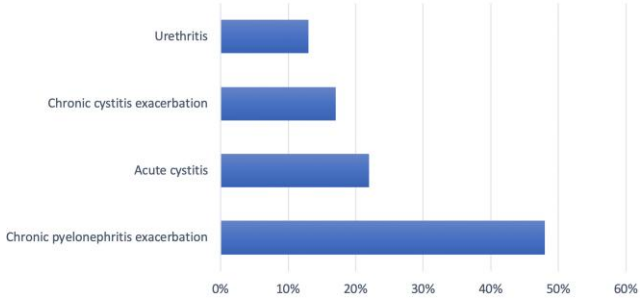
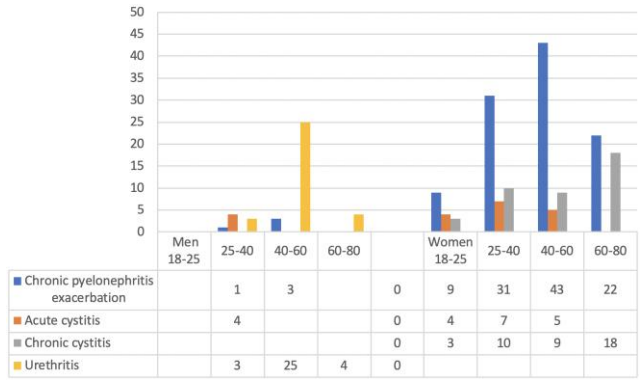


Figure 2: Urinary tract infections structure

The most common reason for antibiotics prescription was a chronic pyelonephritis exacerbation; it is 48%.

Figure 3 shows the UTI disease incidence in men of different age groups. As can be seen, in most cases, urethritis occurs in 40 – 60 years old men, which corresponds to modern data [27].



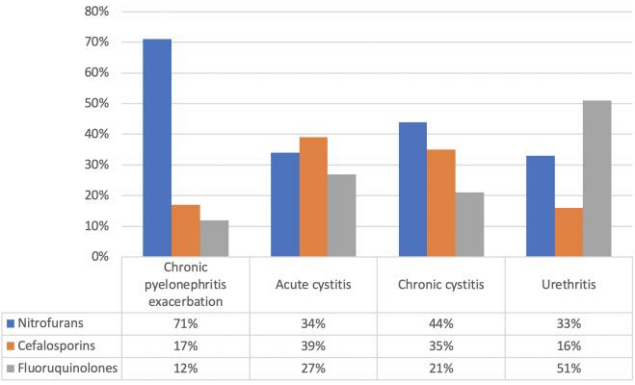
	Men 18-25	25-40	40-60	60-80		Women 18-25	25-40	40-60	60-80
Chronic pyelonephritis exacerbation		1	3		0	9	31	43	22
Acute cystitis		4			0	4	7	5	
Chronic cystitis					0	3	10	9	18
Urethritis		3	25	4	0				

Figure 3: The incidence of UTIs in different age groups

In women of all age groups, the chronic pyelonephritis exacerbation is most common, which corresponds to the literature data (Figure 3) [3].

When selecting antimicrobial therapy, the Protocols for diagnosis and treatment indicate the need for a microbiological study to determine sensitivity to antibacterial drugs, that was not performed in 100% of cases, and initial treatment was empirically prescribed, namely, in 34% of cases there were used drugs from the cephalosporin group (Ceftriaxone), nitrofurans (Furazidin)-42%, fluoroquinolones (Levofloxacin)-24%.

Figure 4 shows the prescription of different antibiotics for urinary tract infections treatment.



	Chronic pyelonephritis exacerbation	Acute cystitis	Chronic cystitis	Urethritis
Nitrofurans	71%	34%	44%	33%
Cefalosporins	17%	39%	35%	16%
Fluoruquinolones	12%	27%	21%	51%

Figure 4: Prescription frequency of different antibiotics for urinary tract infections treatment

In case of chronic pyelonephritis exacerbation, in most cases (71%) (Figure 4) there were used nitrofurans 100 mg, 4 times a day, the treatment was 7-10 days, although according to the literature they are not the first-choice drugs (Table 1) [3].

Table 1: Dosing Schedule, frequency and treatment course of different antibiotics

Drug	Dosage, mg (day)	Number of cases	Dosage frequency	Administration route	Treatment course, days
Levofloxacin	500	48	1	orally	none
Ceftriaxone	1000	60	2	parenterally	7
	2000	8	1	parenterally	none
Furazidin	100	68	4	orally	10
	100	16	4	orally	7

Cephalosporins (Cefriaxone) were prescribed only in 17% of cases (Figure 4), the drug dosage varied from 1000-2000 mg 1-2 times a day, the course lasted for 7 days, although according to literature data, the 2-3 class cephalosporins are alternative-choice drugs: Cefpodoximum axetil-250 mg each 2 times a day; Cefpodoximum-100 mg 2 times a day; ceftibutenum or cefiximum-400 mg per day; -protected aminopenicillins: amoxicillin/clavulanic acid 500 mg/125 mg 3 times a day [3].

Discussion

Moreover, according to the WHO recommendations, on the outpatient stage, it is preferable to use drugs with a non-invasive administration route since parenteral forms reduce the level of compliance between doctor and patient and reduce treatment compliance in the latter [28]. This situation provokes the development of complications, process chronization and antibiotic resistance growth of pathogens. Also, the parenteral forms are

prescribed when it is impossible to take medications orally (nausea, vomiting), the so-called “switch” therapy: the initial parenteral administration of a drug with further change oral administration after improvement.

Fluoroquinolones (Levofloxacin) were prescribed in 12% of cases (Figure 4) with a dosage of 500 mg a day which corresponds to the literature data, but the therapy course duration is not indicated. Although the selected drugs for community-acquired uncomplicated pyelonephritis from the perspective of evidentiary medicine are fluoroquinolones: ciprofloxacin – 500 mg, 2 times a day; Levofloxacin – 500 mg, once a day; Norfloxacin – 400 mg, 2 times a day; Ofloxacin – 200-400 mg, 2 times a day during 10-14 days (1b level of evidence, recommendation grade B) [3].

In the majority of cases, the multiplicity of furazidine intake does not meet the recommendations based on drug pharmacodynamics [29], which indicates poor knowledge of the drug pharmacodynamics.

In treating acute cystitis, in most cases, alternative medications were prescribed, though according to current recommendations, first-line therapy includes fosfomycin trometamol, pivmecillins and nitrofurantoin macrocrystals, which according to the results of this study were not used at all. Co-trimoxazole is the dosage 160 / 800 mg, 2 times a day for 3 days or trimethoprim (TMP) 200 mg, 2 times a day for 5 days can be used as the first-line therapy in areas, where E. coli resistance < 20 % [3]. In 39% of cases, there were prescribed cephalosporins (ceftriaxone), fluoroquinolones (levofloxacin) - 34% and nitrofurans (furazidin)-27% (Figure 4). Alternative antibiotics are ciprofloxacin-250 mg, 2 times a day, ciprofloxacin with a prolonged action at the dose of 500 mg, 1 time a day, levofloxacin at the dose of 250 mg, 1 time a day, norfloxacin-400 mg, 2 times a day, each of the drugs taken a three-day course [3].

The same situation was observed when prescribing antibiotics for urethritis, preference was given to the second-line drugs, so fluoroquinolones (levofloxacin) were administered in 51% of cases, and the first-line drugs, namely cephalosporins, were prescribed only in 16% of cases (Figure 4). Moreover, the dosage regimen of antibacterial drugs, dosage frequency, treatment course did not meet current recommendations [3].

Documentation analysis has shown that the age-specific features of patients were not taken into account when prescribing antibiotics and selecting a dose, and in no case, the glomerular filtration rate was measured [29].

In conclusion, in most cases (71%), alternative antibacterial drugs were prescribed for initial treatment of urinary tract infections. In majority cases, the dosage regimen of antibacterial drugs, dosage frequency, treatment course did not meet current recommendations. Also, there were prescribed antibiotics with the parenteral administration route, although, according to the WHO recommendations, it is preferable to use drugs with a non-invasive administration route in the outpatient stage.

The results obtained from this PPS study, which was first conducted in municipal hospitals, reveal several specific opportunities for improving the practice of antibacterial drugs use, which may lead to improved treatment results of patient, resistance growth prevention and reduced health care costs.

References

1. Kargabayeva BA, Aldazharova ZhK, Kenesova AA, et al. Statistical book "Health of the population of the Republic of Kazakhstan and health organizations activities in 2016", 2017:356.

2. Kaidar EK, Kenesova AA, Yurchenko IV, et al. Statistical book "Health of the population of the Republic of Kazakhstan and health organizations activities in 2017", 2018:354.

3. Bonkat G (Chair), Bartoletti RR, Bruyère F, Cai T, Geerlings SE, Köves B, Schubert S, Wagenlehner F. Guidelines Associates: Mezei T, Pilatz A, Pradere B, Veeratterapillay R. Guidelines on urological infections. European Association of Urology 2018. Stevenson KB. The economics of antimicrobial stewardship: the current state of art and applying the business case model. ICHE. 2012;33(4):390-397.

