ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Dec 20; 6(12):2277-2282.
https://doi.org/10.3889/oamjms.2018.486
eISSN: 1857-9655
Basic Science

Association of rs1042522 SNP with Clinicopathologic Factors of Breast Cancer Patients in the Markazi Province of Iran

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Citation: Anoushirvani AA, Aghabozorgi R, Ahmadi A, Arjomandzadegan M, Sahraei M, Khalili S, Fereydouni T, Khademi Z. Association of rs1042522 SNP with Clinicopathologic Factors Of Breast Cancer Patients in the Markazi Province of Iran. Open Access Maced J Med Sci. 2018 Dec 20; 6(12):2277-2282. https://doi.org/10.3889/oamjms.2018.486

Keywords: Breast cancer; TP53; Nested-PCR; Chemotherapy

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Received: 19-Aug-2018; **Revised:** 13-Oct-2018; **Accepted:** 14-Oct-2018; **Online first:** 14-Dec-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: The nucleotide changes in different genetic loci increased the incidence risk of breast cancer.

AIM: The aim of present study was to investigate genotype distribution at codon 72 of the TP53 gene (rs1042522) in breast cancer patients to achieve a potential diagnostic marker related to some demographic feathers.

METHODS: In our case-control study, blood samples were collected from a total of 34 patients harboured breast cancer. DNA was extracted, and nested-PCR was performed. Products were digested with AccII and subsequently were sequenced. Results were compared with samples characteristics.

RESULTS: The PCR results indicated the correct implementation of extraction and amplification protocol. The genotypic distribution at codon 72 of TP53 in control group was 20%, 62.4% and 16.6% for Arg (wildtype), Arg/Pro (heterozygous) and Pro (homozygous variant) respectively. Also, this distribution in the patient group was 23.52% homozygous, 50% heterozygous, and 26.47% another homozygous variant (Adjusted odds ratio: 1.12 and 95%CI = 0.57 to 2.2, P = 0.03). The absence of Arg at codon 72 of TP53 is relevant with age higher than 40 years and metastasis to other organs.

CONCLUSION: Polymorphism at codon 72 of TP53 was associated with high-grades of breast cancer risk and different responses to chemotherapy treatment. It is recommended genotype distribution of codon 72 of TP53 before chemotherapy.

Introduction

Breast cancer (BC) is one of the most common types of cancer that causes mortality rates each year. Despite advances in early diagnosis and proper treatment for this disease, it remains the main cause of death among women [1], [2], [3]. The most common sites of BC metastasis are bones, lunge and liver. Metastasis of BC to the brain less occur. The growth of cancer cells in BC is considered in three stages. BCs stage I or II are in the initial stage. If the tumour extends to the underlying muscles of the chest wall or skin, BC is type III. This Stage also includes BCs involving inflammatory system, in which case the breast is red or swollen [4], [5], [6]. BC stage IV refers to tumours that metastasise to the outside of the breast and the lymph nodes, as well as to the brain, bones, skin, and other organs. The basics of cancer genetics is the occurrence of changes in signalling pathways and genes regulatory networks [5]. Changes in different codons of the genes, including

BRCA1, *BRCA2*, and *TP53* etc. increase the risk of developing breast cancer. In some cases due to a mutation in *TP53*, the occurrence of BC can be accompanied by brain tumours and leukaemia. *TP53* (also known as *BCC7*, *LFS1*) is a TSG. This gene is encoded on the human chromosome 17 at position p13.1 (HGNC ID: 11998). Different transcripts are reported for this gene (Figure 1). Some of these transcripts are not protein coding (ENSG00000141510). This gene is very important in the coordination of the cell cycle as well as in preventing the development of cancer [2].

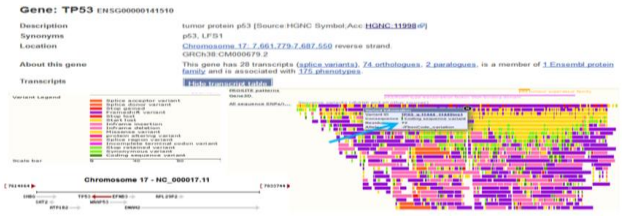

Figure 1: View of the TP53 chromosome position and the Ensemble databank of variants and SNPs of the TP53 gene. The codon 72 is shown by the blue arrow

In addition, the *TP53* gene has a variety of anti-cancer activities, such as apoptosis, genome stability (preventing the appearance of mutation in the genome) and preventing angiogenesis [3]. P53 Protein plays a major role in the initiation of apoptosis. The amount of P53 protein increases when the cell is exposed to stress [3], [6]. The defects in DNA repair are often the result of mutations, which may lead to the loss of DNA repairing proteins activity. DNA polymorphisms may also alter the structure of repairing enzymes and reduce their repair ability. Key polymorphisms in some genes, including *TP53*, may increase the risk of BC and may lead to a difference in treatment. Several SNPs have been recorded for this gene. One of these polymorphisms is the nucleotide change in codon 72 of this gene which can lead to a missense amino acid residue conversion Arg (CGC) to Pro (CCC). Rs1042522 is one of 25 important SNPs reported to increased risk for BC [7]. The G and C of this polymorphism allele encode an Arg and Pro respectively at position 72 of the P53. It has been reported that alteration of this codon is likely to be associated with an increased risk of some of the diseases [8], [9], [10], [11]. This association is related to population type. African population with two copies of G (G;G) in rs1042522 in TP53 were 2.15 times more susceptible to die due to rectal cancer than those with (C;G) or (C;C) at mentioned polymorphism. Also in Caucasians, there was no association between survival and rs1042522. In Asadi et al., study were detected no association between rs1042522 C > G polymorphism and Risk of Colorectal Cancer in the Iranian Azari Population [12]. In his another study, also was detected No association between rs1042522 C > G SNP and neuroblastoma risk using TaqMan genotyping [13]. There has been no report on the relationship between the rs1042522 polymorphism in *TP53* and tumour grade, age, Body mass index (BMI) and the response rate to chemotherapy in BC patients of Markazi province of Iranian population. The aim of present study was to evaluate the polymorphism at codon 72 of *TP53* in patients with BC and investigate the relationship between genotype distributions with above factors in patients with BC in aforementioned Iranian population.

Materials and Methods

In present case-control study, samples were collected from Ayatollah-Khansari hospital in Arak city. This study was performed between November 2016 and June 2017. A total of 34 pathological and clinical data were classified for the collected breast cancer samples (median age: 48 years). Also, normal samples from non-tumour individuals were equal numbers of patients group. Three ml of blood was collected from each sample in CBC test vial. The study was approved by the Research Ethics Committee of Arak University of Medical Sciences (Ethics number *618B/3090*). Participants of present study agreed with and signed a consent form.

DNA extraction was performed from 500 µl whole blood using the Diatom DNA Kit (IsoGene, Moscow, Russia) based on its instruction. Samples were loaded on 1% agarose gel, (YTA, Iran).

In Polymerase chain reaction, 50 mM MgCl2 (Cinagene, Iran), 10X Buffer (Cinagene, Iran), 10 mM dNTP (Cinagene, Iran), 1unit Taq polymerase (Cinagene, Iran), and specific primers at a suitable concentration of 10 pmol were used. Annealing temperature for the primers [9] was optimised at 52.7°C.

In this study, PCR products of the first stage were used in a thermocycler machine (Eppendorf, Germany) as a template in the second stage PCR using a pair of internal primers in order to more specificity and enhancing the amplified bands. A non-template sample was used as a negative control. The sequence of these primers is shown in Table 1.

Table 1: The used primer sequence in this study

Primer ID	Sequence (5'-3')
p53 F	GCTCTTTTCACCCATCTACAG
p53 R	TGAAGTCTCATGGAAGCCAGC
P53 Inner-F	TCCCCCTTGCCGTCCCAA
P53 Inner-R	CGTGCAAGTCACAGACTT

Restriction enzyme digestion was performed by *Acc*II (Vivantis, Malasia) at 37°C for 3-16 hours. Production of one fragment of 300bp indicated the presence of the wildtype allele (Arg). The presence of the variant allele (Pro) was determined by the observation of two fragments of 160 bp and 140bp. The produced amplicons were sent for sequencing.

The sequencing was carried out with the ABI Applied Biosystem machine-Model 3730XL (Macrogene, South Korea). Sequencing results were analysed using Chromas, Mega 4.0, Blast and Blat software.

Validation of genotypes was evaluated by comparison of sequences results with data banks such as UCSC. Chi-sq Hardy-Weinberg equilibrium (HWE) test calculator were used for biallelic markers including SNPs. The difference between the groups was determined by one-way ANOVA GraphPad prism 7.0. P values less than 0.05 were considered statistically significant.

Results

The results of the amplification reaction indicated the accurate of the extracted DNA, the correct implementation of the temperature protocol and used primers. Figure 2 shows the 300bp amplicon of the *TP53* gene.

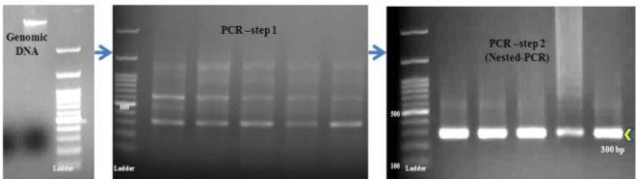

Figure 2: Steps of Nested-PCR steps in this study. The yellow arrow shows the 300 bp amplicon of TP53 gene

In below figure columns 1-4 indicated bands of heterozygous samples that have three bands (300, 160 and 140 bp). Also, Figure 3b shows the sequence analysis of the 8 homozygous amplicons.

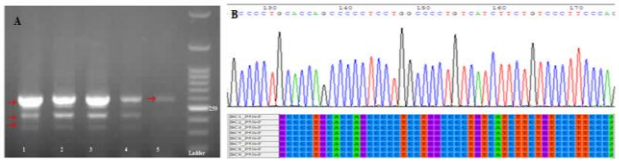

Figure 3: A) Agarose gel of digestion reaction. Column 1-4 showed heterozygote samples (Arg/Pro) and column 5 showed a homozygote wildtype (Arg); B) Nucleotide sequences from the sequencing of clinical samples (Chromas and Mega4 software)

The genotypic distribution in the patient group was 23.52% homozygous wildtype (Arg), 50% heterozygous (Arg/Pro), and 26.47% another homozygous variant (Pro). These genotypes in the control group were 20%, 62.4% and 16.6% for homozygous wildtype, heterozygous and homozygous variant respectively.

The C allele freq = 0.48; G allele freq = 0.52 was the frequency of changes in codon 72 of the *TP53* gene in patient group (HWE calculator, p < 0.05). The Chi-square in control group was not significant (X2 = 6.8, p > 0.05).

Table 2: different patterns from digestion reaction using *MboII* enzyme

Pattern of RFLP	Band (bp)	Genotype	Patients (%)	Odds Ratio	%90 CI	%95 CI	%99 CI	P value
Pattern A	300	GG homozygote	23.52	1.12	0.64-1.98	0.57-2.2	0.46-2.72	< 0.05
Pattern B	300, 160, 140	CG heterozygote	50	1.07	0.66-1.71	0.61-1.87	0.51-2.23	< 0.05
Pattern C	160, 140	CC homozygote	26.47	1.41	0.79 -2.51	0.71-2.8	0.57-3.48	< 0.05

The Figure 4 showed that there are a significant association between the changing at codon 72 (Arg72 Pro) of *TP53* gene sequences and the Age > 40, BMI > 22, WHR > 0.8, metastasis to other organs, positive ER and PR status and less response to chemotherapy in BC samples than the control group. These associations are statistically significant.

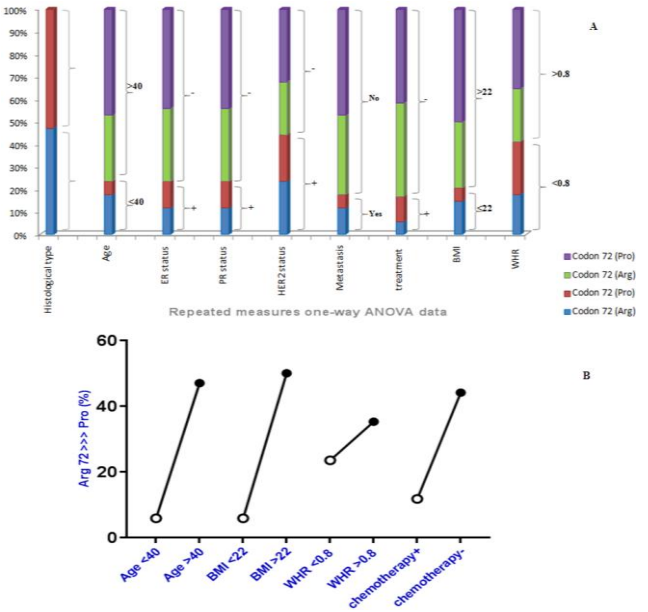

Figure 4: A) This chart shows the relationship between amplicons sequences and the characteristics of the used clinical samples; B) GraphPad Prism 7.0 software showed association between the Pro at codon 72 of TP53 gene and the Age > 40, BMI > 22, WHR > 0.8 and negative response to chemotherapy (One-way ANOVA test, P = 0.03)

Discussion

The incidence rates of BC increase with an ascending trend so that its frequency has doubled over the past 20 years. In Iran, BC is the most common type of cancer after stomach and oesophagus cancers. Therefore, diagnosis at first stages and study of the molecular mechanisms of BC are necessary [1], [2], [3]. Molecular events that regulate cell survival, apoptosis, growth and differentiation of the cell, played an important role in the overall kinetics of tumour growth as benign or

malignant [14]. Mutation of *TP53* is the most common genetic change in human cancers and is associated with poor response to treatment including chemotherapy and radiotherapy. Recent studies have shown the probably beneficial and valuable effects of gene therapy with the goal of *TP53* as a complementary therapy in cancers. Studies in 1997 and 1998 found that p53 plays a critical role in controlling of the cell cycle since its normal function not forces the cell to stop the cell cycle so that it can enter a period of interruption to repair the DNA damage. If the cell could not repair damage to the cell, it would commit suicide to prevent mutations in the cells [6], [7]. Injury to the *TP53* gene occurs during the life-span of the individual, but in rare cases, involved about 1% the cases of sporadic BCs [15], [16], [17], [18]. In a study in 2009, numbers of 1836 articles from 1986 to 2008 were reviewed for involved genes in BC [3]. In the *TP53* gene, the importance of codon 72 has been shown to identify changes in the various populations. This codon site is within the active site of the p53 protein. In studies conducted in 2006 and 2007, it was found that using some of the pathological characteristics of BC, such as the tumor size, negative status of PR, positive status of the lymph nodes and high differentiation can be predicted that a patient will be more likely to develop metastasis [19], [20], [21], [22]. Other studies showed significant differences in the prevalence of codon 72 in *TP53* polymorphism in endometriosis in a Brazilian population [23].