4. Davey P, Brown E, Charani E, Fenelon L, Gould IM, Holmes A, Ramsay CR, Wiffen PJ, Wilcox M. Interventions to improve antibiotic prescribing practices for hospital inpatients. Cochrane Database of Systematic Reviews. 2013(4). https://doi.org/10.1002/14651858.CD003543.pub3

5. Nathwani D. Antimicrobial prescribing policy and practice in Scotland: recommendations for good antimicrobial practice in acute hospitals. Journal of Antimicrobial Chemotherapy. 2006; 57(6):1189-96. https://doi.org/10.1093/jac/dkl137 PMid:16624876

6. Sundén F, Håkansson L, Ljunggren E, Wullt B. Escherichia coli 83972 bacteriuria protects against recurrent lower urinary tract infections in patients with incomplete bladder emptying. The Journal of urology. 2010; 184(1):179-85. https://doi.org/10.1016/j.juro.2010.03.024 PMid:20483149

7. Van der Starre WE, Van Nieuwkoop C, Paltansing S, Van't Wout JW, Groeneveld GH, Becker MJ, Koster T, Wattel-Louis GH, Delfos NM, Ablij HC, Leyten EM. Risk factors for fluoroquinolone-resistant Escherichia coli in adults with community-onset febrile urinary tract infection. Journal of Antimicrobial Chemotherapy. 2010; 66(3):650-6. https://doi.org/10.1093/jac/dkq465 PMid:21123286

8. Pitout JD. Infections with extended-spectrum β-lactamase-producing Enterobacteriaceae. Drugs. 2010; 70(3):313-33. https://doi.org/10.2165/11533040-000000000-00000 PMid:20166768

9. Carlet J, et al. Ready for a world without antibiotics? The pensières Antibiotic Resistance Call to Action. ARIC. 2012; 1:11. https://doi.org/10.1186/2047-2994-1-11 PMid:22958833 PMCid:PMC3436635

10. BioMerieux. International experts join forces against superbugs at the 4th World Forum on Healthcare-Associated Infections and Antimicrobial Resistance, 2018. http://www.biomerieux.com/en/4thworld-forum-healthcare-associated-infections-and-antimicrobialresistance (accessed Feb 7, 2018).

11. Ansari F, Erntell M, Goossens H, et al. The European surveillance of antimicrobial consumption (ESAC) point-prevalence survey of antibacterial use in 20 European hospitals in 2006. Clin Infect Dis. 2009; 49:1496-504. https://doi.org/10.1086/644617 PMid:19842976

12. Zarb P, Amadeo B, Muller A, et al. Identification of targets for quality improvement in antimicrobial prescribing: the web-based ESAC point prevalence survey 2009. J Antimicrob Chemother. 2011; 66:443-49. https://doi.org/10.1093/jac/dkq430 PMid:21084362

13. Zarb P, Amadeo B, Muller A, et al. Identification of targets for quality improvement in antimicrobial prescribing: the web-based ESAC point prevalence survey 2009. J Antimicrob Chemother. 2011; 66:443-49. https://doi.org/10.1093/jac/dkq430 PMid:21084362

14. Robert J, Péan Y, Varon E, Bru JP, Bedos JP, Bertrand X, Lepape A, Stahl JP, Gauzit R. Point prevalence survey of antibiotic use in French hospitals in 2009. Journal of antimicrobial chemotherapy. 2012; 67(4):1020-6. https://doi.org/10.1093/jac/dkr571 PMid:22258928

15. Talaat M, Saied T, Kandeel A, El-Ata G, El-Kholy A, Hafez S, Osman A, Razik M, Ismail G, El-Masry S, Galal R. A point prevalence survey of antibiotic use in 18 hospitals in Egypt. Antibiotics. 2014; 3(3):450-60. https://doi.org/10.3390/antibiotics3030450 PMid:27025755 PMCid:PMC4790372

16. Xie DS, Xiang LL, Li R, Hu Q, Luo QQ, Xiong W. A multicenter point-prevalence survey of antibiotic use in 13 Chinese hospitals. Journal of infection and public health. 2015; 8(1):55-61. https://doi.org/10.1016/j.jiph.2014.07.001 PMid:25129448

17. Gharbi M, Doerholt K, Vergnano S, Bielicki JA, Paulus S, Menson E, Riordan A, Lyall H, Patel SV, Bernatoniene J, Versporten A. Using a simple point-prevalence survey to define appropriate antibiotic prescribing in hospitalised children across the UK. BMJ open. 2016; 6(11):e012675. https://doi.org/10.1136/bmjopen-2016-012675 PMid:27810974 PMCid:PMC5129034

18. Zarb P, Goossens H. European Surveillance of Antimicrobial Consumption (ESAC): value of a point-prevalence survey of antimicrobial use across Europe. Drugs. 2011; 71: 745-55. https://doi.org/10.2165/11591180-000000000-00000 PMid:21504250

19. Malcolm W, Nathwani D, Davey P, et al. From intermittent antibiotic point prevalence surveys to quality improvement: experience in Scottish hospitals. Antimicrob Resist Infect Control. 2013; 2:3. https://doi.org/10.1186/2047-2994-2-3 PMid:23320479 PMCid:PMC3573889

20. Pristas I, Barsic B, Butic I, et al. Point prevalence survey on antibiotic use in a Croatian infectious disease hospital. J Chemother. 2013; 25:222-28. https://doi.org/10.1179/1973947812Y.0000000065 PMid:23906076

21. Zarb P, Coignard B, Griskeviciene J, et al. The European Centre for Disease Prevention and Control (ECDC) pilot point prevalence survey of healthcare-associated infections and antimicrobial use. Euro Surveill. 2012; 17:20316. https://doi.org/10.2807/ese.17.46.20316-en PMid:23171822

22. Versporten A, Sharland M, Bielicki J, et al. The antibiotic resistance and prescribing in European children project: a neonatal and pediatric antimicrobial web-based point prevalence survey in 73 hospitals worldwide. Pediatr Infect Dis J. 2013; 32:e242-53. https://doi.org/10.1097/INF.0b013e318286c612 PMid:23838740

23. Versporten A, Bielicki J, Drapier N, et al. The Worldwide Antibiotic Resistance and Prescribing in European Children (ARPEC) point prevalence survey: developing hospital-quality indicators of antibiotic prescribing for children. J Antimicrob Chemother. 2016; 71:1106-17. https://doi.org/10.1093/jac/dkv418 PMid:26747104

24. Lestner JM, Versporten A, Doerholt K, et al. Systemic antifungal prescribing in neonates and children: outcomes from the Antibiotic Resistance and Prescribing in European Children (ARPEC) Study. Antimicrob Agents Chemother. 2015; 59:782-89. https://doi.org/10.1128/AAC.04109-14 PMid:25403672 PMCid:PMC4335832

25. De LM, Dona D, Montagnani C, et al. Antibiotic prescriptions and prophylaxis in Italian children. Is it time to change? Data from the ARPEC project. PLoS One. 2016; 11:e0154662. https://doi.org/10.1371/journal.pone.0154662 PMid:27182926 PMCid:PMC4868290

26. European Centre for Disease Prevention and Control- Point prevalence survey validation protocol - Version 2.1.

27. Kozlov SN, Korolev SV, Andreeva IV, Belikov AN, Grinev AV, Evstaf'ev VV, Kirpicheva NN, Serdjuckaja MV, Stecjuk OU, Fokin AA. Podhody k diagnostike i lecheniju ostrogo uretrita u muzhchin: rezul'taty mnogocentrovogo nabljudatel'nogo issledovanija. Klinicheskaja mikrobiologija i antimikrobnaja himioterapija. 2011; 13(1):19-32.

28. WHO. The evolving threat of antimicrobial resistance: options for action // WHO, 2013:130.

29. Gupta K, Hooton TM, Naber KG, Wullt B, Colgan R, Miller LG, Moran GJ, Nicolle LE, Raz R, Schaeffer AJ, Soper DE. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: a 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clinical infectious diseases. 2011; 52(5):e103-20. https://doi.org/10.1093/cid/ciq257 PMid:21292654

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Children's Perception and Belief about Medicines: Effectiveness and Its Autonomy

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Abstract

BACKGROUND: The use of medicines in children is usually always under the supervision of parents. Children are considered not to understand the concept of medicine properly. Children's perceptions of medicine are mostly formed from everyday experience. This can have an impact on children's beliefs about medicines which they are also required to be active and rational medicine users.

AIM: This study aims to look at children's perceptions and beliefs about medicines, especially in the perspective of medicine efficacy and children's autonomy in using them.

METHOD: The study was conducted with an analytical method with a cross-sectional approach using a questionnaire instrument in grade V elementary school children in Padang City, Indonesia. The total sample size obtained was 503 students.

RESULTS: Children still think that medicine efficacy is influenced by taste, colour, size, medicine price, a place to buy medicine and how to get medication. 10.1% of children have stored the medicine at home, and 48.5% of children always depends on waited for their parents when they wanted to take medicine. Regarding children's access to medicines, 11.5% of children have bought their own over-the-counter medicine to a pharmacy or medicine store without the parents' knowledge. 31.4% of children have taken medicine at home without the parents' knowledge.

CONCLUSION: The results of this study indicate that children's perceptions of medicine efficacy are still very limited. Even though the child has used the medicine alone in a limited way, the child's autonomy in using the medicine still needs to be monitored by the parent. Therefore, this is the reason for the need for medical education given to children, especially in schools as an integral part of health education.

Introduction

The use of medicines both for medicinal purposes and for prevention has become a habit for everyone, including children [1], [2], [3]. Unlike adults, the use of medicines in children is usually always under the supervision of parents. In general, parents see that children do not understand the concept of medicine enough that at times, can harm children [4]. Therefore, various preventive measures are taken to protect children from medicines. For example, the message "keep out of reach of children" which is often found on medicine packaging for children.

Conversely, children also tend to look at medicines very carefully. Some studies have found that children know that medicines can be harmful to the body because they cause harmful effects. Older children are usually more able to understand the risks of these adverse effects than younger children. This is related to the level of cognitive development of children which is in line with their age level [5].

Children get information about medicines generally only from experience using medicines or from observing families using medicines. Experience that is not good at using medicines can affect their behaviour later. A study showed that 79% of children stated that the taste of the medicine was the main factor that made them unwilling to take medication [6]. In this way, all children build confidence and develop

expectations about medicines that will affect their future behaviour in the use of medicines. Therefore, children need basic information about medicines before they use them independently [7]. Some medical information may be obtained by children from various sources such as parents, teachers or professionals such as pharmacists and doctors [8], [9], [10]. Some are also from media such as television, the internet or directly from medicine packaging. But the information they obtain is still very limited and fragmented, so it has not been able to improve children's understanding of medicines.

A study shows that medicine advertisements such as on TV do not have an effect on increasing children's knowledge about medicines [11]. Some studies show that children's knowledge of medicines is still very superficial [5], [10], [11], [12]. This is certainly very worrying because it can have a bad impact on children's attitudes or behaviour about medicines [13]. Medicines can be viewed as something that is not at risk for health, so children are vulnerable to drug abuse or medicine incidents. Some medicine incidents can occur such as medicines that are mistakenly taken, excessive doses, etc. [14], [15]. Or it could be the other way around that medicines are seen as something very dangerous so that children become afraid of taking medication. Children according to their age often perceive medicines based on their physical appearance. As a result, due to the limitations of this knowledge, children often associate the efficacy of the medicine with formulation factors and the way or source to obtain it. Several studies show that children assume that the efficacy of the medicine is related to size, taste, source obtained etc. [16]. This wrong perception if it is not corrected, it can affect the behaviour of children in using medicines, especially when they grow up. Moreover, children who have used their own medicine through self-medication practice. Self-medication practices have begun when children aged 10-14 years and many of them without the knowledge of parents [17].

An international survey conducted in 28 countries stated that children had already practised self-medication especially for medication for headaches [2], [13]. Children are mostly a good reflection of the culture that surrounds them. Behaviour related to medicines and self-care develops in the early stages of their lives. Therefore, studying the knowledge and use of medicines in childhood is very important to increase children's trust in the concept of benefits and risks of medicine [18]. This kind of thing will certainly have a decisive influence on the behaviour and use of medicines later in the adult stage.

This study aims to look at children's perceptions and beliefs about medicines, especially in the perspective of medicine efficacy and children's autonomy in using them.

Material and Methods

Study Design

This research was conducted in the city of Padang, West Sumatra Province, Indonesia from June to December 2018 with a cross-sectional survey method. The location of the study was conducted in 10 primary schools spread over 3 sub-districts namely North Padang, South Padang and Bungus Teluk Kabung which represented the different socio-economic status of the community, namely people living in the city centre, middle and suburbs. The sample in this study was elementary school-age children in class V. Sampling uses the stratified random sampling method.

The first stage is the selection of schools using the probability proportional to size (PPS) method, which is based on a database of the number of elementary school students in the sub-district as a size that is used as the basis for opportunities in selecting samples. From selected schools, student samples were selected using the simple random sampling method. The sample size was calculated using the Lemeshow formula to obtain as many as 503 samples.

The inclusion criteria from the sample are domiciled in the city of Padang, fifth-grade elementary school students; children have used medicines because of illness or other reasons and are willing to become respondents. Whereas the exclusion criteria are moving schools or houses outside the city of Padang and difficult to contact

Instrument

This research is a quantitative study using an instrument in the form of a closed questionnaire to see children's perceptions and beliefs about medicines and their use. This questionnaire was made based on similar research that has been previously modified, which has been adapted to the conditions in Indonesia. The questionnaire was validated at the research location, namely in the city of Padang, Indonesia. This questionnaire consists of 3 parts, namely the first part contains the sociodemographic characteristics of the respondent, the second part contains the perceptual aspects, and the third part contains aspects of trust or behaviour. In the perception, section consists of 2 subsections, namely medicine efficacy related to medicine formulation and medicine efficacy related to the price and source of the medicine obtained. For the belief or behaviour section divided into 3 subsections, each of which is access gets medicine, saves medicine at home and autonomy takes medicine.

Perception is answered with one of 3 choices, namely yes, no and doesn't know. Whereas the part of trust or behaviour consists of answers never, never or

do not know. Specifically, for answers in the perception section, for each correct answer given a score of 1 and the wrong or not knowing given a score of 0. The draft questionnaire that has been prepared, asked for opinions of community pharmacists and clinical pharmacy related to content validation and then validation test 30 elementary school students. Before the research began, the research team who served as enumerators were gathered to be given training on how to collect data on students. Filling out questionnaires by students is done in the classroom with the help of enumerators. Completing this questionnaire takes about 30 minutes.

Data analysis

The collected data is coded and then sent to the SPSS database for Windows version 21. Univariate analysis (descriptive) includes frequency, percentage, average and standard deviation. Bivariate analysis between dependent variables (perceptions) and independent variables (respondents' sociodemographic characteristics) was determined using the Chi-Square test. If the Chi-Square test does not meet the requirements, the Kolmogorov Smirnov test is used. Data is normally distributed. The level of significance was set at $p < 0.05$.

Ethical considerations

Ethical approval was obtained from the Ethics number: 464/KEP/FK/2018 Committee of the Medical Faculty of Andalas University, Padang. Each parent of the student selected as a sample was also asked for his consent to permit his child to be included in this study. Before starting the research, prior permission was submitted to the Education Office of the City of Padang, West Sumatra. After that, a research implementation letter was submitted to each selected school through the Chair of the Public Health Study Program, Faculty of Medicine, Andalas University.

Results

Sociodemographic characteristics of respondents are shown in Table 1.

Respondents, in general, were children aged 10-11 years (73.4%), male sex (52.3%) and 43.3% had received 10 (ten) major achievements at school. Respondents also generally reside in the city centre, namely in North Padang and the middle region in South Padang (80.0%). Judging from the income of his family, 48.1% of respondents came from families that had a moderate income. Meanwhile, 32.6% of respondents have family members who work in the health sector. People who are considered the most accompanying children when they are sick are 85.3%. Also, 24.7% said that they had been hospitalised.

Table 1: Characteristics of sociodemography of children

Sociodemography of respondents	Variables	Amount	%
Age	10 – 11	369	73.4
	12 – 14	134	26.6
Gender	Female	240	47.7
	Male	263	52.3
Residence	North Padang	201	40.0
	South Padang	201	40.0
	Bungus Teluk Kabung	101	20.0
Family income	≤ Rp 2.500.000	197	39.2
	Rp 2.500.000 – 5.000.000	242	48.1
	≥Rp 5.000.000	64	12.7
Families as health workers	Yes	164	32.6
	No	339	67.4
Companion during illness	Father	46	9.1
	Mother	429	85.3
	Another Brother/Sister	28	5.6
Sources of information for medicine	Parents	335	66.6
	Friends/another people	14	2.8
	School Teacher	48	9.5
	Medicines advertisement in the newspaper	10	2.0
	Medicines advertisement on TV	67	13.3
	Internet	29	5.8
Achievements	Top 10	218	43.3
	Not included in the top 10	285	56.7
Have been treated at the Hospital	Ever	124	24.7
	Never	379	75.3

For medicine information, children get more from their parents, which is around 66.6%.

The children's perceptions of medicine efficacy can be seen in Table 2. Here, perceptions of medicine efficacy were divided into 2 types, namely children's perceptions of the relationship of efficacy to aspects of medicine formulation and children's perceptions of the relationship of efficacy to aspects of price and source of medicines obtained. The medicine formulation here is in terms of taste, colour and size. From this study, it was found that children's perceptions of medicine efficacy, in general, seemed very superficial. Only 46.1% of children whose perceptions are correct; there is no relationship between the efficacy of the medicine and the taste of the medicine. As many as 39.4% said they did not know what the efficacy relationship with medicine colour was, as *well* as 36.6% stated that they did not know what the efficacy relationship with tablet size.

Table 2: Child's perception of medicine efficacy

Statement	Amount (%)		
	Yes	No	Don't know
Efficacy relationship with medicine formulation			
The taste of the medicine (sweet or bitter) affects the efficacy of the medicine	133 (26.4)	232 (46.1)	138 (27.4)
Medicine color (colored or white) affects medicine drug efficacy	113 (22.5)	192 (38.2)	198 (39.4)
Tablet size (large or small) affects medicine efficacy	142 (28.2)	177 (35.2)	184 (36.6)
Efficacy relationships with prices and sources of medicine drugs are obtained			
Medicine prices (expensive or cheap) affect medicine efficacy	162 (32.2)	218 (43.3)	123 (24.5)
Where to buy medicine (pharmacies or medicine drug stores) affect the efficacy of the medicine	247 (49.1)	144 (28.6)	112 (22.3)
How to get medication (using prescription or without a prescription) affects the efficacy of the medicine	358 (71.2)	80 (15.9)	65 (12.9)

However, only 38.2% of the perceptions were true that there was no correlation between efficacy with medicine colour and 35.2% stated that there was no relationship of efficacy with tablet size. Regarding the medicine efficacy relationship with medicine prices, 43.3% stated that it was not related to the efficacy with medicine prices, and then 49.1% stated

that the place to buy medicines was related to medicine efficacy and almost most children. 71.2% stated that how to get the medicine that is, by prescription or without a doctor's prescription, is associated with medical efficacy.

Comparison of the results of this study with previous studies specifically for taste relationships and medicine size is presented in Table 3. From Table 3, the results are similar to previous studies.

Table 3: Comparison of several results of studies on the perception of medicine in children in several countries

Statement	% Statement correct answer from the statement				
	Results of this study	Malaysia (Dawood 2015)	Armenia (Bush 2010)	Nepal (Bush 2010)	USA (Bush 2010)
The taste of the medicine (sweet or bitter) does not affect the efficacy of the medicine	46.1	37.7	30	57	63
Tablet size (large or small) does not affect medicine efficacy	35.2	66.1	35	55	22

*Age of children in Malaysia 11-12 years. Armenia 10-13 years. Nepal 9-13 years and USA 10-12 years. This study alone in children 10-14 years.

To see the relationship between the sociodemographic characteristics of respondents with perceptions about the efficacy of the medicine above, bivariate analysis was performed using the chi-square test. The results of the analysis are presented in table 4, and the results show that there are only two variables that have a significant relationship ($P < 0.05$) with the child's perception of the efficacy of the medicine, namely the area where the respondent lives and the child companion when sick.