The present study showed that the frequency of changes in codon 72 of the *TP53* gene in the studied population was common and totally 76.7 % of the studied patients had this change. Also, there are associations between a polymorphism at microRNA binding site in BRCA2 with BC susceptibility [24], [25]. Also, there are an association of polymorphisms in FCGR2A and FCGR3A with a degree of Trastuzumab in the Adjuvant treatment of ERBB2/HER2- positive BC [26]. A study results at 2017 showed a significantly association between six SNPs including FGFR2 (rs2981582), HCN1 (rs981782), MAP3K1 (rs889312), TOX3 (rs3803662), ZNF365 (rs10822013), and RAD51B (rs3784099), with breast cancer risk [25]. A study in South Korea on persons with gastric cancer that treated with cisplatin and paclitaxel chemotherapy indicated that rs1042522 (G/G and C/G) genotypes compared to rs1042522 (C;C) were significantly associated with lower response rate to the chemotherapy treatment (35.7 vs. 66.7%, p-value 0.019) [28]. Another study at 2014 on patients with NSCLC showed that G/G genotype of rs1042522 was more resistant to the first-line chemotherapy drugs, such as cisplatin [29]. In the present study, we evaluated all changes in studied amplicon compared to the control samples by sequencing method. The results of our study indicated the relationship between the rs1042522 polymorphism with age, BMI, metastasis, positive status of PR and ER and treatment response rate in these patients (Table 3).

Table 3: Some demographic features of the studied samples and differences in genotypes distribution of *TP53* at codon 72 in this study (P value < 0.05)

Characteristic	Detail	Total no.	No. Of patients (%)	Gg arg(%)	Dif.	Gc arg /pro(%)	Dif.	Cc pro(%)	Dif.
Age	<40	8	23.52	8.82	-16.8	11.76	-38.24	2.94	-22.06
	>40	26	76.47	14.070	-10.3	38.23	-11.17	23.52	-1.48
Menopausal	No	16	47.05	11.76	-10.3	23.52	-26.48	11.76	-13.24
age	<52	3	8.82	2.94	-13.24	2.94	-47.06	2.94	-22.06
	>52	15	44.11	8.82	-22.06	17.64	-32.36	17.64	-7.36
Er status	Positive	8	23.52	5.88	-19.12	11.76	-38.24	5.88	-19.12
	Negative	26	76.47	17.64	-7.36	35.29	-14.71	23.52	-1.48
Pr status	Positive	8	23.52	5.88	-19.12	11.76	-38.24	5.88	-19.12
	Negative	26	76.47	17.64	-7.36	35.29	-14.71	23.52	-1.48
Her2 status	Positive	15	44.11	11.76	-13.24	20.58	-29.42	11.76	-13.24
	Negative	19	55.88	11.76	-13.24	26.47	23.43	17.64	-7.36
Metastasis	Yes	6	17.64	5.88	-19.12	8.82	-41.18	2.94	-22.06
	No	28	82.35	17.64	-7.36	41.17	-8.83	23.52	-1.48
Treatment	Chemo-therapy+	6	17.64	2.94	-22.06	8.82	-41.18	5.88	-19.12
	Chemo-therapy-	30	88.23	8.82	-16.8	41.17	-8.83	29.41	4.41
Bmi	>22	7	20.58	8.82	-16.8	8.82	-41.18	2.94	-22.06
	<22	27	79.41	14.70	-10.3	38.23	-11.77	26.47	1.47
Whr (waist to hip ratio)	<0.8	14	41.17	8.82	-16.8	20.58	-29.42	11.76	-13.24
	>0.8	20	58.82	11.76	-13.24	2.94	-47.06	17.64	-7.36
Histological type	Invasive ductal carcinoma	34	100	23.52	-1.48	50	0	26.47	1.48

dif: the difference between expected and observed genotypes in HWE equation; p2: GG, 2pq: G/C, q2: CC)

In the present study, we investigated that rs1042522 SNP may be associated with susceptibility to BC among Iranian women with higher age and BMI. The distribution of C Allele in individuals with age of higher than 40 years was 1.2 times more than lower ages. Also, the frequency of G allele is associated to lower BMI and WHR. Our data showed there was a direct association between C/G and C/C genotype of rs1042522 with a negative response to chemotherapy (1.13 times).

Based on the new issue of “personalised medicine” in modern medicine, patients can be treated according to their molecular feathers. Therefore, it can be inferred that determination of *TP53* variants for BC patients is suitable before the starting the treatment protocol. Cancer is a multi-factorial disease and, in addition to genetic, environmental factors such as smoking, family income etc. also contributes to its development. The results of this study revealed the lack of significant correlation between these factors including family income, Educational level, the age of marriage and cigarette smoking with the studied polymorphism (data not shown). However, any of these factors may be effective in the overall survival rate and severity of the disease.

However any of these factors may be effective in overall survival rate and severity of the disease. Performance of molecular diagnostic tests for the evaluation of cancer genes in medical centers is critical. Hence, the determination of polymorphism in the mentioned codon can be used as a potential candidate diagnostic marker for high risk BC.

Acknowledgements

The authors of this article thank for the research and technology deputy of Arak University of medical sciences.

Author contributions

The first two authors contributed to this study and have equal role: clinical sample preparation; AA: designed study, performed the experiments, analysed data, interpreted data and manuscript preparation; TA: clinical sample preparation, SA, MA and MS: performed the experiments

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Dec 20; 6(12):2283-2289.
https://doi.org/10.3889/oamjms.2018.414
eISSN: 1857-9655
Basic Science

Pseudomonas aeruginosa - Modified Hodge Test (PAE-MHT) and ChromID Carba Agar for Detection of Carbapenemase Producing *Pseudomonas Aeruginosa* Recovered from Clinical Specimens

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Citation: Othman HB, Abdel Halim RM, Abdul-Wahab HEE, Abol Atta H, Shaaban O. *Pseudomonas aeruginosa* - Modified Hodge Test (PAE-MHT) and ChromID Carba Agar for Detection of Carbapenemase Producing Pseudomonas Aeruginosa Recovered From Clinical Specimens. Open Access Maced J Med Sci. 2018 Dec 20; 6(12):2283-2289. https://doi.org/10.3889/oamjms.2018.414

Keywords: CRPA; ChromID Carba Agar; MHT; PAE-MHT; PCR

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Received: 22-Sep-2018; **Revised:** 08-Oct-2018; **Accepted:** 09-Oct-2018; **Online first:** 15-Dec-2018

Funding: This research did not receive any financial support

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

Abstract

AIMS: This study aims to evaluate the ability of ChromID Carba agar, and *Pseudomonas aeruginosa* modified Hodge test (PAE-MHT) for detection of carbapenemase-producing *P. aeruginosa* and to determine the associated carbapenemase gene classes by PCR.

METHODS: One hundred Carbapenem-resistant *P. aeruginosa* (CRPA) isolates were tested for: i) carbapenemases production by ChromID carba agar, Modified Hodge test (MHT) and (PAE-MHT) and ii) detection of some carbapenemase genes by PCR.

RESULTS: All (100%) of the isolates showed growth on ChromID Carba agar with 100% sensitivity. Using MHT, 54% of isolates were positive, 3% were indeterminate, and 43% were negative, demonstrating 58.9% sensitivity and 80% specificity. On performing PAE-MHT, 91% of the strains were positive, 3% were intermediate, and 6% were negative, demonstrating 97.9% sensitivity and 80% specificity. The most prevalent gene was *blaKPC* (81%), followed by *blaVIM* (74%); *blaIMP* was detected in only one isolate, and *blaOXA-48* in 34% of the isolates.

CONCLUSIONS: We conclude that PAE-MHT and ChromID Carba are sensitive, specific, simple and cost-effective screening tests for detection of CRPA isolates compared to the traditional MHT.

Introduction

Carbapenemase-producing P. aeruginosa strains are resistant to almost all β-lactams. Carbapenem-resistant *P. aeruginosa* (CRPA) can be produced as a different consequence mechanism, such as decreased bacterial outer membrane permeability, overexpression of AmpCs or expression of carbapenemases [1].

Carbapenemases production in *P. aeruginosa* belong to the Ambler class A (KPC- and GES-type β-lactamases) and most commonly the Ambler class B (Metallo-β-lactamases) (MBLs) of the VIM, IMP, SPM, GIM, AIM, DIM, FIM, and NDM types [1], also OXA beta-lactamase genes in *Pseudomonas aeruginosa* have been identified [2].

Several inhibitor-based tests have been developed for the detection of carbapenemases in *P. aeruginosa*. However, misdetection of newly emerging isolates with a combination of carbapenemases could occur with these methods [3].

Using a novel indicator strain, *K. pneumoniae* ATCC 700603, the MHT was optimised for more accurate and reliable detection of carbapenemase production in *P. aeruginosa*, and this test was named the *P. aeruginosa* MHT (PAE-MHTs) [3]. Chromogenic media containing a carbapenem (chromID Carba, chromID KPC, chromID ESBL) are convenient tools for the screening and rapid detection of carbapenemase-producing Gram-negative bacilli (CPGNB). Among these media, chromID Carba demonstrated the highest sensitivity and specificity. Different genotypic methods have been applied for the detection of carbapenemases encoding genes in clinical isolates of *P. aeuroginosa* [4].

Thus, we performed ChromID Carba agar chromogenic medium, MHT and PAE-MHT to evaluate their ability for detection of CRPA and compared the results to PC.

Methods

The study included 100 carbapenem-resistant *P. aeruginosa* isolates (imipenem and/or meropenem) detected by disk diffusion method according to CLSI, 2015 [5] among different isolates recovered from different specimens referred to Central Microbiology Laboratory for routine culture and sensitivity. The selected CRPA isolates had been referred from the following departments of Ain Shams University Hospitals; burn unit (55%), ICU (22%), surgery department (16%), and from the internal medicine department (7%). They were recovered from {pus (71%), respiratory (17 %), urine (11%) and only one strain (1%) from blood specimens. All isolates were subjected to:

A) Phenotypic detection of carbapenemases producing P. aeruginosa

1. ChromID carba agar

After, overnight incubation of blood agar plates at 36 ± 1ºC, isolates were suspended in one mL of 0.9% sterile saline solution and the inoculum was adjusted to a density of a 0.5 McFarland standard (10^8 CFU/ mL). Then, ten µL (10^6 CFU/ mL) of this suspension was streaked by 10 µL standard loop onto ChromID Carba agar (bioMérieux, France).

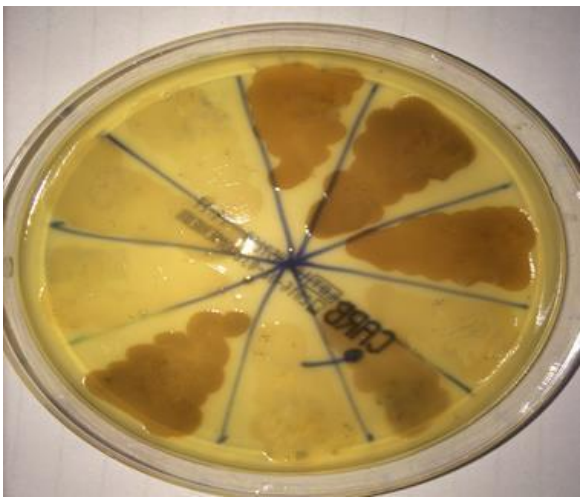

Figure 1: P. aeruginosa on ChromID Carba. Five strains are colourless, and five show brown pigmentation

All plates were incubated in ambient air at 36 ± 1°C for 24 hours. Some carbapenemase producing Pseudomonas strains were colourless, and some showed brown pigmentation (Figure 1). Non-carbapenemase producing Pseudomonas strains showed no growth [4].

2. Modified Hodge test (MHT)

Modified Hodge test was performed according to CLSI [6]. An inoculum of the indicator organisms (E. coli ATCC 25922), was adjusted to a 0.5 McFarland turbidity standard then a 1/10 dilution was made by adding 1ml of 0.5 McFarland turbidity tube to 9ml sterile saline and inoculated on the surfaces of Mueller-Hinton agar plates by swabbing. After the plates had been allowed to stand for 10 min at room temperature, a disk of meropenem, 10 µg (Oxoid, UK) was placed in the centre of each plate. Subsequently, four colonies of the test organisms, grown overnight on blood agar plate, were inoculated onto the plate in a straight line from the edge of the disk to the periphery of the plate (without touching the disc) by swabbing. A positive result is indicated by the enhanced growth of the indicator strain towards a meropenem disk, clover leaf-type indentation at the point of intersection of the isolate with the indicator strain. Whereas, no enhanced growth of the indicator strain towards a meropenem disk (no clover leaf-type indentation at the point of intersection of the isolate with the indicator strain is considered negative. Indeterminate results showed inhibition of the growth of the indicator strain produced by the test isolate (Figure 2 & 3).

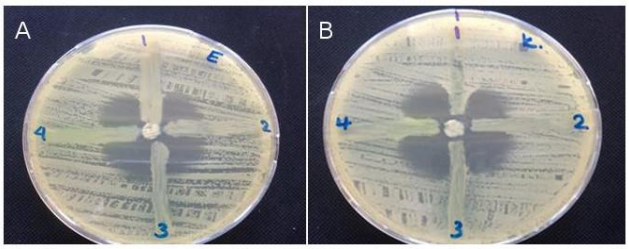

Figure 2: A) MHT; isolates 1, and 4 show positive results, isolates 2, and 3 show negative results; B) PAE-MHT; isolates 1, 2, 3, 4 show positive results

3. Pseudomonas aeruginosa modified Hodge test (PAE-MHT)

The same procedure as MHT was performed but *K. pneumonia* ATCC 700603 was used as indicator strain [7]. Interpretation of results was the same as in MHT (Figure 2 & 3).