Table 4: Sociodemographic relationships with respondents' perceptions

No.	Variables	Mean (SD)	Median (min-max)	P-value
Age	10-11	2.11 (1.58)	2.0 (0-6)	0.444
	12-14	1.96 (1.36)	2.0 (0-6)	
Gender	Female	2.08 (1.43)	2.0 (0-6)	0.752
	Male	2.07 (1.61)	2.0 (0-6)	
Residence	North Padang	2.42 (1.59)	2.0 (0-6)	0.000*
	South Padang	1.86 (1.48)	2.0 (0-6)	
	Bungus Teluk Kabung	1.81 (1.35)	2.0 (0-6)	
Family income	≤ Rp 2.500.000	1.94 (1.46)	2.0 (0-6)	0.132
	Rp 2.500.000 – 5.000.000	2.10 (1.54)	2.0 (0-6)	
	≥ Rp 5.000.000	2.38 (1.65)	2.0 (0-6)	
The family as a health worker	Yes	2.12 (1.60)	2.0 (0-6)	0.535
	None	2.05 (1.52)	2.0 (0-6)	
Companion during illness	Father	2.78 (1.49)	3.0 (0-6)	0.000*
	Mother	1.97 (1.51)	2.0 (0-6)	
	Another Brother/Sister	2.54 (1.48)	2.5 (0-5)	
Information source for medicines	Parents	1.94 (1.48)	2.0 (0-6)	0.068
	Friend/another people	2.71 (1.54)	3.0 (0-6)	
	School teacher	2.19 (1.75)	2.0 (0-6)	
	Medicines advertisements in newspaper	1.90 (1.52)	1.5 (0-4)	
	Medicines advertisements on TV	2.43 (1.63)	2.0 (0-6)	
	Internet	2.31 (1.20)	2.0 (0-5)	
Achievements	Top 10	2.09 (1.49)	2.0 (0-6)	0.725
	Not include top 10	2.06 (1.56)	2.0 (0-6)	
Having been treated in a hospital ever	Ever	2.02 (1.43)	2.0 (0-6)	0.933
	Never	2.09 (1.56)	2.0 (0-6)	

Children living in the downtown area of Padang City showed a higher average perception score of 2.42 (SD 1.59) on a scale of 6 than children living in the suburbs of South Padang and Bungus Teluk Kabung, respectively 1.86 (1.48) and 1.81 (1.35). Whereas the child accompanied by the father when sick had a higher average perception score of 2.78 (1.49) than if accompanied by mothers and other siblings, namely 1.97 (1.51) and 2.54 respectively (1.48).

The child's behaviour in taking medication can be seen in the results in Table 5. The behavior of children taking medicine in this table are grouped on 3 things, the behaviour of how children access to medicines, store medicines at home and autonomy children using medicines. Almost most of the children, which amounted to 83.9%, said they were obedient in taking medication. Only 10.1% said that they had never obeyed their medication. Whereas related to the autonomy of children in taking medicine. The percentage was almost the same, namely, some children (48.5%) stated that they had to depends on their parents when they wanted to take medicine and others (47.1%) also stated that they did not always depend on their parents when you want taking medication.

Table 5: Child behaviour in medicine

Statement	Amount (%)		
	Yes	No	Don't know
Medicine saving behaviour			
Have children ever refused (not obeyed) to take medicine when they were sick?	51 (10.1)	422 (83.9)	30 (6.0)
Autonomy for medicine use			
Do children always depend on parents when taking medication	244 (48.5)	237 (47.1)	22 (4.4)
When sick, children have taken their medication without being accompanied by parents or family	239 (47.5)	233 (46.3)	31 (6.2)
Access to get medicine			
Have children ever bought over-the-counter medicine at a pharmacy or medicine store, without the knowledge of parents?	58 (11.5)	428 (85.1)	17 (3.4)
Have children ever taken the medicines themselves that will be taken from the medicine storage, without being told by parents?	158 (31.4)	316 (62.8)	29 (5.8)

The same pattern of answers is also shown in statements about the experience of children taking their medication without being accompanied by parents or family. As many as 47.5% of children stated that they never took medicine without being accompanied by a parent, but as many as 46.3% said they had taken their medication without being accompanied by a parent. Regarding children's access to medicines, the results showed that 85.1% of children never bought their over-the-counter medicines to pharmacies or medicine stores without the parents' knowledge even though 11.5% of them had instead bought over-the-counter medicines without parental knowledge and 10.1% had kept it at home.

As many as 62.8% of children also stated that they never took their medication at home without the parents 'knowledge but instead 31.4% had taken medicines at home without the parents' knowledge.

Discussion

Medicine perception in children, especially about medicine efficacy, is usually formed when looking at the physical appearance of the medicine itself. Children's perceptions of medicine efficacy can be explained in the following two categories. Namely, children's perceptions of the relationship or relationship of efficacy with formulation factors and medicine sources obtained.

The formulation factor is in the form of physical attributes consisting of taste, colour and size. The taste, colour and size of medicine has no relation to the efficacy or efficacy of the medicine. The percentage of children who attributed the efficacy of the medicine to their taste, colour and size were 26.4%; 22.5% and 28.2%. These results are lower than those found by previous researchers in Spain, namely 61.3% for taste and 53.0% for size [8]. Older children know that medicine efficacy is not influenced by the colour of medicine [8]. The results of other studies also support these findings that many children assume that taste, colour and size are related to medicine efficacy [16].

While the relationship between the efficacy of the medicine and the price and source of the medicine can be explained as follows. A total of 43.3% of children stated that there was no relationship between the price of the medicine and the efficacy of the medicine. In contrast, 32.2% of children stated that prices affected the efficacy of the medicine. Other researchers found the same fact that some children believe that prices influence the efficacy of medicines [16].

However, for the relationship of efficacy with the source of the medicine obtained, the children turned out to be more confident that the official place of medicine such as the pharmacy influenced the efficacy of the medicine which was equal to 49.1%. 37.8% of children in Spain also stated the same [8] and by other researchers [16]. Likewise, getting prescription medication or without a doctor's prescription according to the child. This factor has the greatest effect on medicine efficacy, which is 71.2%. The same results were also shown by previous studies that 60.7% of children stated that prescription medicines were better in efficacy [8]. This shows that the child's trust in the doctor is higher so that the child believes that each medicine given by a doctor is very helpful in healing the disease. If the results of this study are taste and size factors compared to some previous studies [19] can be summarised as in Table 3. From this table, it can be concluded that children's perceptions of each country are different about medicine efficacy. This difference can be caused because of the method of research conducted. However, the results obtained from this study are not very different from the results of previous studies.

From this analysis, two factors significantly influence ($P < 0.05$) on the respondents' average perceptions of medicine efficacy. This factor is the address of the respondent's residence and the child's companion when sick. Children living in the city centre who described a high socio-economic status (SES) in North Padang had a better perception than children living in the suburbs with low SES status, namely in South Padang and Bungus Teluk Kabung. Previous research has shown that SES affects children's level of knowledge about medicines [10], [19].

Child companion when sick has a role in shaping the child's perception of medicine efficacy. Parents are the main source of medical information from children as in Table 1, so it is reasonable that children accompanied by parents when sick have an average score of perception of medicines that is better than other siblings. Overall it can be concluded that children's perceptions of medicine efficacy are still very superficial. This can be seen from the very small average score of perceptions, which is around 2 on a scale of 6. This is thought to be due to the low and limited knowledge of children about medicines. Nearly all studies conducted by other researchers about children's knowledge related to medicine show the fact that children's knowledge of medicines is indeed very limited and fragmented [8], [9], [10], [12], [20], [21], [22]. The low level of children's knowledge can also have an impact on children's beliefs in medicine. In this research, children's trust in medicines is explained by the behaviour of children using medicines and how children can remember the medicines they have used.

Children's behaviour in using medicines here can be categorised into three subsections, namely medication adherence, the autonomy of medicine use and access to obtaining medicines. In Table 5, it can be seen how children's behaviour towards medicines. The percentage of children who answered did not know small enough that was 3.4-6.2%. This certainly illustrates that children can still remember their experiences of using medicines. In general (83.9%) children said they were never stored the medicine at home. Only about 10.1% of children who never store the medicine at home

This shows that children are not afraid of medicines and can realise that medicines can cure if used regularly. This is in line with other studies that show that medicines should be taken when sick and when needed [12], [16], regarding the autonomy of children in using medicines. Some children state that they are dependent on their parents and others do not depend on their parents. The percentage comparison is almost the same between the two. As many as 48.5% of children always depends on parents or their parents when they are taking medicine, and on the other hand, 47.1% of children say they don't always wait for their parents when they are taking medication. As many as 47.5% of children never took their medication without the knowledge of their parents and

as much as 46.3% said they had taken their own medication without the parents' knowledge. Judging from these results it can be concluded that the autonomy of children in using medicines is quite large.

In developed countries like the United States, children are convinced of themselves as active medicine users, and they are reported to have more autonomy in using medicines. Another study said that 44% of children aged 9-16 years always carry their medicine when summer camp activities and 25% of them aged 9-12 years use it themselves without the knowledge of parents [5], for access to medical treatment, almost most of the children. Around 85.1% stated that they had never bought over-the-counter medicines without the parent's knowledge. However, there are around 11.5% of children who have bought over-the-counter medicines themselves. The behaviour of buying medicines on their own if not accompanied by sufficient knowledge is very vulnerable to harming children, especially with risks such as the occurrence of side effects or possibly medicine poisoning.

To access medicine at home, children are generally equal to 62.8%, never taking their own medicine from the place of storage. However, there are around 31.4% of children who have taken their medication without parents' knowledge. This shows that medicine storage at home is not too strict in its supervision and can be accessed by children. The same thing was also shown by other researchers [10], [17], [19].

In line with the results above where children are more dependent on parents when using medicines. The same thing is shown by the child's ability to remember the medication he has used. Children think that the medical problem is entirely a matter of parents so that children feel they do not need to remember or know what medicines they are taking. In contrast, in developed countries like Finland, children are very familiar with medicines [2], [12].

Approximately 13.3% of children get medical information through TV advertisements which are the second-largest source of medicine information after parents as noted in Table 1. While in Finland, children generally receive medicine information from professionals such as pharmacists and doctors and media such as the internet and medicine packaging [10], [12], [20].

Unlike in Greece, children get a lot of medical information from their school teachers [10]. Also, children who have experience using medicines when sick usually know the name of a commonly used medicine [10], [23]. This has to do with the limited knowledge of children about medicines. Previous studies state that children knowledge of benefits. Risks and use of medicine are still low and very limited [24].

Based on the results above, it can be concluded that the child's perception of the efficacy of medicine is still very superficial. Children cannot understand that the taste, colour, size, price, place of purchase and how to get the medicine are not at all related to the efficacy of the medicine. Likewise, the autonomy of children in using medicines cannot be completely removed without the knowledge of parents.

The limitations and fragmentation of children's knowledge about medicines can have an impact on the child's low belief in medicines that can be beneficial on the one hand and on the other, they can be harmful to the body. Therefore, we recommend that it is very important to apply medical education to children, especially in schools. With this medicine education children are expected to be able to obtain sufficient knowledge about medicine so that it can become a provision for children to fulfill the demands of being active and rational medicine addicts. In the end children can also be expected to be agents of change for their families in using rational medicines.

Limitation of research: This research is only limited to children who attend elementary school in grade V only so they have not described school children in general.

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Reference

1. Cruz MJ, Dourado LF, Bodevan EC, Andrade RA, Santos DF. Medication use among children 0-14 years old: population baseline study. J Pediatr (Rio J). 2014; 90(6):608-615. https://doi.org/10.1016/j.jped.2014.03.004 PMid:24953722

2. Hansen D, Holstein B, Due P, Currie C. International survey of self-reported medicine use among adolescents. Ann Pharmacother. 2003; 37:360-366. https://doi.org/10.1345/aph.1C111 PMid:12639163

3. Ylinen S, Hämeen-Anttila K, Sepponen K, Lindblad AK, Ahonen R. The use of prescription medicines and self-medication among children - a population-based study in Finland. Pharmacoepidemiol Drug Saf. 2010; 19(10):1000-1008. https://doi.org/10.1002/pds.1963 PMid:20712023

4. Geissler PW, Meinert L, Prince RJ, Nokes C, Aaagaard-Hansen J, Jitta J, et al. Self-treatment by Kenyan and Ugandan schoolchildren and the need for school-based education. Health Policy and Planning. 2001; 16(4):362-371. https://doi.org/10.1093/heapol/16.4.362 PMid:11739361

5. Hämeen-Anttila K, Bush PJ. Healthy children's perceptions of

medicines: a review. Research in Social and Administrative Pharmacy. 2008; 4(2):98-114. https://doi.org/10.1016/j.sapharm.2007.05.002 PMid:18555964

6. Sharaideh R, Wazaify M, Albsoul-Younes AM. Knowledge and attitude of school children in Amman/Jordan toward the appropriate use of medicines: A cross-sectional study. Saudi Pharmaceutical Journal. 2013; 21(1):25-33. https://doi.org/10.1016/j.jsps.2012.01.001 PMid:23960817 PMCid:PMC3745049

7. Bush PJ, Ozias JM, Walson PD, Ward RM. Ten guiding principles for teaching children and adolescents about medicines. US Pharmacopeia. Clin Ther. 1999; 21(7):1280-1284. https://doi.org/10.1016/S0149-2918(00)80030-2

8. Aramburuzabala. P. Children's Knowledge of Medicines. Implications for Health Education. Educacao Sociedade & Culturas. 2013; 38:135-149.

9. Bankar MA, Sujata SD. Promoting the proper use of medicines in rural school children of India. Int J Basic Clin Pharmacol. 2013; 2(4):375-380. https://doi.org/10.5455/2319-2003.ijbcp20130806

10. Bozoni K, Kalmanti M, Koukouli S. Perception and knowledge of medicines of primary schoolchildren: the influence of age and socioeconomic status. Eur J Pediatr. 2006; 165(1):42-49. https://doi.org/10.1007/s00431-005-1760-6 PMid:16222526

11. Almarsdottir AB, Zimmer C. Children's knowledge about medicines. Childhood. 1998; 5(3):265-281. https://doi.org/10.1177/0907568298005003003

12. Hämeen-Anttila K, Juvonen M, Ahonen R, Bush PJ, Airaksinen M. How well can children understand medicine related topics? Patient Educ Couns. 2006; 60(2):171-178. https://doi.org/10.1016/j.pec.2004.12.011 PMid:15939568

13. Geissler PW, Nokes K, Prince RJ, Achieng'Odhiambo R, Aagaard-Hansen J, Ouma JH. Children and medicines: self-treatment of common illnesses among Luo schoolchildren in western Kenya. Social science & medicine. 2000; 50(12):1771-83. https://doi.org/10.1016/S0277-9536(99)00428-1

14. Holstein BE, Hansen EH, Due P, Almarsdo AB. Self-reported medicine use among 11- to 15-year-old girls and boys in Denmark 1988 - 1998. Scand J Public Health. 2003; 31:334-341. https://doi.org/10.1080/14034940210165082 PMid:14555369

15. Lindell-Osuagwu L, Sepponen K, Farooqui S, Kokki H, Hämeen-Anttila K, Vainio K. Parental reporting of adverse drug events and other drug-related problems in children in Finland. European journal of clinical pharmacology. 2013; 69(4):985-94. https://doi.org/10.1007/s00228-012-1426-z PMid:23093040

16. Menacker F, Aramburuzabala P, Minian N, Bush PJ, Bibace R. Children and medicines: What they want to know and how they want to learn. J Soc Adm Pharm.1999; 16(1):38-52.

17. Sloand ED, Vessey JA. Self-medication with common household medicines by young adolescents. Issues in Comprehensive Pediatric Nursing. 2001; 24(1):57-67. https://doi.org/10.1080/014608601300035625

18. Geest S, Geissler PW. Should medicines be kept away from children? African considerations. Tropical Medicine and International Health. 2003; 8(2):97-99. https://doi.org/10.1046/j.1365-3156.2003.00991.x PMid:12581432

19. Dawood OT, Ibrahim MIM, Abdullah AC. Chidrens knowledge and beliefs about medicines. Journal of child Health Care. 2015; 19(1)73-83. https://doi.org/10.1177/1367493513496911 PMid:23975718

20. Kärkkäinen S, Hämeen-Anttila K, Vainio K, Kontturi S, Patrikainen R, Keinonen T. Fourth graders' perceptions about medicines and medicine use. Health Educ. 2014; 114(1):43-57. https://doi.org/10.1108/HE-03-2013-0009

21. Whatley B, Williams SE, Gard PR, MacAdam A. Healthy children's identification and risk perception of medicines in England. Res Social Adm Pharm. 2012; 8(5):478-483. https://doi.org/10.1016/j.sapharm.2011.11.004 PMid:22264962

22. Dawood OT, Ibrahim MIM, Abdullah AC. Factors influencing chidrens knowledge and attitudes toward medicines in Malaysia. JMH. 2011; 8(4)288-298. https://doi.org/10.1016/j.jomh.2011.04.005

23. Desai C, Girdhar AO, Shah UH. Knowledge and Awareness about Medicines among Primary Schoolchildren in Ahmedabad. India. Regional Health Forum. 2005; 9(2):1-8.

24. Syofyan S, Dachriyanus D, Masrul M, Rasyid R. The Knowledge and Attitudes about the Benefits. Risks and Use of Medicine in Aged Primary Students in Indonesia. Open Access Maced J Med Sci. 2019; 7(11):1860-1866. https://doi.org/10.3889/oamjms.2019.347 PMid:31316674 PMCid:PMC6614253

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Review Article

Celiac Trunk and Hepatic Artery Variants in Pancreatic and Liver Resection Anatomy and Implications in Surgical Practice

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Abstract

The presence of celiac trunk or hepatic arterial anomaly influences preservation of vascular arterial system and achievement of an R0 resection in the liver and pancreatic resection. The objective of this study is to review the literature, to describe the anomalous arterial variations of the celiac trunk and hepatic artery reiterating the importance of identification of the anomaly.

Introduction

The first description of normal and aberrant celiac trunk anatomy was published in 1756 by Haller. Lipshutz seems to have been the first who suggested a classification of the celiac trunk into four types. Later, Adachi presented a more detailed classification, while the two most commonly used classifications were suggested by Morita and Michels [1], [2], [3], [4], [5]. Knowledge of the variant vascular anatomy of upper gastrointestinal region is valuable to hepato-biliary surgeons to limit operative complications due to unexpected bleeding and to the achievement of R0 resection during liver or biliary tree resection or pancreaticoduodenectomy [6], [7]. It is defined typical 'normal', the hepatic artery that arises from celiac trunk and divides into three main branches- the right hepatic, left hepatic and middle hepatic supplying the right, left and the quadrate lobe of the liver respectively. 'Aberrant' hepatic is a hepatic arising otherwise than from a typical celiacal hepatic and is of two types accessory and replaced. The term 'accessory' hepatics should be used only in those cases where the normal celiacal right or left hepatic is present, and there is an additional artery from other sources. When the normal celiacal right or left hepatic artery is missing the replacing vessel coming from another source supplying the right or left lobe is to be termed as a 'replaced' right or left hepatic artery. The coeliac artery, also known as the celiac trunk (CT) or coeliac axis is the first ventral branch of the abdominal aorta. It is a short wide branch about 1.25 cm long, that arises from the aorta at the level of T12–L1 vertebrae immediately below the aortic hiatus of the diaphragm. The normal branching pattern of the CT and the three main branches are left gastric artery (LGA), common hepatic artery (CHA) and splenic artery (SA). The celiac axis and its branches supply the derivatives of foregut, i.e. stomach, spleen, pancreas, liver and part of the duodenum. The most common classical type of branching of CT is known as trifurcation and was first described by Haller in 1756 as tripus Halleri. The incidence of different celiac and hepatic artery anomalies was calculated depending on Uflacker's and Michel's classifications. According to

the Michels' studies about the anatomical vascular anomaly, celiaco-mesenteric arterial aberrations is described in 22%-48% of observations [8], [9]. The literature suggests that the normal pattern of hepatic artery anatomy is observed in 55 to 79% of patients [11], [12], [13]. Since Michels published his first report, several studies have reported not only common and rare hepatic artery variants, but also different classifications [14] (Table 1).