B) Genotypic detection of carbapenemase encoding genes

All isolates were subjected to molecular identification of carbapenemase encoding genes using conventional PCR for (*blaKPC*) [8] and conventional multiplex PCR for (*blaVIM, blaIMP, and*

blaOXA-48) [9]. DNA extraction was done using Boiling method [10].

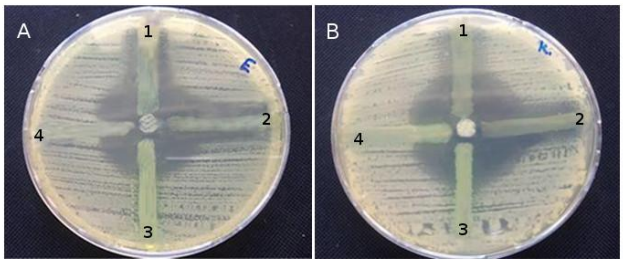

Figure 3: A) MHT; isolates 1, 2 show indeterminate results, isolate 3 shows a negative result, isolate 4 shows positive results; B) PAE-MHT; isolates 1, 2 show indeterminate result, Isolate 3 shows a negative result, isolate 4 shows a positive result

All PCR reactions were performed with My Taq HS Red mix (Bioline, UK) 2 X and carried out on a Thermal Cycler 2720 (Applied Biosystem, USA) instrument. The primers (Table 1) and the components of the master mix were added to templet DNA in a two separate reaction mixture; one for *blaKPC* gene and the other for *blaIMP, blaVIM* and *blaOXA-48* genes in a final volume of 25 µL for each reaction mixture.

Table 1: Primers sequences of carbapenemase encoding genes

Primer name	Primer sequence	PCR product size (bp)	Refrences
KPC (forward) KPC (reverse)	5′-ATGTCACTGTATCGCCGTCT-3′; 5′-TTTTCAGAGCCTTACTGCCC-3′	893-bp	[8]
IMP-A (forward) IMP-B (reverse)	5′-GAA GGY GTT TAT GTT CAT AC-3′ 5′-GTA MGT TTC AAG AGT GATGC-3′ (where Y = C or T and M = A or C)	587-bp	[9]
VIM2004A (forward) VIM2004B (reverse)	5′-GTT TGG TCG CAT ATC GCA AC-3′ 5′-AAT GCG CAG CAC CAG GATAG-3′	382-bp	[9])
Oxa 48A (forward) Oxa 48B (reverse)	5′-TTGGTGGCATCGATTATCGG-3′ 5′-GAGCACTTCTTTTGTGATGGC-3′	744-bp	[31]

Stock solution of primers = 100 pmol/1 µL; Working solution of primers = 4 pmol/1 µL.
Stock solution of primers = 100 pmol/1 µL; Working solution of primers = 4 pmol/1 µL.

The amplification program for *blaKPC* was composed of initial denaturation at 95°C for 15 min, 38 cycles of denaturation for one minute at 94°C, annealing at 62°C for one minute, and an extension step at 72°C for one minute, then final extension for ten minutes at 72°C. As for *blaIMP, blaVIM* and *blaOXA*-48, it was composed of an initial denaturation step at 94°C for 5 min, followed by 30 cycles of DNA denaturation at 94°C for one minute, primer annealing at 54°C for one minute, and primer extension at 72°C for one and half minute.

For detection of the amplified Products; ten µl were examined by 1.2% agarose gel in Tris-boric acid EDTA buffer (TBE). A DNA size marker was included for comparison. Electrophoresis was performed at 80 volts, and the gel was then stained by ethidium bromide (0.5 µg/ml) for 30 minutes. Then, it is visualised using a UV transilluminator and photographed. *BlaKPC, blaIMP, blaVIM,* and *blaOXA-48* genes gave bands at 893, 587, 382, and 744 bp. Respectively (Figure 4).

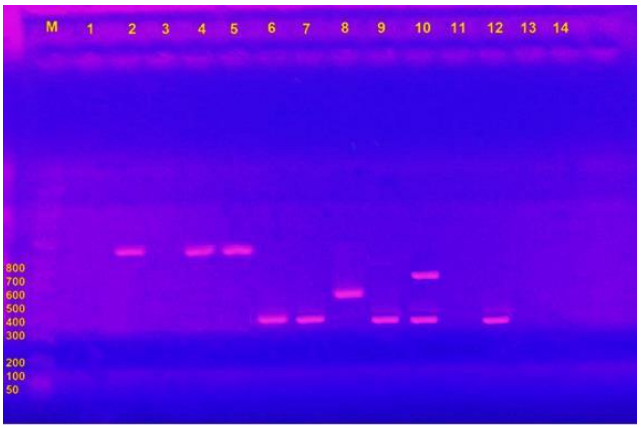

Figure 4: Gel electrophoresis of PCR products; Lane M is DNA ladder; Lanes 2, 4 & 5 are positive for KPC (893 bp); Lanes 6, 7, 9 & 12 are positive for VIM (382 bp); Lane 8 is positive for IMP (587 bp); Lane 10 is positive for for both oxa 48 (744 bp) with VIM (382bp); Lanes 1, 3, 11, 13 & 14 are negative

Results

All (100%) of the CRPA isolates showed growth on ChromID Carba agar after overnight incubation at 36 ± 1°C indicating carbapenemase production. Detection of carbapenemase by MHT showed that; (54%) of isolates were positive, three (3%) produced indeterminate results and (43%) were negative. On performing PAE-MHT, (91%) of the strains were positive, three (3%) produced indeterminate results and (6%) were negative. False positive results were 20%, and false negative results were 2.1%.

Results of PCR for detection of the four carbapenemases encoding genes (*blaKPC, blaVIM, blaIMP, blaOXA-48*) on 100 CRPA isolates showed that; *blaKPC, blaVIM, blaIMP* and *blaOXA-48* were detected in 81%, 74%, 1%, and 34% of the isolates respectively. The four carbapenemase genes were positive in (95%) of CRPA isolates. Five strains were negative for all the four carbapenemase genes included in the study.

PCR results showed that 19% isolates were positive for only one gene; nine (%) isolates for *blaKPC* and ten (10%) isolates for *blaVIM*. Fifty seven (57%) strains were positive for two genes; *blaKPC* + *blaVIM* 41 (41%) strains, *blaKPC* + *blaIMP* one (1%) strain, *blaKPC* + *blaOXA-48* 11 (11%) strains and *blaVIM* + *blaOXA-48* four (4%) strains. Nineteen strains (19%) were positive for three genes (*blaKPC* + *blaVIM* + *blaOXA-48*) collectively. No strains were positive for the four genes collectively (Table 2).

Table 2: The distribution of carbapenemase encoding genes among 95 PCR-positive isolates (Per isolate)

Carbapenemasegenes	n	(%)
One gene only detected:		
KPC only	9	(9.5)
VIM only	10	(10.5)
Total	19	(20)
Two genes detected:		
KPC + VIM	41	(43.2)
KPC + IMP	1	(1)
KPC + OXA-48	11	(11.6)
VIM + OXA-48	4	(4.2)
Total	57	(60)
Three genes detected:		
KPC + VIM + OXA-48	19 (20)	
Total	19 (20	

In our study, the performances of MHT, PAE-MHT and ChromID Carba were compared to PCR, which is recommended as a standard gold method for detection of carbapenemase production [11]. MHT gave 58.9% sensitivity, 80% specificity, 20% false positive rate and 41.1% false negative rate. On the other hand, PAE-MHT showed higher sensitivity (97.8%), specificity was 80%.

When results of ChromID Carba were compared to PCR results, 95 strains, that were PCR positive, showed growth on the ChromID Carba. The remaining five isolates that showed growth on ChromID Carba were negative by PCR. Thus, the sensitivity of ChromID Carba was 100% (compared to PCR), but the specificity could not be calculated as the test didn't show any negative results.

Regarding the five isolates that were PCR negative but showed growth on the ChromID Carba, three of them gave positive results by both MHT and PAE-MHT while the remaining two were negative.

In the present study, 3% of the CRPA isolates gave indeterminate results by both MHT and PAE-MHT; 2% were only MBL producers (*blaVIM*), and 1% was both KPC and MBL (*blaVIM*) producer.

Discussion

Carbapenemase-producing *P. aeruginosa* strains are resistant to almost all β-Lactams. So, their spread in hospital settings is extremely dangerous. Also, carbapenemase genes are usually located on transferable genetic determinants such as plasmids, leading to their rapid dissemination. Hence, detection of carbapenemases is important for early implementation of infection control measures [1].

On performing MHT, 54% of our strains were positive, 3% of showed indeterminate result and 43% were negative. In a previous study, among the 32 meropenem resistant *P. aeruginosa*, only 9 (28.1%) were positive for carbapenemases by MHT. However, 16 (50.0%) were MBL producers by EDTA disk synergy test [12].

In our study, the sensitivity of MHT was 58.95%, and specificity was 80%. False positive results were detected in 20% of the strains, and 41.1% were a false negative. The false positive results might be due to the co-production of CTX-M and hyperproduction of AmpC which may result in the minor hydrolysis of carbapenems [13].

The false negative results might be related to the presence of NDM type of MBLs [11]. This was consistent with our results, where 4/10 (44%) strains carrying only MBL genes, gave negative result by MHT. Also, MHT does not distinguish between carbapenemase types and lack sensitivity for MBL detection unless it is performed on MacConkey's agar or Zn^{+2} added to the carbapenem disk [14]. Moreover, it noted that the sensitivity and specificity of the test for detecting low-level Metallo-β-lactamase production are not known, and no data exists on the usefulness of this test for the detection of carbapenemase production in non-fermenting gram-negative bacilli like Pseudomonas [5]. On the other hand, our study revealed that six out of nine strains carrying *blaKPC* (66%) were negative for MHT and all the eleven strains (100%) carrying both *blaKPC* and blaOXA-48 were also negative for MHT.

A study carried on 64 *P. aeruginosa* isolates (42 carbapenemase producers and 22 carbapenemase non-producers) revealed that the MHT showed 78% sensitivity and 57% specificity. When the test was repeated on 15 strains, ten carbapenemase producers and five carbapenemase non-producers, nine strains out of 15 showed variable results over the test period (repeatability; 40%) [7].

In our study, 3% of the CRPA isolates produced indeterminate results by MHT; 2% were MBL producers (*blaVIM*), and 1% was both KPC and MBL (*blaVIM*) producer. This could be justified by a possible secretion of a substance such as colicin, a bacteriocin-peptide released by some GNB, which may inhibit the growth of the indicator strain and interfere with the results of the test [15].

Indeterminate results by MHT in 12% of MBL *P. aeruginosa* producers, which were not obtained for KPC producers, were observed in a previous study. Similarly, they reported a high proportion of indeterminate results (22% and 43% of carbapenemase and non- carbapenemase producers respectively) which occurred at a rate ten times higher in *P. aeruginosa* than that for Enterobacteriaceae. They optimised the MHT for more accurate and reliable detection of carbapenemase production in *P. aeruginosa* by using a novel indicator strain, *K. pneumoniae* ATCC 700603, and named this test the *P. aeruginosa* MHT (PAE-MHT). First, the test was performed using 64 *P. aeruginosa* isolates (42 carbapenemase producers and 22 carbapenemase non-producers), and it demonstrated 100% sensitivity and 98% specificity for detection of carbapenemase activity without indeterminate results [7].

In the present study, 91% of the strains of CRPA isolates were positive by PAE-MHT, 3%

demonstrated indeterminate results and 6% were negative. The sensitivity of PAE-MHT was (97.81%), specificity was (80%), false positive results were 20%, and false negative results were 2.1%.

The false positive results in our study might be explained as previously mentioned in MHT by the overproduction of cephalosporinases as AmpC enzymes or some ESBL enzymes as (CTM-X) that may have minor hydrolytic activity on carbapenemases [7], [13].

In the current study, two strains showed false negative results with PAE-MHT; one was carrying *blaKPC* only while the other was carrying both *blaKPC* and *blaOXA-48*. This may be explained by the possibility of failure of the phenotypic expression of these genes [16].

On performing PAE-MHT, 3% of our CRPA isolates showed indeterminate results which may be due to the production of substances by the tested isolates that inhibit the growth of the indicator strain as reported for MHT [17]. This was discordant with Pasteran et al., who reported no indeterminate results [7].

All of the (100) CRPA isolates, in our work, showed growth on ChromID Carba and 95% of isolates that were proved to carry carbapenemase genes by PCR showed growth on ChromID Carba, so the estimated sensitivity of the ChromID Carba was 100%. However, the test showed false-positive results in five isolates that were negative by PCR.

False positive results with ChromID Carba have different explanations. Simner et al. reported that growth on a chromogenic medium only signifies carbapenem resistance (e.g. AmpC producer with porin loss may grow) but does not confirm the production of a carbapenemase. In their study, ChromID Carba agar demonstrated 89.8% sensitivity in detecting carbapenemase producing GNB but still could grow carbapenemase negative GNB (included 16.7 % of ESBL producers and 6.7% of AmpC producers) [4].

The second explanation could be the presence of other carbapenemase genes not included in our study as out of our five PCR negative isolates (positive by ChromID Carba), three strains were positive in both MHT and PAE-MHT, but the remaining two strains were negative by both tests.

One limitation of the ChromID Carba agar which is mentioned by the manufacturer and several studies[18], [19], [20], is the limited detection of OXA-48 producers, because of the low hydrolysing activity of this enzyme on carbapenem. However, in our study, this was not observed because all OXA-48 producing strains showed growth on Chrom ID Carba. This could be attributed to the finding that all OXA-48 isolates in our study were co-producer of other carbapenemases (KPC or VIM).

Diene and Rolain reported that the VIM carbapenemases enzymes, composed of around 33 variants, have been widely described in *P.aeruginosa* and lesser found in enterobacterial species [21].

Carrër et al. reported that most carbapenemases in clinical isolates of GNB including *P. aeruginosa* are IMP/VIM and KPC [22].