Table 1: Michels and Hiatt classifications (from CT Angiography for Delineation of Celiac and Superior Mesenteric Artery Variants in Patients, Corinne B. Winston et al.) [28]

Hepatic arterial anatomy	Michels classification	Hiatt classification
Normal anatomy	Type I	Type I
LHA branch LGA	Type II	Type II
RHA branch SMA	Type III	Type III
Type I and II association	Type IV	Type IV
LHA accessory LGA	Type V	Type II
RHA accessory SMA	Type VI	Type III
LHA accessory LGA + RHA accessory SMA	Type VII	Type IV
LHA accessory LGA+ RHA branch SMA	Type VIII	Type IV
CHA branch SMA	Type IX	Type V
RHA and LHA branch LGA	Type X	------
CHA aorta branch	--------	Type VI

"------"= liver variation not present in the corresponding classification column. RHA=right hepatic artery; LHA=left hepatic artery; SMA=superior mesenteric artery; LGA = left gastric artery; CHA=common hepatic artery

Material and Methods

Study selection

A systematic search of the literature from PUBMED databases published between 1766 and 2018 was performed. The following search terms were used: celiac trunk, hepatic artery, anomaly, variants, normal hepatic artery, aberrant, accessory, Michels classification. A manual search was also carried out.

Inclusion and exclusion criteria

We retrospectively analysed 100 articles about Hepatic Artery (H.A) and Celiac Trunk Anatomic Variants (CTAV) using Pubmed research. Michels, Uflacker and Hiatt classifications were analyzed (Table 1, 2, 3). The inclusion criteria were: (1) papers are written in English; and (2) manuscript with 100 o most patients. Exclusion criteria: Abstracts, case reports, reviews, low-quality studies and non-comparative studies, and intraoperative data which were unable to be extracted from the published studies were excluded. Here, we describe the most numerous medical records research articles and Michels classification images (Figure 1-9).

Results

Da Fonseca-Neto et al., [15] retrospectively analysed 479 medical records of transplanted adult patients in the 13 years. It was identified as normal hepatic arterial anatomy in 416 donors (86.84%). The other 63 patients (13.15%) showed some variation.

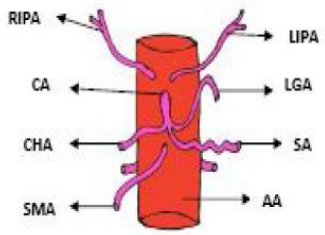

Figure 1: Usual branching pattern of celiac trunk. LGA, left gastric artery; SA, splenic artery; CHA, common hepatic artery; SMA, superior mesenteric artery; RIPA, right inferior phrenic artery; LIPA, left interior phrenic artery; CA, celiac artery; AA, abdominal aorta

According to the Michels classification, the most frequently observed abnormalities were: right hepatic artery branch of superior mesenteric artery (Type III, n = 27, 5.63%); left hepatic artery branch of the left gastric artery (Type II, n = 13, 2.71%); right hepatic artery arising from the superior mesenteric artery associated with the left hepatic artery arising from the left gastric artery (Type IV, n = 4, 0.83%). Similarly, about Hiatt classification, the most prevalent changes were: right hepatic accessory artery or substitute of the superior mesenteric artery (Type III, n = 28, 6.05%)), followed by liver ancillary left artery or replacement of gastric artery left (Type II, n = 16, 3.34. Fourteen donors (2.92%) showed no anatomical abnormalities defined in classifications, the highest frequency being hepatomesenteric trunk identified in five (01.04%).

*H. Gümü*s et al., [16] Evaluated 820 patients who underwent angiography of the abdominal aorta. Anatomical findings were grouped according to the Michels classification. Several variations and/or anomalies were noted in 33.2% of the patients (n = 272). The most common abnormality was Michels type III (10.1%), followed by type V (7.3%), type II (4.7%) and others.

Kuo-Hsien Chiang et al., [17], reviewed 405 patients who underwent upper abdominal arteriography between Aug. 1998 and Sep. 2004. Hepatic artery anatomy was analyzed and classified according to Suzuki's classification. Single hepatic artery group included 283 of 405 cases (69.9 per cent). Double hepatic artery group included 114 cases (28.1 per cent). Multiple hepatic artery group included 8 cases (2.0 per cent). Seventeen patterns were identified in this study.

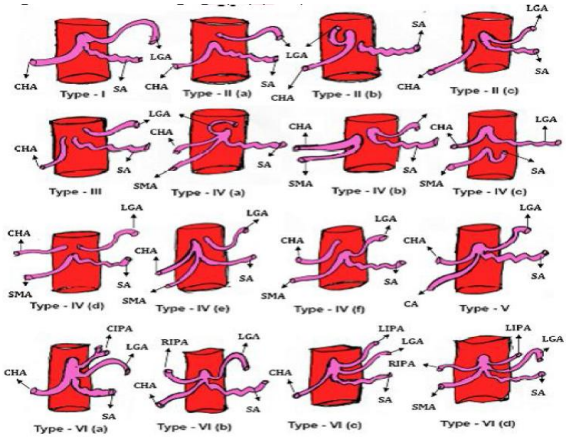

Figure 2: Schematic diagram showing variation in the origin of celiac trunk and its branches. LGA, left gastric artery; SA, splenic artery; CHA, common hepatic artery; SMA, superior mesenteric artery; RIPA, right inferior phrenic artery; LIPA, left interior phrenic artery; CA, celiac artery; AA, abdominal aorta

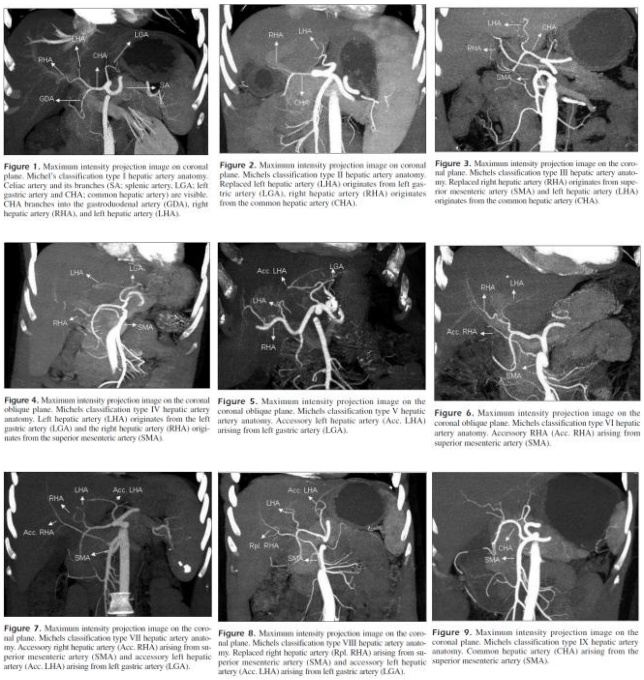

Figure 3: Variations of the celiac trunk and hepatic arteries: a study with 64- detector computed tomographic angiography, H. GÜMÜS et al by European Review for Medical and Pharmacological Sciences (Fig. 1-9)

MS Ugurel et al., [18] analysed retrospectively 100 patients. There was a coeliac trunk trifurcation in 89% and bifurcation in 8% of the cases. The coeliac trunk was absent in 1%, a hepatosplenomesenteric trunk was seen in 1%, and a splenomesenteric trunk was present in 1%. Hepatic artery variation was present in 48% of patients. Coeliac trunk and/or hepatic arterial variation was present in 23 (39.7%) of the 58 patients with normal renal arteries, and 27 (64.3%) of the 42 patients with accessory renal arteries. There was a statistically significant correlation between renal artery variations and coeliac trunk–hepatic arterial system variations (p50.015). They concluded that MDCT angiography permits a correct and detailed evaluation of hepatic and renal vascular anatomy.

Ahmed M. Osman MD et al., [19] retrospectively analysed 1285 Egyptian patients using triphasic CT or CT aortic angiography. The incidence of different celiac and hepatic artery anomalies was calculated depending on Uflacker's and Michel's classifications. They found about 90.5% of the patients showing normal trifurcation pattern of the celiac trunk (Uflacker type I) with the commonest variant was gastro-splenic trunk (Uflacker type V) with 4.3% incidence. The bifurcation pattern was representing 7.7% of cases. Regarding the hepatic artery, 74.3% of the cases showed the normal origin of the hepatic arteries (Michel type I) with the commonest anomaly was Michel type III with 12.5% incidence. Some cases are discovered not previously described by either Uflacker's or Michel's classifications. They concluded that good imaging quality of MSCT angiography had proved effectiveness in depicting different celiac trunk and hepatic artery variants.

MK Tharao et al., [20] studied one hundred and two subhepatic regions in adult Kenyans by gross dissection for the pattern of arterial blood supply. Measurements were made for the distance of hepatic bifurcation from the liver. The common hepatic artery (CHA) originated from the celiac trunk in 95.1% and the superior mesenteric artery (SMA) in 4.9% of cases. The mean distance of bifurcation from the liver was 2.6 cm, and the CHA gave rise to 2 hepatic branches in 93.1% of cases. The right hepatic artery (RHA) passed anterior to the CHD in 48%, posterior in 41.2% and 7.8% was related to the common bile duct (CBD). Accessory RHA and left hepatic arteries (LHA) were observed in 10.8% and 9.8% of the cases.

Jonathan R. Hiatt et al., [21], recorded of 1000 patients who underwent liver harvesting for orthotopic transplantation between 1984 and 1993 were reviewed. Arterial patterns in order of frequency included the normal Type 1 anatomy (n = 757), with the common hepatic artery arising from the celiac axis to form the gastroduodenal and proper hepatic arteries and the proper hepatic dividing distally into right and left branches; Type 3 (n = 106), with a replaced or accessory right hepatic artery originating from the superior mesenteric artery; Type 2 (n = 97), with a replaced or accessory left hepatic artery arising from the left gastric artery; Type 4 (n = 23), with both right and left hepatic arteries arising from the superior mesenteric and left gastric arteries, respectively; Type 5 (n = 15), with the entire common hepatic artery arising as a branch of the superior mesenteric; and Type 6 (n = 2), with the common hepatic artery originating directly from the aorta.

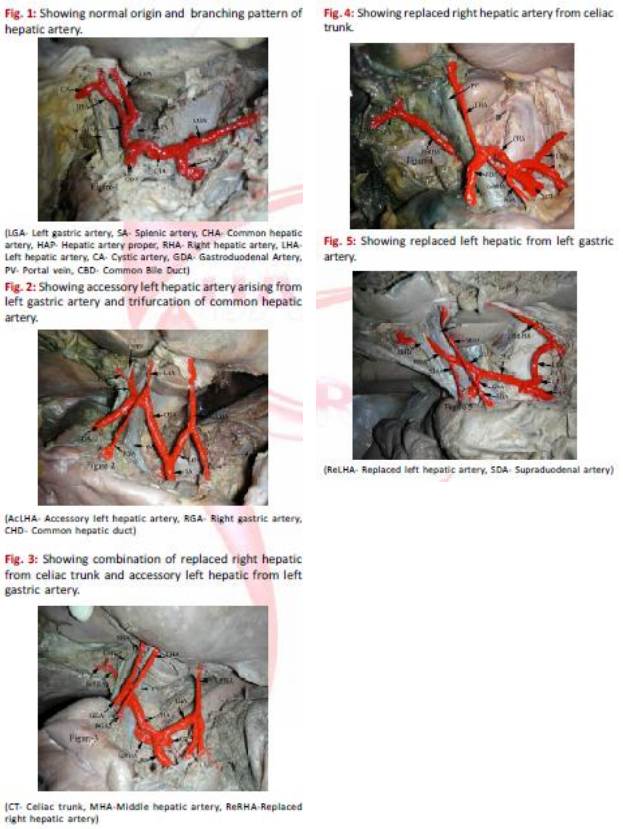

Figure 4: Variants in celiac trunk. From Kavitha Kamath. B. Int J Anat Res 201 [8]

A. Ahmed et al., [22], do a retrospective study in which 1000 post-contrast CT scans of the abdomen between July 2011 and June 2012. Out of the 10 Mitchel's hepatic arterial variations, 9 were seen. Type I was most common, and 678 (67%) patients had it. Type II variation was seen in 178 (17%) patients, type III in 34 (3.4%) type V in 25 (2.5%) patients.

Kavitha Kamath. B. [23], studied extrahepatic branching pattern of hepatic arteries in 40 embalmed cadavers. Classical textbook pattern of hepatic arterial anatomy was seen in 30(75%) cases, and ten (25%) cases showed the presence of aberrant hepatic arteries. 12 aberrant hepatic arteries were seen in these ten cases, eight (20%) cases with single aberrant hepatic artery and two (5%) with a combination of aberrant right and left hepatics. Aberrant right hepatic arteries were seen in four (10%) cases, and all of them were replaced right hepatics arising directly from the celiac trunk. Aberrant left hepatic arteries were seen in eight (20%) cases, of which six (15%) were accessory, two (5%) were replaced, and all of them arose from the left gastric artery.

Azhar Perwaiz et al., [24] analysed 200 consecutive pancreaticoduodenectomies finding Fifty-three patients (26.5%) that had arterial anomalies. The complexity of the surgery was determined by the course of these arteries. The mean duration of surgery was 420 ± 32.0 minutes in patients with arterial anomalies versus 370 ± 38.5 minutes in those with normal arterial anatomy (P = 0.005). They concluded that during pancreaticoduodenectomy, arterial anomalies could increase operative complexity but do not usually compromise the safety of the procedure or its oncological outcome.

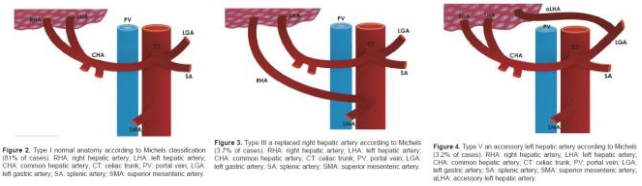

Figure 4: Variants in celiac trunk. From George Noussios J Clin Med Res. 2017;9(4):248-252)

George Noussios et al., [25], retrieved twenty articles. A total of 19,013 patients were analysed]. 81% of the cases displayed normal anatomy. A replaced right hepatic artery (RHA) arose from the superior mesenteric artery (SMA) in 3.7% of cases, while a replaced left hepatic artery (LHA) stemmed from the left gastric artery in 3% of cases. Both a replaced RHA and a left one were found in 0.8% of cases, while an accessory LHA and an accessory RHA were present in 3.2% and 1.6% of cases, respectively. A common hepatic artery (CHA) originating from the SMA appeared in 1.2% of cases. Finally, 784 cases (4.1%) encountered in were rare unreported anomalies or more commonly unclassified anomalies.

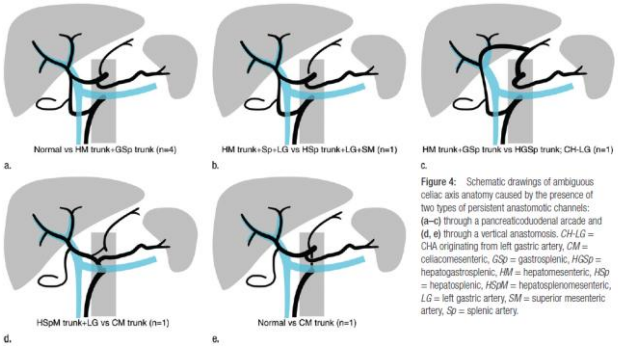

Figure 5: Variants in celiac trunk. From Soon-Young Song. Radiology: Volume 255: Number 1—April 2010 [27]

Rafael Lo´pez-Andujar et al., [26], investigated the surgical anatomy of the extrahepatic arterial vascularisation prospectively in 1,081 cadaveric donor livers, transplanted from January 1991 to August 2004. The vascular anatomy of the hepatic grafts was classified according to Michels description. Anatomical variants of the classical pattern were detected in 30% of the livers (n 320). The most common variant was a replaced left artery arising from the left gastric artery (9.7%) followed by a replaced right hepatic artery arising from the superior mesenteric artery (7.8%).

Soon-Young Song et al., [27], retrospectively

evaluated 5002 patients who underwent spiral CT and digital subtraction angiography (DSA). Of 15 possible types of CA variation, 13 types were identified. A normal CA was noted in 4457 (89.1%) of the 5002 patients. Twelve types of Celiac Axis (CA) variation were identified in 482 (9.64%) patients. In the remaining 63 (1.26%) patients, the CA anatomy was classified as ambiguous because the Common Hepatic Artery (CHA) was absent owing to separate origins of the hepatic arteries and the gastroduodenal artery (*n* = 55) or because the origin of the CHA could not be determined owing to persistent anastomotic channels (*n* = 8). Seven CHAs originating from the normal CA had a retroportal (*n* = 6) or transpancreatic (*n* = 1) course. All eight CHAs originating from the left gastric artery passed the fissure of the ligamentum venosum. The 148 CHAs originating from the superior mesenteric artery showed diverse relationships with the pancreas-being supra-, trans-, or intrapancreatic- and the superior mesenteric-portal venous axis-being pre- or retroportal. The 20 CHAs originating from the aorta had a normal suprapancreatic periportal course.

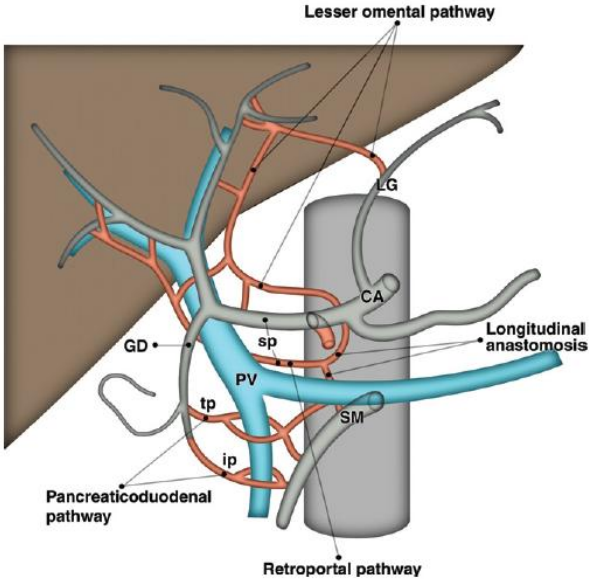

Figure 6: Schematic drawing illustrates hypothetical anatomic model to summarize possible anatomic courses of celiac axis and CHA variations. Gray indicates normal arterial anatomy. Red indicates possible anatomic course. CA, celiac axis; GD, gastroduodenal artery; ip, intrapacreatic course; LG, left gastric artery; PV, portal vein; SM, superior mesenteric artery; sp, suprapancreatic course; tp, transpancreatic course

Corinne B. Winston et al., [28], analyzed a CT Angiography (CTA) database from August 2001 to February 2003 that included 394 consecutive CT angiograms in 371 patients, all of whom were under consideration for surgical resection for a suspected pancreatic or hepatobiliary neoplasm One hundred eighty-eight (51%) of the 371 patients had classic arterial anatomy identified at abdominal CT Angiography (CTA). One hundred sixty-two (44%) patients had a single arterial variant identified, and 21 (6%) patients had more than one arterial variant seen. The most common variant identified was a replaced right hepatic artery originating from the superior mesenteric artery (SMA), which was seen in 54 (15%) patients. The second most common variant was a replaced left hepatic artery originating from the left gastric artery, seen in 30 (8%) patients. Variations in the origin of the common hepatic artery were seen in 12 (3%) patients. In six (2%) patients, the common hepatic artery originated from the SMA, and in six (2%) patients, the common hepatic artery originated from the abdominal aorta. A “double hepatic artery,” as described by Fasel et al., and Covey et al., refers to one or both hepatic arteries originating from the celiac axis or the aorta. This was seen in 15 (4%) of the patients in our study population. The right hepatic artery originated from the celiac axis in 13 (4%) patients and from the aorta in one (< 1%) patient. The left hepatic artery originated from the celiac axis in one (< 1%) patient. Accessory hepatic arteries were identified in 16 (4%) patients. We showed some exmples of variants of hepatic artery (From Kavitha Kamath. B. Int J Anat Res 2015) (Figure 1, 2, 3, 4, and 5). We reported the figures reconstruction of celiac axis variants (From Dilli Babu E., Int J Anat Res 2013) (Figure 1, and 2).