Among our 100 CRPA isolates, carbapenemase genes (*blaKPC, blaVIM, blaIMP, blaOXA-48*) were detected in 95% of the isolates. Only five isolates produced negative results for all of these four carbapenemases. PCR results revealed that *blaKPC* was the most prevalent gene as it was detected in 81% of the isolates, followed by *blaVIM* which was detected in 74% of the isolates, blaOXA-48 in 34% of the isolates, while *blaIMP* was found in one per cent of the isolates. Fifty seven (57%) of the strains was positive for two genes*; (41%: blaKPC + blaVIM), (1%: blaKPC + blaIMP), (11%: blaKPC + blaOXA-48) and (4%: blaVIM + bla OXA-48).* Nineteen strains (19%) were positive for three genes *(blaKPC + blaVIM + blaOXA-48).*

The five isolates with negative PCR results may carry other carbapenemase genes not included in our study (as mentioned previously) or may be due to the presence of another mechanism of carbapenem resistance, e.g. efflux pump and porin loss [23].

Comparable to our results Pasteran et al., reported that in 74 carbapenemase-producing *P. aeruginosa* isolates, 36 (48.6%) isolates carried *blaKPC*, 21 (28.37%) *blaVIM*, 9% *blaIMP* and 8 (10.8%) carried *blaSPM* [3]. Huang et al. found that out of 135 Enterobacteriaceae isolates, 72 isolates (53%) were carbapenemase producers, and out of 221 *P. aeruginosa* isolates, 55 (25%) isolates were carbapenemase producers confirmed by PCR. *BlaOXA-48* carbapenemase was the predominant carbapenemase gene (82%) found in Enterobacteriaceae, while *blaVIM* gene was largely predominated (93%) in *P. aeruginosa* [20].

In contrast to our results, some studies reported that in Pseudomonas species, carbapenemases are mostly MBLs of VIM, IMP, SPM, GIM, AIM, DIM and NDM types and to lesser extent Ambler class A carbapenemases of the KPC and GES types[24], [25], [26].

Koutsogiannou et al. reported that all of MBL positive CRPA isolates (49 isolates) were carrying the *blaVIM* gene and were spread in all hospital wards, especially among the non-ICU patients [27].

Simner et al. found that out of 49 carbapenemases producing GNB (Enterobacteriaceae, *P. aeruginosa* and Acinetobacter) 16 isolates carried the *blaKPC* gene (one *P. aeruginosa*), 12 carried the *blaVIM* gene (five *P. aeruginosa*), and four isolates carried *blaIMP* (one

P. aeruginosa). One of these GNB isolates (E. cloacae) carried both *blaVIM* and *blaOXA-48* [4].

Previous studies revealed association of MBLs (VIM/IMP) with KPC (*blaKPC + blaVIM and blaKPC + blaIMP*) in certain CRPA clones (ST564) in different areas of the world, indicating that this clone is a successful, worldwide multidrug-resistant clone with the ability to acquire relevant carbapenemases [24], [28].

Peshattiwar and Peerapur reported that the percentage of MBL (detected by a phenotypic method; IMP-EDTA DDST) in the imipenem resistant *P. aeruginosa* isolates was 62.5%. They concluded that carbapenem resistance in *P. aeruginosa* is predominantly via MBL production [29]. A nearly comparable rate was noted by Noyal et al., that attributed 50% of imipenem resistant *P. aeruginosa* isolates to the production of MBL [12]. Upadhyay et al. reported a high prevalence of MBL 56 (46.6%) among 120 AmpC producing isolates [30].

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Dec 20; 6(12):2290-2294.
https://doi.org/10.3889/oamjms.2018.454
eISSN: 1857-9655
Basic Science

Isolation and Genotyping of Acanthamoeba from Soil Samples in Markazi Province, Iran

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Citation: Meighani M, Eslamirad Z, Hajihossein R, Ahmadi A, Saki S. Isolation and Genotyping of Acanthamoeba from Soil Samples in Markazi Province, Iran. Open Access Maced J Med Sci. 2018 Dec 20; 6(12):2290-2294.
https://doi.org/10.3889/oamjms.2018.454

Keywords: Acanthamoeba; Soil; PCR; Molecular method; Genotype

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Received: 28-Aug-2018; **Revised:** 25-Oct-2018; **Accepted:** 27-Oct-2018; **Online first:** 16-Dec-2018

Funding: This research did not receive any financial support

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

Abstract

AIM: A previous study confirmed the contamination of water sources with this parasite in Arak, Markazi Province, Iran. The current study investigated soil sources and determined the predominant genotype of Acanthamoeba in this region of Iran.

MATERIAL AND METHODS: Forty-eight soil samples, collected from different regions of Arak, Markazi province, Iran, were evaluated in this study. The samples were processed and identified by culturing on a specific medium, performing PCR assay, and sequencing the PCR products. Finally, using the NCBI database, the genotypes were determined.

RESULTS: Of 48 soil samples, 33.3% and 31.25% were contaminated with Acanthamoeba according to the culture and molecular assays, respectively. The majority of these isolates belonged to the T4, T5 and T6 genotypes of Acanthamoeba.

CONCLUSION: The genotypes of most isolates from soil samples in Arak similar to other regions of Iran belong to T4 genotype of this parasite. New sequence accession numbers include MG066681 and MG298785-MG298794.

Introduction

Acanthamoeba is described as a free-living amoeba with a widespread distribution in the water and soil of various regions [1]. Host infection occurs through the entry of parasite cysts into the nose and eyes. Depending on the host conditions, this can lead to various diseases, such as granulomatous amebic encephalitis (GAE) or keratitis [2]. Water and soil are important reservoirs of this parasite, and to date, parasites have been isolated from different environmental sources in Iran [3]. For instance, 46.25% of environmental samples collected from Tehran were contaminated with this parasite; also, all soil samples were contaminated [4]. Also, 71.6% and 26% of water and soil samples from south of Iran were contaminated with Acanthamoeba, respectively [5].

A limited number of studies have examined water sources in different regions of Iran. In Isfahan, 45.16% of water sources were contaminated with this parasite, and a higher prevalence was found in environmental water sources in comparison with tap water [6]. Similar results have been reported from West Azerbaijan Province (Northwest of Iran) [7] and Shiraz [8]. Moreover, 70.3% of surface water samples

were contaminated with Acanthamoeba in North of Iran (Gilan Province) [9].

Morphological and molecular studies have revealed that the T4 strain is the predominant Acanthamoeba genotype in the environmental sources of Iran [3]. Considering the high prevalence of pathogenic Acanthamoeba strains in the environment, it is recognised as a dangerous organism. Also, it seems that fine airborne dust plays a role in the dissemination and transmission of this parasite [10]. The contamination of water sources with Acanthamoeba was confirmed by a previous study conducted in Markazi Province [11]; however, there is no information regarding the prevalence and isolates of this parasite in the soil of Markazi Province.

Therefore, this study aimed to assess the contamination status and genotypes of this parasite in soil samples from this region.

Material and Methods

Forty-eight soil samples were collected from Arak, capital of Markazi Province, located on the crossroad of northern, eastern, southern, and western provinces of Iran (Arak, Iran; 34°00' N 49°40' E; Figure 1). Because of the presence of various industries, this city has many immigrants. In this study, the soil of parks and gardens were collected for analysis. For this purpose, approximately 50 g of soil was collected in sterile bags and transferred to the Parasitology and Mycology Laboratory, Arak University of Medical Sciences.

Figure 1: Geographic Location of Arak city in Iran

The soil samples were prepared using a 250-µm sieve and then a 0.45-µm nitrocellulose filter, as described in Figure 2. The nitrocellulose filter was transferred to a non-nutrient agar (NNA) plate, coated with E. coli (killed) at a temperature of 28°C. After monitoring the plates for four weeks, the surface of positive cultures was rinsed with sterile Page's saline. The parasites were centrifuged for 5 minutes at 1500 × g after collecting and washing them with phosphate buffer. These samples were used for molecular analysis.

According to the literature, the phenol-chloroform method was used for DNA extraction [12, 13]. For PCR amplification, genus-specific primers were used. To amplify a nearly 500-bp fragment of Acanthamoeba-specific 18S ribosomal DNA, reverse primer JDP2 (5′- TCTCACAAGCTGCTAGGGGAG TCA-3′) and forward primer JDP1 (5′-GGCCCAGATCGTTTACCGTGAA-3′) were used [14].

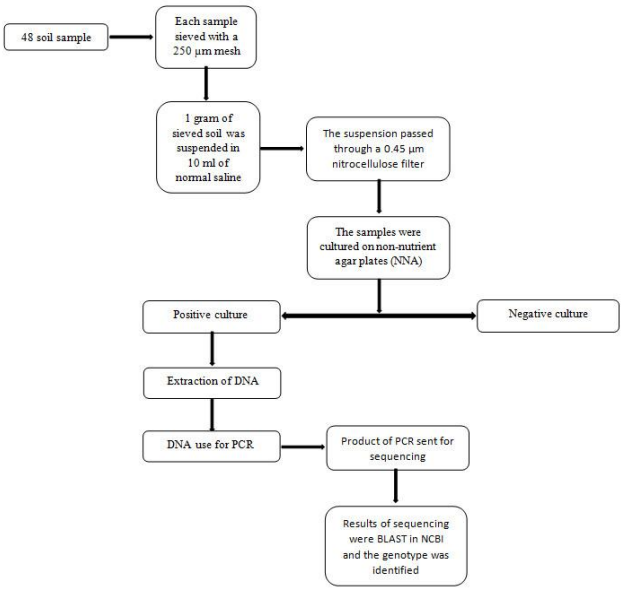

Figure 2: Preparation and testing steps on soil samples

A Master Mix Kit (CinnaGen Co.) was used to optimise the PCR reactions. A final volume of 50 µL was used for amplification in an Eppendorf thermocycler with incubation at 95°C for 3 minutes, followed by 35 cycles at 94°C for 45 seconds, at 55°C for 45 seconds, at 72°C for 45 seconds, and incubation at 72°C for 10 minutes. Using electrophoresis on 1.5% agarose gel, the products were assessed and then visualised under ultraviolet light. After sequencing the PCR products from positive samples, homology analysis was conducted through comparison of the sequences with Acanthamoeba DNA sequences available in the GenBank. The sequences generated in the current study were submitted to the GenBank database.

Results

Sixteen out of 48 soil samples contained Acanthamoeba isolates according to the culturing method (16 samples, 33.3%). Molecular evaluation of the positive samples confirmed 15 (31.25%) isolates.

The eleven sequences (11 sequences) reported in the current study were submitted to the GenBank (accession numbers, MG066681 and MG298785-MG298794). Using the BLAST tool, homology of sequences was compared with the

sequences available in the GenBank. The specificity of the detected isolates is presented in Table 1.

Table 1: Comparison of Acanthamoeba spp. isolates from Arak soil samples with available strains found in the GenBank database