Discussion

Modifications of the hepatic branch and the celiac axis, occur in 25% to 75% of cases. According to Michels classification, the most frequent change according to the literature is the type III present in from 6-15.5% of cases. It seems the most important because it has the potential to affect surgical procedures being indispensable its identification. The second most common is type II, reported in literature between 2.5-10%. Type IV is described with an incidence of 1-7.4%. The types VII, VIII, IX and X are rarely described in literature. [1], [2], [3], [4], [5], [6], [7], [8], [9], [10], [11], [12], [13], [14] This is demonstrated from the figures (from George Noussios J Clin Med Res. 2017; 9(4):248-252) (Figure 2, 3, and 4). We finded also schematic drawings of ambiguous celiac axis anatomy caused by the presence of two types of persistent anastomotic channels (From Soon-Young Song, MD Radiology: Volume 255: Number 1—April 2010) (Figure 4, and 7).

Conclusion

The precise knowledge of the celiac trunk and hepatic artery variants and anomalies is very important pre-surgical, prelaparoscopic or even pre-interventional. Unknowledge these variants produces different technical difficulties, or challenges. It is

essential in order to avoid damage, vascular surgical complications. and decrease the morbidity of patients [15], [16], [17], [18], [19], [20], [21], [22], [23], [24], [25], [26], [27], [28].

References

1. Haller A. Icones anatomicae in quibus aliquae partes corporis humani delineatae proponuntur et arteriarum potissimum historia continetur. Gottingen, 1756.

2. Lipshutz B. A Composite Study of the Coeliac Axis Artery. Ann Surg. 1917; 65(2):159-169. https://doi.org/10.1097/00000658-191702000-00006 PMid:17863663 PMCid:PMC1426316

3. Adachi B. Das Arteriensystem der Japaner. Vol.2. verlag der Kaiserlich- Japanischen Universitat zuKyoto, 1928.

4. Morita M. Reports and conception of three anomalous cases on the area of the celiac and the superior mesenteric arteries. Igaku Kenkyu (Acta Med). 1935; 9:159-172.

5. Michels NA. Blood supply and anatomy of the upper abdominal organs, with a descriptive atlas. Lippincott, Philadelphia. 1955;139-143.

6. Thapa PB, Yonjen TY, Maharjan DK, Shrestha SK. Anatomical Variations of Hepatic Artery in Patients Undergoing Pancreaticoduodenectomy. Nepal Med Coll J. 2016; 18(1-2):62-7.

7. Tharao MK, Saidi H, Kitunguu P, Ogengo JA. Variant anatomy of the hepatic artery in adult Kenyans. Eur J Anat. 2007; 11(3):155-161.

8. Kamath BK. A study of variant hepatic arterial anatomy and its relevance in current surgical practice. Int J Anat Res. 2015; 3(01):947-53. https://doi.org/10.16965/ijar.2015.124

9. Dilli Babu E, Khrab P. Coeliac trunk variations: review with proposed new classification. Int J Anat Res. 2013; 1(3):165-70.

10. Koops A, Wojciechowski B, Broering DC, Adam G, Krupski-Berdien G. Anatomic variations of the hepatic arteries in 604 selective celiac and superior mesenteric angiographies. Surg Radiol Anat. 2004; 26:239-44. https://doi.org/10.1007/s00276-004-0229-z PMid:14968265

11. Covey AM, Brody LA, Maluccio MA, Getrajdman GI, Brown KT. Variant hepatic arterial anatomy revisited: digital subtraction angiography performed in 600 patients. Radiology. 2002; 224:542-7. https://doi.org/10.1148/radiol.2242011283 PMid:12147854

12. Michels NA. Newer anatomy of the liver and its variant blood supply and collateral circulation. Am J Surg. 1966; 112:337-47. https://doi.org/10.1016/0002-9610(66)90201-7

13. Soin AS, Friend PJ, Rasmussen A, Saxena R, Tokat Y, Alexander GJ, et al. Donor arterial variations in liver transplantation: management and outcome of 527 consecutive grafts. Br J Surg 1996; 83:637-41. https://doi.org/10.1002/bjs.1800830515 PMid:8689206

14. López-Andújar R, Moya A, Montalvá E, Berenguer M, De Juan M, San Juan F, Pareja E, Vila JJ, Orbis F, Prieto M, Mir J. Lessons learned from anatomic variants of the hepatic artery in 1,081 transplanted livers. Liver transplantation. 2007; 13(10):1401-4. https://doi.org/10.1002/lt.21254 PMid:17902125

15. Lima HC, Rabelo P, Amorim AG, Lacerda CM. Anatomic Variations of Hepatic Artery: A Study In 479 Liver Transplantations. ABCD. Arquivos Brasileiros de Cirurgia Digestiva (São Paulo). 2017; 30(1):35-7. https://doi.org/10.1590/0102-6720201700010010 PMid:28489166 PMCid:PMC5424684

16. Gümüs H, et al., Variations of the celiac trunk and hepatic arteries: a study with 64-detector computed tomographic angiography European Review for Medical and Pharmacological Sciences 2013; 17:1636-1641.

17. Kuo-Hsien C, et al. Angiographic Evaluation of Hepatic Artery Variations in 405 cases, Chin J Radiol. 2005; 30:75-81.

18. Ugurel MS, Battal B, Bozlar U, Nural MS, Tasar M, Ors F, Saglam M, Karademir I. Anatomical variations of hepatic arterial system, coeliac trunk and renal arteries: an analysis with multidetector CT angiography. The British journal of radiology. 2010; 83(992):661-7. https://doi.org/10.1259/bjr/21236482 PMid:20551256 PMCid:PMC3473504

19. Osman AM, Abdrabou A. Celiac trunk and hepatic artery variants: A retrospective preliminary MSCT report among Egyptian patients. The Egyptian Journal of Radiology and Nuclear Medicine. 2016; 47(4):1451-8. https://doi.org/10.1016/j.ejrnm.2016.09.011

20. Tharao MK, Saidi H, Kitunguu P, Julius OA. Variant anatomy of the hepatic artery in adult Kenyans. European Journal of Anatomy. 2019; 11(3):155-61.

21. Hiatt JR, Gabbay J, Busuttil RW. Surgical anatomy of the hepatic arteries in 1000 cases. Annals of surgery. 1994; 220(1):50-52. https://doi.org/10.1097/00000658-199407000-00008 PMid:8024358 PMCid:PMC1234286

22. Ahmed A. Hepatic Arterial Variations And Its Implications On Hepatobiliary Surgery Awais Ahmed, Rashed Nazir, Rayyan Pervez, Umar Amin, Sadia Babar, Atif Rana. Department of Radiology, Shifa International hospital, Islamabad, Pakistan. European Congress of Radiology 2013.

23. Covey AM, Brody LA, Maluccio MA, Getrajdman GI, Brown KT. Variant hepatic arterial anatomy revisited: digital subtraction angiography performed in 600 patients. Radiology. 2002; 224(2):542-7. https://doi.org/10.1148/radiol.2242011283 PMid:12147854

24. Perwaiz A, Singh A, Singh T, Chaudhary A. Incidence and Management of Arterial Anomalies in Patients Undergoing Pancreaticoduodenectomy. JOP. J Pancreas. 2010; 11(1):25-30.

25. Noussios G, Dimitriou I, Chatzis I, Katsourakis A. The main anatomic variations of the hepatic artery and their importance in surgical practice: review of the literature. J Clin Med Res. 2017; 9(4):248-252. https://doi.org/10.14740/jocmr2902w PMid:28270883 PMCid:PMC5330766

26. López-Andújar R, Moya A, Montalvá E, Berenguer M, De Juan M, San Juan F, Pareja E, Vila JJ, Orbis F, Prieto M, Mir J. Lessons learned from anatomic variants of the hepatic artery in 1,081 transplanted livers. Liver transplantation. 2007; 13(10):1401-4. https://doi.org/10.1002/lt.21254 PMid:17902125

27. Song SY, Chung JW, Yin YH, Jae HJ, Kim HC, Jeon UB, Cho BH, So YH, Park JH. Celiac axis and common hepatic artery variations in 5002 patients: systematic analysis with spiral CT and DSA. Radiology. 2010; 255(1):278-88. https://doi.org/10.1148/radiol.09090389 PMid:20308464

28. Winston CB, Lee NA, Jarnagin WR, Teitcher J, DeMatteo RP, Fong Y, Blumgart LH. CT angiography for delineation of celiac and superior mesenteric artery variants in patients undergoing hepatobiliary and pancreatic surgery. American Journal of Roentgenology. 2007; 189(1):W13-9. https://doi.org/10.2214/AJR.04.1374 PMid:17579128