Isolate sequence	Isolate Accession number	Name of Isolate	Genotype	Name of similar isolates in BLAST	Accession number of similar sequence (Genotype)	Region of origin	Reference
AGGTGAAATTCTTGGATTTATG AAAGATTAACTTCTGCGAAAGC ATCTGCCAAGGATGTTTTCATT AATCAAGAACGAAAGTTAGGG GATCGAAGACGATCAGATACC GTCGTAGTCTTAACCATAAACG ATGCCGACCAGCGATTAGGAG ACGTTGAATACAAAACACCACC ATGTATAATTCAGTATATGGCC AGTCAAATGGTTGTA TATTGTTTGTACATG	MG298785	Acs2	ND	Arc-T01	MF470298.1 (ND)	United Kingdom	Asif, Unpublished
				Arc-B08	MF470259.1 (ND)	United Kingdom	Asif, Unpublished
				Arc-NB03	MF470254.1 (ND)	United Kingdom	Asif, Unpublished
				BYB 2017-2	MF113385.1 (T2)	USA	Martin-Perez et al.2017[15]
				Arc-V13	MF470280.1 (ND)	-	Asif, Unpublished
				Arc-E08	MF470243.1 (ND)	-	Asif, Unpublished
				Arc-S07	MF470242.1 (ND)	-	Asif, Unpublished
				RG-HD186	LC184519.1 (ND)	-	Rasti et al.2016, Unpublished
GGCCCAGATCGTTTACCGTGA AAAAATTAGAGTGTTCAAAGCA GGCAGATTCAATTTTCTGCCAC CGAATACATTAGCATGGGATAA TGGAATAGGACCCTGTCCTCCT ATCTTCAGTTGGTTAACTTGTA GAGGATCAGGGTAATGATTAAT AGGGATAGTTGGGGGCAT	MG066681	AcAS4	T4	SSH40	KU885380.1 (T4)	Spain	Reyes-Batlle et al. 2016[20]
CTGGGGCCCAGATCGTTTACC GTGAAAAAATTAGAGTGTTCAA AGCAGGCAGATTCAATTTTCTG CCACCGAATACATTAGCATGGG ATAATGGAATAGGACCCTGTCC TCCTTTTTTCAGTTGGTTAATAA CAGAGAGGATCAGGGTAATGA TTAATAGGGATAGTTG	MG298786	Acs5	T6	CRIB-25	EU273827.1 (T6)	France	Thomas V. (2008) [21]
GGGGTTGGCCCAGATCGTTTA CCGTGAAAAAATTAGAGTGTTC AAAGCAGGCAGATCCAATTTTC TGCCACCGAATACATTAGCATG GGATAATGGAATAGGACCCTGT CCTCCTATTTTCAGTTGGTTTTG GCAGCGCGAGGACTAGGGTAA TGATTAATAGGGATAGTTGGGG GCATTAATA	MG298787	Acs7	ND	EGM3	EF050490.1 (ND)	India	Anand et al. Unpublished
AAAATTAGAGTGTTCAAAGCAG GCAGATTCAATTTTCTGCCACC GAATACATTAGCATGGGATAAT GGAATAGGACCCTGTCCTCCT CTTTTCAGTTGGTTAATTTACGT GTGAGGATCAGGGTAATGATTA ATAGGGATAGTTGGGGGCATT A	MG298788	Acs9	ND	Arc-SK07	MF470308.1 (ND)	-	Asif, Unpublished
				Arc-NB06	MF470264.1 (ND)	-	Asif, Unpublished
				T2/6C	JQ669661.2 (ND)	USA	Crary, Unpublished
				T5-1	EF378672.1 (T5)	-	Wildschutte et al. 2007 [23]
				250GILLE	GQ087290.1 (T2)	France	Year et al. 2007 [24]
ATTTTGGCCCAGATCGTTTACC GTGAAAAAATTAGAGTGTTCAA AGCGGGCAGATATTTTTCCTGC CACCGAATACATTAGCATGGGA TAATGGAATAGGACCCTGACCT CCTATTTTCAGTTGGTTTTGTTT ACAGCGAGGTTATATCAGGGTA ATGATTAATAGGGATAGTTGGG GGCATTAAT	MG298789	Acs10	T5	FL1	JQ418506.1 (T5)	Brazil	Otta et al. (2012) [25]
GGCCCAGATCGTTACCGTGAA AAAATTAGAGTGTTCAAAGCGG GCAGATATTTTTCCTGCCACCG AATACATTAGCATGGGATAATG GAATAGGACCCTGACCTCCTAT TTTCAGTTGGTTTTGTTACAGC GAGGTTATATCAGGGTAATGAT TAATAGGGATAGTTGGGGGCA TTA	MG298790	Acs11	ND	AG-2012 clone AR551	JQ678613.1 (ND)	Spain	Garcia et al. (2013) [26]
TGAGATGGCCCAGATCGTTTAC CGTGAAAAAATTAGAGTGTTCA AAGCAGGCAGATCCAATTTTCT GCCACCGAATACATTAGCATGG GATAATGGAATAGGACCCTGTC CTCCTATTTTCAGTTGGTTTTG GCAGCGCGAGGACTAGGGTAA TGATTAATAGGGATAGTTGGGG GCATTAAT	MG298791	Acs12	T4	JSS-2	KM189416.1 (T4)	Jamaica	Todd CD. (2015) [27]
				JWS-37	KM189412.1 (T4)	Jamaica	Todd CD. (2015) [27]
				JSS-24	KM189408.1 (T4)	Jamaica	Todd CD. (2015) [27]
TTTTTGGCCCAGATCGTTTACC GTGAAAAAATTAGAGTGTTCAA AGCAGGCAGATCCAATTTTCTG CCACCGAATACATTAGCATGGG ATAATGGAATAGGACCCTGTCC TCCTATTTTCAGTTGGTTTTGG CAGCGCGAGGACTAGGGTAAT GATTAATAGGGATAGTTGGGG GCATTAATA	MG298792	Acs13	T4	A29	KT934544.1 (T4)	Venezuela	Wagner (2015) [28]
ATCGTTTACCGTGAAAAAATTA GAGTGTTCAAAGCGGGCAGAA ACTTTTTCCTGCCACCGAATAC ATTAGCATGGGATAATGGAATA GGACCCTGACCTCCTATTTTCA GTTGGTTTTGTTTACAGCGAGG TTCATCAGGGTAATGATTAATA GGGATAGTTGGGGGCATTAA	MG298793	Acs14	T5	P7	JQ268238.1 (T5)	Brazil	Alves Dde S. (2012) [29]
				JWS-26	KM189414.1(T5)	Jamaica	Todd CD. (2015) [27]
TGAAAAAAATTAGAGGGTTCAA AGCAGGCAGATTCAATTTTCTG CCACCGAATACATTAGCATGGG ATAATGGAATAGGACCCTGTCC TCCTATTTTCAGTTGGTTTTGG CAGCGCGAGGACTAGGGTAAT GATTAATAGGGATAGTTGGGG GCA	MG298794	Acs16	T4	SSH40	KU885380.1 (T4)	Spain	Reyes-Batlle M. (2016) [20]

ND: not determined.

The genotyping study of these 11 positive specimens showed that 4 (36.4%), 2 (18.2%) and one (9.1%) sequence belonged to T4, T5 and T6 genotypes of Acanthamoeba, respectively. Phylogenic tree with the neighbor-joining method was shown in Figure 3.

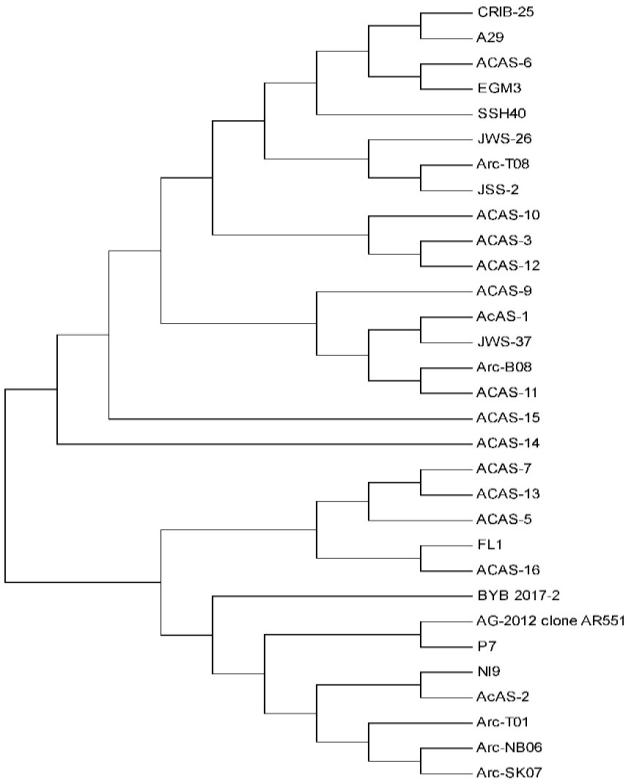

Figure 3: Phylogenic tree constructed with the neighbour-joining tree using nucleotide sequences of DF3 region of the 18S rRNA gene by MEGA5 software

Discussion

Presence of Acanthamoeba spp. in natural resources of Iran has been confirmed in several studies. This organism is involved in dangerous infections of the nervous system and eyes [3]. The present study showed that 33.3% of soil samples from different parts of Arak were contaminated with free-living amoeba, and molecular examination confirmed that 31.25% of these contaminants were related to Acanthamoeba. Since previous studies have shown that water sources in Arak are contaminated with this parasite, the current results were not unexpected. However, the rate of soil contamination and the genotypes of the identified parasites should also be taken into consideration. In this study, seven out of eleven molecular-positive samples were contaminated with T4, T5 and T6 Acanthamoeba genotypes, while genotype of others cases was not determined.

The geographic location and climatic conditions of Markazi province have exposed it to the phenomenon of fine airborne dust. Airborne dust is a source of many microorganisms and has the potential to transfer Acanthamoeba. Therefore, identification of pathogenic microorganisms that can be transmitted by

soil and dust is important for proper planning and prevention of their spread. Strains of Acanthamoeba have been identified and reported in environmental sources from some regions in Iran. In the majority of these reports, the most prevalent Acanthamoeba genotype was T4 in water sources, comprising 62.96%, 91.7%, 83.3%, and 71.6% of water source samples from Shiraz [8], Tehran [30], Mazandaran [31], and Ahvaz [5], respectively. Also, the results of a systematic review showed that Acanthamoeba genotypes T4, T5, and T2 comprised 39%, 17%, and 16% of Acanthamoeba in water sources of Iran, respectively [32].

Some similar studies have examined soil and dust in Iran; T4 was the most common genotype of this parasite in soil and dust specimens in our country. Rezaeian et al., (2008) reported Acanthamoeba contamination in 100% of soil samples and 45.9% of dust samples; however, they did not investigate the parasite genotypes [4]. Niyyati and colleagues (2009) identified the first case of pathogenic genotype Acanthamoeba in dust samples collected from hospital wards of Iran. The isolated strains were related to genotypes T4, T5, and T11 (84.6%, 7.6%, and 7.6%, respectively) [10]. In another study conducted in South of Iran, three genotypes of Acanthamoeba, T2, T5, and T4, were isolated from soil, and T4 (86.6%) was the predominant genotype [5]. Another study demonstrated that 17.3% of soil samples were molecularly positive for T4 genotype Acanthamoeba [33]. Moreover, the soil samples in East Azerbaijan were contaminated with T3, T4, T5, and T11 genotypes of Acanthamoeba [34].

Comparison of genotypes obtained in the current study with other studies from Iran indicates that the genotypes of most isolates from soil samples belong to the unclassified group in Arak. All of these genotypes have been reported in environmental samples from other parts of the world, whereas T4 genotype was dominant in other regions of Iran. Future research should determine the causes of genetic differences between the isolates in our study and other research in Iran.

The results of this study confirmed that soil from parks and gardens has the potential for transgenic transmission of Acanthamoeba to humans. Therefore, individuals, especially children and immunocompromised people, are more likely to develop parasitic infections.

Acknowledgement

This study was done as a MSPH thesis of Islamic Azad University (Mrs. Mehri Mighanee) and was approved by the Ethics Committee of Arak University of Medical Sciences (IR.ARAKMU.REC.1395.178). The authors wish to thank the deputy of research at Arak University of Medical Sciences for their support.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Dec 20; 6(12):2295-2300.
https://doi.org/10.3889/oamjms.2018.456
eISSN: 1857-9655
Basic Science

Role of CD10 Marker in Differentiating Malignant Thyroid Neoplasms from Benign Thyroid Lesions (Immunohistochemical & Histopathological Study)

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Citation: Gabal SM, Salem MM, Mostafa RR, Abdelsalam SM. Role of CD10 Marker in Differentiating Malignant Thyroid Neoplasms from Benign Thyroid Lesions (Immunohistochemical & Histopathological Study). Open Access Maced J Med Sci. 2018 Dec 20; 6(12):2295-2300.
https://doi.org/10.3889/oamjms.2018.456

Keywords: Malignant thyroid neoplasms; Benign thyroid lesions; CD10; Immunohistochemical

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Received: 27-Aug-2018; **Revised:** 09-Nov-2018; **Accepted:** 10-Nov-2018; **Online first:** 19-Dec-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: CD10 was initially recognised as a cell–surface antigen expressed by acute lymphoblastic leukaemias, and hence it's early designation as Common Acute Lymphoblastic Leukemia Antigen (CALLA). Also, it has been proven to be reactive in various non-lymphoid cells and tissue and different types of neoplasms.

AIM: To evaluate the immunohistochemical expression of CD10 in malignant thyroid neoplasms and different benign lesions and to assess whether CD10 can be used as a malignancy marker in thyroid pathology or not.

MATERIAL AND METHODS: A total of 83 archived, formalin fixed, paraffin embedded tissue blocks of 83 cases of malignant thyroid neoplasms and different benign lesions. The samples were immunohistochemically analysed for CD10 expression. A p-value of less than 0.05 was considered statistically significant.

RESULTS: CD10 was expressed in 91% of the studied malignant thyroid neoplasms and 58% of benign thyroid lesions. It was expressed in 26 of 28 (92.9%) conventional papillary carcinomas, ten of 10 (100%) follicular variants of papillary carcinoma, seven of nine (77.8%) minimally invasive follicular carcinomas, two of three (66.7%) widely invasive follicular carcinomas, and seven of 7 (100%) undifferentiated carcinomas, seven of 11 (66.7%) adenomatous nodules and eight of 15 (53.3%) follicular adenomas. No statistically significant correlations were detected between CD10 expression and patients' age, sex, lymph node metastasis, tumour stage and capsular invasion.

CONCLUSION: CD10 shows strong sensitivity (91.2%) and moderate specificity (42.3%) in the diagnosis of malignancy overall and shows strong sensitivity (86.4%) and moderate specificity (42.3%) in the diagnosis of malignancy in the follicular-patterned lesions. So, CD10 might be useful in differentiating malignant from benign thyroid lesions (good positive test) and in the diagnosis of follicular variant of papillary carcinoma.

Introduction

Thyroid cancer represented the most common endocrine malignancy [1]. Thyroid carcinoma accounts for about 1% of all cancers, and its incidence has notable geographic variation [2]. Papillary thyroid carcinoma (PTC) is the commonest form of malignant thyroid tumour accounting for 75% to 85% of all thyroid cancer cases [3].

There are two main obstacles to the diagnosis of thyroid lesions especially the follicular-patterned ones which encompass four entities: adenomatous nodule, follicular adenoma (FA), follicular carcinoma (FC), and follicular variant of papillary thyroid carcinoma (FVPTC) [4]; the discrimination of minimally invasive follicular carcinoma from follicular adenoma or adenomatous nodule and the correct diagnosis of follicular carcinoma [5], [6], [7]. FC is differentiated from FA when there is a capsular, vascular, or extra-thyroid invasion or if there are nodal or distant metastases [8]. Furthermore, the differential diagnosis of follicular cell-derived hyperplastic and neoplastic lesions may be very problematic, and the matter is rarely solved by immunohistochemistry [9].

Moreover, some of the encapsulated thyroid nodules showing follicular morphology may exhibit diffuse or focally intermediate nuclear features of

papillary carcinoma. Thus, it may become another source of controversy [10]. As long as the lesion shows capsular and/or vascular invasion, it is possible to make a diagnosis of well-differentiated carcinoma without further subtyping. On the contrary, if there is no invasion, follicular adenoma (FA) and papillary carcinoma follicular variant (PCFV) should be considered in the diagnosis. This crucial issue cannot be solved by morphology only and even immunohistochemically for some nodules. In this situation, these cases are stated as 'well-differentiated tumour of uncertain malignant potential' (WDTUMP) [10].

Several immunohistochemical markers such as HBME1, CK19 and galectin 3 have been used to overcome this problem [10], [11]. These antibodies can be valuable, especially when used together, but all display some disadvantages and limitations [10], [11], [12], [13], [14], [15]. So, more reliable markers are still required to differentiate between benign and malignant thyroid neoplasms.

Structurally, CD10 is known as a single-chain, 90-110-kDa cell surface zinc-dependent metalloprotease that inactivates many bioactive neuropeptides [16]. Lately, it has been established to be reactive in various non-lymphoid cells and tissues and different types of neoplasms [17]. In thyroid pathology, it was positive in thyroid marginal zone non-Hodgkin lymphoma [18]. The utility of CD10 marker in differentiating different benign and malignant thyroid lesions has been demonstrated in some reports [17], [19].

Material and Methods

This cross-sectional study included 83 cases with different benign and malignant thyroid lesions obtained through the collection of archived paraffin blocks, from the Department of Pathology, Faculty of Medicine, Cairo University, Cairo, Egypt, during the period from June 2016 till September 2017. Cases with deficient clinical data, tiny biopsies or poorly fixed specimens were not included in the study. The medical records which included clinical and histopathological data such as age, gender, site and size of the tumours were revised.

Each paraffin block was re-cut by rotator microtome at 5 µ thickness then mounted on glass slides to be stained by Haematoxylin and Eosin (H&E) for histopathological re-evaluation by two pathologists. Histopathologic examination of H&E stained slides was performed under low power than high power for confirming the diagnosis. Histological classification for thyroid tumours was done according to WHO classification 2004 [20].

Cases included 11 adenomatous nodules, 15 follicular adenomas, 12 follicular carcinomas, 38 papillary carcinomas and 7 undifferentiated carcinomas.

Paraffin blocks were cut at 5 µ thickness then mounted on charged slides and stained manually for immunohistochemistry. The sections were deparaffinized in xylene, for 10 min, then dehydrated in descending series of ethanol (100%, 96%, 70%), followed by washes in TBS (0.05 mmol/L Tris-buffer physiological saline, pH 7.4-7.6), for 5 min.

Antigen retrieval was achieved by heating the samples without boiling in 10 mmol/L sodium citrate buffer, pH 6.0 (200 mL) in a microwave oven. This treatment was conducted twice for 7 min. The sections were washed in TBS buffer for 30 min.

The endogenous peroxide was blocked by 0.3% hydrogen peroxide in methanol for 5 min. The sections were washed in TBS for 15 minutes. To inhibit non-specific background staining; the samples were incubated in a superblock for 5-10 minutes at room temperature.

The primary antibody was monoclonal mouse CD10 antibody clone GM003 (Genemed, South San Francisco, CA, USA) was purchased from SNF medical Company, LOT NO.L 60125051, at 1:50 dilution for one hour at room temperature. The dilution was based on dilution experiments. The antibody was diluted with 20 mmol/L TBS, pH 7.4 (10 mmol/L $CaCl_2$, 0.1% NaN_3 and 1% BSA). The sections were incubated in the diluted antibody. The incubation took place in incubation boxes overnight. The secondary antibody (4.5 µL biotinylated anti-mouse antibody in 1 mL of 1% BSA) was pipetted onto the sections and incubated in the moist box for 30 min. The secondary antibody was washed in TBS buffer for 15 min.

The final staining was done in diaminobenzidine tetrahydrochloride (DAB) solution (49 mL TBS-buffer, 34 mg imidazole, 17 µL 30% hydrogen peroxide and 1 mL 30% DAB), for 5-15 min. The slides were washed with distilled water, 70% ethanol for 1 min, then in distilled water. The nuclei were stained with Mayer's hematoxylin for 30 seconds as a counterstain. The extra stain was washed with tap water. The slides were then transferred through ascending ethanol series, and xylene before mounting.

Positive control for cases stained for CD10 was done using sections obtained from tonsils, which exhibited a strong intensity of CD10 immunostaining and the negative control was obtained by omitting the primary antibody. Tumour tissue sections were examined and scored under LEICA ICC50HD microscope at low power than high power magnification by two independent pathologists who were not informed of the histological diagnosis. The sections were regarded as positive when immunoreactivity was observed in the cytoplasm and

cell membrane. For each case, 10 high power fields were evaluated. Immunoreactivity was graded as 0 (negative) when less than 10% of tumour cells were positive, 1 (weak) when 10-49% of tumour cells were positive and 2 (strong) when 50% or more of tumour cells was positive. The immunoreactivity interpreted based on the percentage of the stained cells irrespective of the intensity of the staining.

Data were collected, coded and analysed using the Statistical Package for Social Science (SPSS version 21.0). Data presented in the form of mean, standard deviation and percentage. For categorical variables, differences were analysed using Chi-square (X^2) test and Fisher's-exact test. Cohen's kappa is used for measurement of the agreement. A p-value of less than 0.05 was considered significant. Sensitivity, specificity, and diagnostic accuracy were assessed. Sensitivity means the true positive rate; specificity means the true negative rate.

Results

Clinicopathological characteristics of the studied cases and their correlation with CD10 expression are summarised in (Table 1).

Table 1: Clinicopathological characteristics of the studied cases and its correlation with CD10 expression

Parameter	Number (%)	CD10 (Negative) expression	CD10 (weak)	CD10 (strong)	P value
Age					
< 45 years	54 (65.1%)	5 (13.9%)	8 (22.2%)	23 (63.9%)	0.197
≥ 45 years	29 (34.9%)	0 (0.0%)	6 (28.6%)	15 (71.4%)	
Gender					
Male	15 (18%)	0 (0.0%)	5 (45.5%)	6 (54.5%)	0.141
Female	68 (82%)	5 (10.9%)	9 (19.6%)	32 (69.6%)	
Category					
Malignant	57 (68.7%)	5 (8.8%)	14 (24.6%)	38 (66.7%)	0.001
Benign	26 (31.3%)	11 (42.3%)	6 (23.1%)	9 (34.6%)	
Pathologic tumour stage (T)					
T1	18 (31.6%)	2 (11.1%)	3 (16.7%)	13 (72.2%)	0.180
T2	20 (35.1%)	2 (10.0%)	2 (10.0%)	16 (80.0%)	
T3	16 (28.1%)	1 (6.3%)	8 (50.0%)	7 (43.8%)	
T4	3 (5.3%)	0 (0.0%)	1 (33.3%)	2 (66.7%)	
Lymph Node (LN) Metastasis					
N0	43 (75%)	5 (11.6%)	9 (20.9%)	29 (67.4%)	0.275
N1	14 (25%)	0 (0.0%)	5 (35.7%)	9 (64.3%)	
Stage					
Stage I	37 (64.9%)	5 (13.5%)	8 (21.6%)	24 (64.9%)	0.778
Stage II	9 (15.8%)	0 (0.0%)	3 (33.3%)	6 (66.7%)	
Stage III	7 (12.3%)	0 (0.0%)	2 (28.6%)	5 (71.4%)	
Stage IV	4 (7.0%)	0 (0.0%)	1 (25.0%)	3 (75.0%)	
Papillary carcinoma cases					
With capsular invasion	17 (44.7%)	0 (0.0%)	7 (41.2%)	10 (58.8%)	0.2
Without capsular invasion	21 (55.3%)	2 (9.5%)	4 (19.0%)	15 (71.5%)	

CD10 immunostaining was identified in 26 of 28 (92.9%) conventional papillary carcinomas, ten of 10 (100%) follicular variants of papillary carcinoma, seven of nine (77.8%) minimally invasive follicular carcinomas, two of three (66.7%) widely invasive follicular carcinomas, seven of seven (100%) undifferentiated carcinomas, eight of 15 (53.3%) follicular adenomas and seven of 11 (63.6%) adenomatous nodules (Table2).

Table 2: Immunoreactivity of CD10 in each diagnostic category

Diagnosis	Total case number	CD10 (Negative) expression	CD10 (weak)	CD10 (strong)
Conventional PC	28	2 (7.1%)	8 (28.6%)	18 (64.3%)
Follicular variant of PC	38	0 (0.0%)	3 (30.0%)	7 (70.0%)
Minimally invasive FC	9	2 (22.2%)	0 (0.0%)	7 (77.8%)
Widely invasive FC	3	1 (33.3%)	2 (66.7%)	0 (0.0%)
Undifferentiated carcinoma	7	0 (0.0%)	1 (14.3%)	6 (85.7%)
Follicular adenoma	15	7 (46.7%)	2 (13.3%)	6 (40.0%)
Adenomatous nodule	11	4 (36.4%)	4 (36.4%)	3 (27.3%)

Studied cases are categorised into two groups (benign and malignant). Ninety-one per cent of malignant cases showed positive CD10 expression and the remaining 9% showed negative CD10 expression, while 58% of benign cases showed positive CD10 expression and the remaining 42% showed negative CD10 expression.

To evaluate the value of CD10 immunostaining in the diagnosis of malignancy, we performed sensitivity, specificity and diagnostic accuracy. CD10 shows strong sensitivity (91.2%), 95% Confidence Interval (95% CI) (80.7 to 97%), moderate specificity (42.3%), 95% CI (23.35% to 63.08%) and diagnostic accuracy (75.9%), 95% CI (65.27% to 84.62%). Cohen's k was run to determine if there was an agreement between CD10 and the diagnostic tool (by H&E). There was moderate agreement $k = 4$, $p < 0.01$.

Besides, we separate a subset of 48 thyroid lesions with a follicular growth pattern (15 follicular adenomas, 12 follicular carcinomas, 11 adenomatous nodules and 10 follicular variants of papillary carcinoma). Sensitivity, specificity, and diagnostic accuracy of CD10 for the diagnosis of malignancy in these lesions were 86%, 95% CI (65.09% to 97.09%), 42%, 95%CI (23.35% to 63.08%) , and 62.5%, 95% CI (47.35% to 76.05%) respectively.

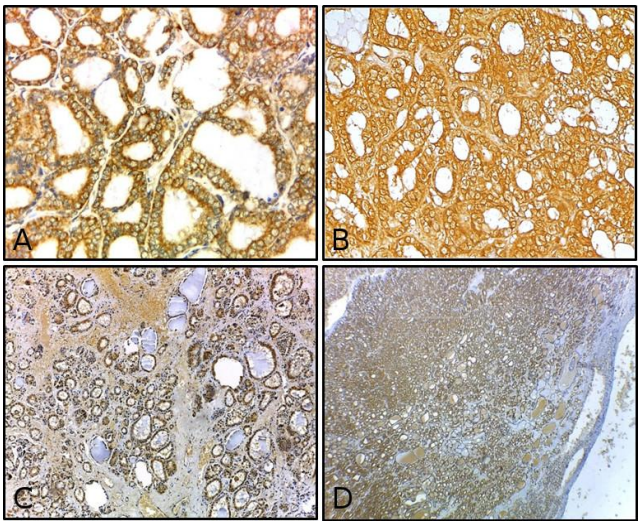

Figure 1: Follicular-patterned lesions; A: Follicular carcinoma showing strong CD10 staining (score 2+) (original magnification X 400); B: Follicular variant of papillary carcinoma showing strong CD10 staining (original magnification X 400); C: Adenomatous nodule showing positive CD10 staining (score 2+) (original magnification X 100); D: Follicular adenoma showing positive CD10 staining (score 2+) (original magnification X 100)

All the undifferentiated thyroid tumours and 90% of the differentiated tumours showed positive CD10 staining, but there is no statistically significant relationship between CD10 expression and differentiation of thyroid tumours (p = 0.8) (Table 3).

Table 3: Correlation of CD10 immunohistochemical expression with the differentiation of thyroid tumours

Category	CD10			Total	P value
	Negative	Weak	Strong		
Differentiated thyroid carcinoma	5 (10%)	13 (26%)	32 (64%)	50 (100.0%)	0.8
Undifferentiated thyroid carcinoma	0 (0.0%)	1 (14.3%)	6 (85.7%)	7 (100.0%)	

Moreover, we assess the correlation of CD10 expression with some prognostic factors as lymph node metastasis, tumour stage and capsular invasion. No statistically significant relationships were observed between the previously mentioned parameters and CD10 expression (P-value = 0.27, 0.77, 0.2 respectively) (Table1).

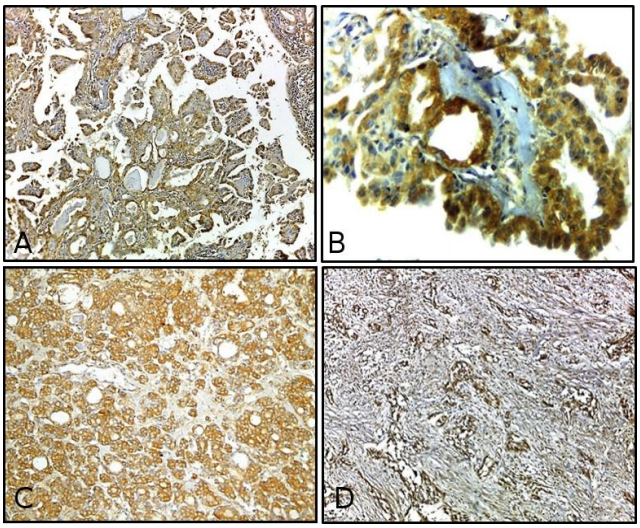

Figure 2: Both differentiated (papillary and follicular carcinomas) and undifferentiated carcinomas showing positive CD10 staining (score 2+); A: Conventional papillary carcinoma (original magnification X 100); B: High power of A (original magnification X 400); C: Follicular carcinoma (original magnification X 100); D: Undifferentiated carcinoma (original magnification X 100)

Discussion

In this study, we aimed to assess the value of CD10 in differentiating benign and malignant tumours of the thyroid especially the follicular patterned thyroid nodules.

The distinction between benign and malignant follicular tumours may be occasionally problematic as it relies mainly on the presence of capsular and/or vascular invasion. If the focus of invasion is small or not evident, right diagnosis becomes difficult. Furthermore, benign follicular tumours are sometimes misdiagnosed as follicular variants of papillary carcinoma. Lloyd and his colleagues assessed the observer variation in the diagnosis of follicular variant of papillary carcinoma; all ten skilled thyroid pathologists agreed in the diagnosis in only 39% of cases [21].

In order to overcome the previous limitations, several markers of malignancy such as CK19, HBME1, and galectin 3 have been studied in thyroid specimens, but unfortunately, all present some disadvantages and limitations [10], [11]. Also, a marker of malignancy may be used as a preoperative diagnostic tool for suspicious lesions. Thus the extent of surgery and the complementary treatments could be planned before thyroid operations.

Originally, CD10 was thought to be a tumour-specific antigen [22], but studies have shown that it is expressed by a variety of cell types including bronchial epithelial cells, renal proximal tubular epithelial cells, cultured fibroblasts, bone marrow stromal cells, breast myoepithelium, biliary canaliculi, fetal intestine, and certain solid tumours [17], [23], [24].

Tomoda *et al.,* 2003 was the first report on the expression of CD10 marker in thyroid neoplasms. They evaluated CD10 expression in 70 thyroid neoplasms and reported that CD10 was negative in benign lesions and pure papillary carcinomas but was positive in 80% and 77% of follicular carcinomas and follicular variant of PTC, respectively. They deduced that CD10 immunostaining could be a useful marker of follicular carcinoma to distinguish it from follicular adenoma and benign hyperplastic nodules and in diagnosing follicular variants of papillary thyroid carcinoma [17].

Another research was done by Yegen *et al.*, who have investigated the staining pattern of CD10 in different benign (*n* = 14) and malignant (*n* = 61) thyroid lesions. They reported their results as follows: CD10 was negative in adenomatous nodules, minimally invasive follicular carcinomas and well-differentiated carcinomas. It was positive in conventional papillary carcinomas (64.2%), follicular variants of papillary carcinoma (16.6%), papillary microcarcinomas (50%), widely invasive follicular carcinomas (11.1%) and follicular adenomas (30%). They concluded that, despite CD10 strong positivity in conventional papillary carcinoma, it could not be used as a useful marker for differentiating benign and malignant thyroid lesion [19].

Mokhtari and Ameri, 2014 reported a significant correlation between CD10 expression and both benign and malignant thyroid lesions (P < 0.001) as they found CD10 positivity in 29.9% of PTC cases, but in none of the thyroid benign lesions (0%) [25].

Chu and Arber have investigated CD10 expression in 505 non-hematopoietic neoplasms

including 55 thyroid tumors [follicular adenoma (n = 24), papillary carcinoma (n = 10), medullary carcinoma (n = 16) and follicular carcinoma (n = 5)] by IHC. They detected that CD10 expression was negative in all the examined thyroid tumours [22].

In like manner, Yasuda et al., have studied the availability of CD10, as a histopathological marker, in different non-hematopoietic neoplasms including thyroid tumours. They reported that CD10 was not present in thyroid tumours and showed no diagnostic value for this group of non-hematopoietic neoplasms [27].

In contrast with the previously mentioned studies, we observed CD10 expression in benign lesions both in FA and adenomatous nodules as well as in carcinomas. Malignant tumours and benign lesions showed 91% and 58% positivity overall respectively. CD10 was identified in 26 of 28 (92.9%) conventional papillary carcinomas, ten of 10 (100%) follicular variants of papillary carcinoma, seven of nine (77.8%) minimally invasive follicular carcinomas, two of three (66.7%) widely invasive follicular carcinomas, seven of seven (100%) undifferentiated carcinomas, eight of 15 (53.3%) follicular adenomas and seven of 11 (63.6%) adenomatous nodules.

Statistically, CD10 shows strong sensitivity (91.2%), 95% CI (80.7 to 97%) and moderate specificity (42.3%), 95% CI (23.35% to 63.08%) in the diagnosis of malignancy overall with diagnostic accuracy (75.9%), 95% CI (65.27% to 84.62%). Furthermore, in the follicular-patterned lesions, it was found that sensitivity, specificity, and diagnostic accuracy of CD10 for the diagnosis of malignancy in these lesions were 86%, 95% CI (65.09% to 97.09%), 42%, 95%CI (23.35% to 63.08%) , and 62.5%, 95% CI (47.35% to 76.05%) respectively.

Our results are not consistent with the results of previous studies. We observed a higher frequency of positivity in both benign and malignant lesions. This discrepancy could be explained by geographic and genetic variability between patients, different sample size, technical variations and different antibodies, clones and brands. A well-known fact in immunohistochemistry is that the specificity and sensitivity of the antibodies may show variations with different clones and brands. Moreover, some studies did not evaluate different malignant thyroid lesions and did not perform CD10 sensitivity and specificity. The strengths of our data are the great variability of the thyroid lesions analysed by IHC using CD10 expression, as well as performing its sensitivity and specificity in the diagnosis of thyroid lesions.

In conclusion, CD10 is highly expressed in malignant tumours (95% of papillary carcinoma, 75% of follicular carcinomas and 100% of undifferentiated carcinoma. CD10 showed strong sensitivity (91.2%) and moderate specificity (42.3%) in the diagnosis of malignancy overall and showed strong sensitivity (86.4%) and moderate specificity (42.3%) in the diagnosis of malignancy in the follicular-patterned lesions. So, CD10 might be useful in differentiating malignant from benign thyroid lesions (good positive test) and in the diagnosis of follicular variant of papillary carcinoma being observed in 100% of these tumours.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Dec 20; 6(12):2301-2305.
https://doi.org/10.3889/oamjms.2018.413
eISSN: 1857-9655
Clinical Science

Use of Fentanyl Patch and Intravenous Morphine for Treatment of Leg Bone Fracture: Treatment Profile, and Clinical Effectiveness

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Citation: Jokar A, Ahmadi K, Hajimaghsoodi L, Ketabi S. Use of Fentanyl Patch and Intravenous Morphine for Treatment of Leg Bone Fracture: Treatment Profile, and Clinical Effectiveness. Open Access Maced J Med Sci. 2018 Dec 20; 6(12):2301-2305. https://doi.org/10.3889/oamjms.2018.413

Keywords: Transdermal fentanyl patches; Morphine; Pain

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Received: 13-Aug-2018; **Revised:** 09-Oct-2018; **Accepted:** 10-Oct-2018; **Online first:** 10-Nov-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Severe pain is one of the major problems in patients with leg bone fracture. Various methods have been proposed to relieve pain. Opioids are one of the most important available medications to control these types of pain. Among the opioids available, fentanyl can be applied for its unique properties as transdermal patches.

AIM: Therefore, the current study aimed to investigate the effect of intravenous morphine and fentanyl skin patch in patients with a lower leg fracture.

METHODS: We entered 60 patients in this randomised, one-blind randomised clinical trial among patients referring to the emergency department of Vali-e-Asr Hospital in Arak with a fracture of the leg. Demographic and clinical data were recorded for patients. The case group (n = 30) received the fentanyl patch in the same area. Patients in the control group (30) received 0.1mcg/kg of morphine intravenously. In both groups, the severity of pain was measured every 20 minutes within two hours after onset of treatment based on VAS criteria and subsequently recorded in the checklist. Data were analysed by SPSS v.22 software package.

RESULTS: The results of the present study demonstrated that the mean visual analogue scale (VAS) pain score at minutes 20, 40, 60 and 80 were statistically lower in intervention group when compared with the control group (p = 0.000).

CONCLUSION: Our results indicated a considerable risk-benefit profile for the treatment of pain in patients suffering from dysphagia, nausea and vomiting, or resistance to other opioids. The use of fentanyl patch is also suitable for patients who are not able to take their medication at their scheduled time.

Introduction

The increasing use of machinery and vehicles has led to vehicle-*related accidents* and serious physical *injuries*, which has become a major health problem for humans. Due to the occurrence of accidents at high speeds, vehicle-*related injuries* are a considerable risk *of* provoking a severe complication and often results in disability, amputation and death of the injured patients. One of the most common causes of amputation is open fractures, one of the most common causes of amputation is open fractures, most commonly occurring in the legs of 21.9% [1] and consisting 25% of open fractures [2].

On the one hand, the type of soft tissue injury causes acute and life-threatening infections in open fractures; on the other, creates chronic and resistant bone infection [3]. Leg bone fractures (tibia and fibula fractures) are the most common bone fracture in the body that occurs in the majority of men at younger ages [4], due to the low soft tissue coverage in anterior and *anteromedial parts* and inadequate blood supply and soft tissue in this area [5]; whereas tibia

nonunion account for the highest percentage of total referrals [6]. Pain is one of the most preventable complications in surgeries, but usually, it is not enough to treat it. Pain can indirectly increase morbidity and mortality, while also contributing to increased costs and lower quality of life.

Pain relief is a challenge after surgery, which requires pain relievers with minimal side effects and the highest level of safety for the patient [7]. Currently, postoperative pain treatment has been highly considered. Some studies have reported a high prevalence of postoperative pain. Severe pain not only causes pain and discomfort but also prevent patients from returning to daily activities, which is considered an important socio-economic factor [8].

Severe pain is one of the major problems for patients with leg bone fracture. Various methods have been proposed to relieve pain associated with bone fracture. Opioids can be mentioned as the most important compounds with *the most availability* regarding *controlling* this type of pain. Pain control using oral and intravenous opioids requires effective dosage and regular use of medications. In most cases, regular use of these compounds may be difficult due to problems with patient's problem and forgetfulness and ultimately leads to insufficient control of the patient's pain.

Therefore, various methods have been introduced for transferring the drug, which can be referred to transdermal adhesives [9]. Among the opioids available, fentanyl can be applied for its unique properties as transdermal patches (adhesives). Fentanyl has been considered as an artificial opioid due to its fatty properties and high analgesic power compared to morphine in the 1990s [10]. *Fentanyl transdermal* patch can be effectively used, especially for patients suffering from dysphagia, nausea and vomiting, or resistance to treatment, or intolerance to other opioids.

Also, this method is appropriate for patients who are not able to take their medication at the prescribed time [11]. Fentanyl is an artificial opiate drug that was first made in 1960 and has been used as part of the anaesthetic regimen for about 30 years. Physical properties of the drug include low molecular weight, high solubility in fat and high power, which has led to the use of fentanyl in *transdermal* drug delivery *systems* [12]. There is a controversy about the effectiveness of the fentanyl skin patch method, where some studies have not yet succeeded in exhibiting this effect well. The researchers attribute the causes of these differences to the differences in the methodology and quality of the studies, as well as sample sizes and comparison groups.

The current study aimed to compare the effectiveness of the *fentanyl transdermal patch* with the effect of intravenous morphine in patients.

Material and Methods

We enrolled 60 (43 man and 17 women) in this, *single-blind* randomised controlled *clinical trial*. The statistical population consisted of patients who referred to the emergency department of Vali-e-Asr Hospital in Arak, with a fracture of the leg. All of them were in the first and second classes (*ASA II and I*) of the American Society of Anesthesiologists (ASA). The primary diagnosis was based on four criteria including localised tenderness, pain, deformity, and crepitation. Patients were entered the treatment groups using a random number table after filling the inclusion criteria. The primary examination of the patient was done by a specialist, and the final diagnosis was performed by the two physicians. Early demographic and clinical characteristics of patients were recorded. The patients' pain was then checked by the VAS criteria and data recorded in the checklist.

In the case group (30 patients), subjects received the fentanyl transdermal patches in the same areas. The *patch placement* includes 1: left deltoid muscle; 2: right deltoid muscle; 3: left chest on the top of the nipple; 4: *right chest* on the top of the nipple.

In the control group (30 patients), subjects also received 0.1 mcg/kg of morphine intravenously.

In both groups, pain intensity was measured every 20 minutes during two hours after treatment based on VAS criteria and finally recorded in the checklist.

All data were analysed by SPSS v.22 software package. To present the results, mean indexes, standard deviation, standard error, the percentage of frequency were applied. Furthermore, tests such as covariance analysis, Chi-square, Independent T-test or its nonparametric equivalents were employed to compare the means. $P < 0.05$ was considered as statistically significant.

Inclusion criteria included the age of over 18 years, patients with leg fractures, *obtaining* an informed consent form,

Exclusion criteria include Patients' *refusal to participate* in the study, the failure to diagnose fractures in the radiographic images, the incidence of complications and the sensitivity to the drug administration, and the history of related diseases, as well as the transferring the patient to the operating room within two hours after entering the emergency room.

The informed consent of the participation in the study was taken optionally, and the confidentiality of the information was retained. This research project was approved by the Ethics Committee of the Research Council of Arak University of Medical Sciences with the number 2792 (ethics code: IR.ARAKMU.REC.1395.298).

Results

Of the 30 patients in the experimental group, 20 were men (66.6%) and the rest were women. Also, the control group consisted of 23 male patients (76.7%) and 17 female patients. To test the homogeneity of the two groups, Chi-square test was applied, where showed no significant difference in both groups (P = 0.22). In the test group, the majority of patients (73.3%) were in the age group of 21-30 years, while the lowest frequency (3. 3%) was in the age group of fewer than 20. Moreover, most of the patients in the control group (70%) were seen in the age group of 20-30 years, and the lowest (6.7%) belonged to the age group of fewer than 20 years old. Based on the Fisher test, the two groups did not exhibit a significant difference in age (P = 0.42). Base on the use of t-test, there was no significant difference between the mean *VAS* pain score at 0 minutes (P = 0.37) in both groups. There was no significant difference between the two groups regarding the vital signs of the patients (Table 1).

Table 1: Comparison of pain intensity and vital signs at zero minute

Variable	Fentanyl	Morphine	Analysis text
VAS	06.9±.66	8±.78	0.370
O2saturation	36.95±20.3	36.95±45.3	0.100
BP	63.13±25.1	73.13±15.1	0.710
T	37±.45	01.37±.13	0.09
RR	36.22±78.4	30.21±36.5	0.140
PR	53.98±60.9	90.95±45.3	0.140

Using t-test, the mean *VAS* pain score at 20 minutes in both groups was significantly different (P = 0.000). A pain score of the morphine group significantly decreased more than the other group. Furthermore, the vital signs of the patients in both groups were evaluated using a t-test, where the findings did not emphasise the significant difference between the two groups (Table 2).

Table 2: Comparison of pain severity and vital signs in the 20 the minute

Variable	Fentanyl	Morphine	Analysis text
VAS	54.8±.66	26.4±.69	0.000
O2saturation	23.96±27.1	23.96±47.1	0.640
BP	73.13±15.1	81.13±54.1	0.770
T	97.36±.17	37±.45	0.730
RR	40.22±78.4	40.22±52.4	1.000
PR	53.96±45.3	46.98±06.9	0.270

The mean *VAS* pain score in both groups was statistically significant at 40 minutes (P = 0.000); in other words, the mean *VAS* pain score of morphine group was markedly decreased as compared to another group. There was no significant difference between the two groups regarding O_2 saturation, RR and PR in both groups. However, we found that the mean of BP and T in both groups was statistically different from t-test (Table 3).

Table 3: Comparison of severity of pain and vital signs in 40 the minute

Variable	Fentanyl	Morphine	Analysis text
VAS	20.5±.92	21.4±.76	0.000
O2saturation	30.93±57.1	23.96±27.1	0.310
BP	24.12±01.5	86.13±49.1	0.000
T	99.36±.19	8.36±.24	0.002
RR	36.21±09.6	40.22±78.4	0.460
PR	20.96±72.3	53.98±06.9	0.190

As shown in Table 4, the mean pain score (VAS) of 60 minutes was lower in the morphine group than in the fentanyl group (P = 0.000). There was no significant difference between the two groups regarding O2saturation, RR, and PR symptoms in both groups. However, the mean values of P and T in both groups were significantly different based on the T-test.

Table 4: Comparison of the severity of pain and vital signs in the 60th minute

Variable	Fentanyl	Morphine	Analysis text
VAS	1.4±.75	16.3±.79	0.000
O2saturation	23.96±7.12	26.96±31.1	0.920
BP	21.12±91.2	91.13±4.1	0.001
T	97.36±.21	86.36±.22	0.005
RR	63.20±78.5	40.22±78.4	0.200
PR	83.95±.70	46.98±65.1	0.140

Table 5 demonstrated a significant difference between the mean *VAS* pain score at 80 minutes in both groups (P = 0.000). Also, there were no significant differences in O2 saturation, RR, and PR symptoms in both groups. However, the mean value of BP and T in both groups was remarkably different (Table 5).

Table 5: Comparison of pain severity and vital signs in the 80th minute

Variable	Fentanyl	Morphine	Analysis text
VAS	06.4±.73	23.3±.77	0.000
O2saturation	36.96±17.2	26.96±31.1	0.760
BP	11.12±68.2	76.13±46.1	0.002
T	97.36±.21	86.36±.22	0.005
RR	93.20±92.5	43.22±80.4	0.280
PR	9.96±89.3	46.98±06.9	0.170

As indicated in Table 6, the mean *VAS* pain score for 100th minutes in the fentanyl group was less than the other group (P = 0.000). On the other hand, there were no significant differences in the O2 saturation, RR, and PR in both groups. However, the mean value of BP and T in both groups revealed a significant difference by using t-test.

Table 6: Severity of pain and vital signs in the 100th minute

Variable	Fentanyl	Morphine	Analysis text
VAS	56.3±.50	13.4±.77	0.001
O2saturation	36.96±21.1	33.96±26.1	0.920
BP	44.12±40.2	89.13±48.1	0.000
T	96.36±.20	85.36±.23	0.005
RR	55.21±01.1	16.22±.89	0.620
PR	64.94±19.5	53.97±6.9	0.009

Our results revealed that the mean *VAS* pain score in the 100th minute in the fentanyl group was not significantly different compared with morphine

group (P = .000). We did not find a significant difference in the vital signs of O2 saturation, RR and PR in patients in both groups. Nevertheless, the mean values of BP and T in both groups were significantly different by using T-test (Table 7).

Table 7: Comparison of severity of pain and vital signs in 120 the minute

Variable	Morphine	Fentanyl	Analysis text
VAS	06.3±.36	03.4±.76	0.000
O2saturation	89.96±22.2	40.96±47.1	0.270
BP	42.12±45.6	82.13±24.5	0.000
T	94.36±.25	46.36±.22	0.050
RR	30.20±85.4	53.22±89.4	0.080
PR	10.95±17.4	60.98±08.9	0.060

We compared the mean *VAS* pain at minutes 0 to 120 in the two groups of fentanyl and morphine. The results exhibited that there was no significant difference between the two groups in only 0 minutes, but in the remaining minutes, this difference was statistically significant (Table 8).

Table 8: VAS pain score at minutes 0 to 120

Variable	Fentanyl	Morphine	Analysis text
VAS0	06.9±.66	8±.78	0.370
VAS20	54.8±.66	26.4±.69	0.000
VAS40	20.5±.92	21.4±.76	0.000
VAS60	1.4±.75	16.3±.79	0.000
VAS80	06.4±.73	23.3±.77	0.000
VAS100	56.3±.50	13.4±.77	0.001
VAS120	06.3±.36	03.4±.76	0.000

Discussion

In this clinical trial study, 60 patients were randomly divided into intervention and control groups during 2017 in Valiasr Hospital. The study showed that the oldest person in the intervention group was 94 years old, and the youngest was 49 years old. Moreover, the oldest person in the control group belonged to a 64 years old individual and the youngest person was 16 years old. Using statistical tests, the mean age of the two groups was not found to be significantly different (p = 0.42).

Furthermore, the mean height of the subjects in the intervention group was determined to be 6.2 ± 1.71. In the intervention group, the mean height of the patients was calculated as 15.60 ± 1.69. There was no statistically significant difference between the mean height of the control and intervention groups (p = 0.12). This study aimed to compare the pain in patients with fracture of the leg in the use of fentanyl skin patches and injectable morphine ampoule. Before the onset of the intervention, by comparing the pain with VAS scores, it was revealed that the mean score of pain in both groups was not significantly different (P-value = 37.7).

Regarding the random sampling and the similarity of pain between the two groups, the pain was accordingly the same between the two groups before each intervention. Based on the results presented in this study, the pain score between the two intervention and treatment groups was statistically significant at 20, 40, 60 and 80 minutes, so that the pain score of the control group (morphine) was lower than that of the intervention group (P = 0.000). In other words, morphine has been able to produce more analgesic effects at these times. The onset of analgesic effect of intravenous morphine (0.1-0.05 mg.kg) is 10-20 minutes after injection, but the onset of analgesic effect of fentanyl skin patch could be started after one hour [13].

Also, fentanyl patch is not predictable, which its effects may be started up to several hours, depending on the body temperature, and the previous dose of the drug, as well as other factors such as the location of use, hemodynamics and the general condition of the patient (fragility, hypovolemia).

Absorption continues for a few hours unpredictably *following* removal *of* the *patch* [14]. These results are consistent with our findings. Also, due to the slow start of fentanyl absorption, patients may use a patch from the day before surgery. Localised blood flow to the patch site can affect the absorption of the drug. Heat blankets, moisture or sepsis, can increase blood flow to the skin, leading to an increase in total systemic absorption [14]. The results of our study demonstrated that the mean score of pain at 100 and 120 minutes was significantly different, where the mean score of pain in the intervention group was significantly lower as compared to the control group (p = 0.00). In other words, patients in the intervention group who used fentanyl skin patch had less pain after one hour than those in the control group. The unique properties of fentanyl include its 75-fold strength compared to morphine, low molecular weight, and lipophilicity, as well as higher skin absorption capacity than morphine.

In*consistent with our* results, these features reduce pain by initiating the effect of fentanyl [15]. In 2004, Clark found that the analgesic effect of fentanyl skin patch was significantly higher than that of morphine [16], which is in agreement with our results. In another study by Hemmati et al., The results showed that fentanyl skin patch significantly reduced the pain of patients with soft tissue tumours compared to placebo [17]. Another study evaluated the safety and therapeutic effect of 12-month use with fentanyl patch. This mentioned study indicated a reasonable risk-benefit profile for managing moderate to severe chronic pain in non-cancer patients treated with fentanyl patch under long-term compared with patients who treated with other opiate drugs. Respiratory depression, drug dependence, and drug discontinuation were rarely observed in patients [18].

Some of the properties of the drug that leads to better tolerance of the drug by the patient, its effectiveness and its relative safety include the need

for repeated administration of the drug, lower *peak plasma concentration*, and lack of liver first pass metabolism [19]. In patients with cancer pain, the use of this drug leads to prolonged and effective analgesia. Four different drug types are available including 25, 50, 75 and 100 micrograms per hour.

The fentanyl patch needs 24 to 72 hours to reach a sustained level of blood, and absorption of remaining fentanyl lasts for several hours after removing the patch. After removing the fentanyl patch, it takes about 17 hours to reduce the plasma concentration of the drug by 50%. Therefore, there is a potential for drug interruption with anaesthetics, sleep apnea and other opioids several hours after removing the patch. Therefore, the risk of drug interruption is not eliminated immediately *following* the removal *of* the *patch*. It is worth noting that the fentanyl patch releases the drug for up to 72 hours and has been proposed as a synthetic drug with short-term analgesia [20].

On the other hand, fentanyl provides an appropriate plasma concentration up to 72 hours, where the blood concentration of the drug gradually increases, leading to a reduced risk of complications. Fentanyl metabolites are not pharmacologically active and are not affected by the liver first pass metabolism or gastrointestinal absorption. Fentanyl with a high tendency and specifically binds to the μ2-opioid receptor. Therefore, the side effects of activating the μ2-opioid receptor such as nausea, vomiting and constipation that is seen with the use of morphine are not seen with this drug. It should be taken into consideration that the complications of accumulation of metabolites are not seen in patients receiving this drug [21].

In summary, this study exhibited that the fentanyl skin patch has a significantly more analgesic effect in patients with fractures after one hour than morphine. The use of transdermal fentanyl is useful especially for patients suffering from dysphagia, nausea, vomiting, or other forms of resistance to other opioids. It is also suitable for patients who are not able to take their medication at their scheduled time.

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Open Access Macedonian Journal of Medical Sciences. 2018 Dec 20; 6(12):2306-2309.
https://doi.org/10.3889/oamjms.2018.436
eISSN: 1857-9655
Clinical Science

The effectiveness of DC Motor Vibrilatory Stimulus (DMV) among Postpartum Women on Giving Breast Milk

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Citation: Nasution SS, Erniyati E, Aizar E. The Effectiveness of DC Motor Vibrilatory Stimulus (DMV) among Postpartum Women on Giving Breast Milk. Open Access Maced J Med Sci. 2018 Dec 20; 6(12):2306-2309. https://doi.org/10.3889/oamjms.2018.436

Keywords: DC Motor Vibrilatory Stimulus; Postpartum women; Breastmilk

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Received: 28-Aug-2018; **Revised:** 20-Oct-2018; **Accepted:** 21-Oct-2018; **Online first:** 25-Nov-2018

Funding: This research was financially supported by the Ministry of Research and Technology and Higher Education of Indonesia under the research grant via TALENTA USU of the Year 2018

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

Abstract

BACKGROUND: The success of a woman in following the exclusive breastfeeding program is determined by the significant effort of a woman in giving breast milk and providing additional food to the baby. This problem can be solved by using the vibration effect on the postpartum mother's breast through DC Motor Vibratory (DMV) system.

AIM: Therefore, the research aims to analyse the effectiveness of DMV among postpartum woman on giving breast milk. This intervention will assist to stimulate the prolactin hormone and to remove the oxytocin hormone of the breast milk.

METHODS: The study is a quasi-experiment, with only post-test design and control group. The sample of the study included 76 postpartum women in University of Sumatera Utara (USU) Hospital and maternity clinic in Medan, Indonesia with accidental sampling technique. The data were collected by using questionnaires, such as demographic questionnaire and observation of breast milk expenditure. The study was analysed by using the Mann-Whitney test.

RESULTS: The Result of the study showed that the DMV could accelerate the process of breastfeeding for a postpartum woman with a significant value of $p < 0.05$.

CONCLUSION: The research concluded that DMV could be applied in the postnatal care process, especially in stimulating breast milk expenditure.

Introduction

The high rate of new-born deaths is commonly based on many factors, including maternal behaviour and the role of health workers. Infection in infants and low birth weight are a major factor in the occurrence of morbidity and mortality [1]. This condition is closely related to the still low health status of mothers and new-borns; low access and quality of maternal and infant health services, especially during pregnancy, childbirth and immediately after birth [2]. Maternal and family behaviour which are preventive and curative is still minimal; community involvement in care, especially the perception of breastfeeding for new-born babies, is still a lot of negatives [3].

Morbidity and mortality rates in infants can be prevented by early breastfeeding, which is immediately after birth and following the exclusive breast milk (ASI) program [4]. Based on data from UNICEF (2006), as many as 30,000 infant deaths in Indonesia and 10 million deaths of infants and toddlers were recorded in the world each year which can be prevented through exclusive breastfeeding for six months, without having to provide additional food and drinks to infants. Breastfeeding is one of the adaptation processes experienced by postpartum mothers. Breast milk (ASI) is a natural nutrient for babies with the most suitable nutritional content for optimal growth [5]. Breastfeeding is revealed to be effective to protect maternal and infant health [6].

The maximum breastfeeding since the baby is

born can meet the nutritional needs accordingly so that the baby will avoid pain and death. The World Health Organization (WHO) recommends that every new-born baby get exclusive breastfeeding for six months, but in some mothers do not give exclusive breastfeeding because the US does not come out or only slightly so that it does not meet the needs of the baby [7]. Postpartum mothers often experience breast swelling after giving birth with nearly 90%, on the second day until the fourth day. This happens due to the lack of breastmilk expenditure and is a strong reason for mothers to stop breastfeeding and provide additional food such as formula milk [8]. The Basic Health Research of Indonesia (RISKESDA) (2010) reported that exclusive breastfeeding for infants aged < 6 months was reported to be less than 40% [3]. Many ways have been used to facilitate postpartum maternal breast milk expenditure such as brash care, oxytocin massage, acupuncture and acupressure [9].

This method is still considered to be less effective as it uses invasive methods, long duration, not practical, must have the skills and techniques in its implementation. Postpartum treatment in optimising breastmilk expenditures uses a lot of non-invasive methods to be considered as alternatives in accelerating breastmilk expulsion, because with effective therapeutic methods with a relatively short time and easy to do by postpartum mothers [10].

Based on that information, a tool needed to be designed as a stimulus therapy to accelerate the release of breast milk postpartum mothers who are simpler, economical, and practical by utilising DC Motor Vibratory which provides a vibration effect for activating prolactin and oxytocin hormones. The DC vibratory motor method is a method of vibration propagation that vibrates the ions in the breast which is directed to the activation of the prolactin and oxytocin hormones which results in the stimulus of the alveoli in flowing milk through the ductulus to the lactiferous duct and into the milk sinus and the nipple hole.

Methods

This research is quantitative research using a quasi-experimental method research design: with the use of Current ASI DMV tools and only post-test design in the intervention and control groups. This study was conducted on postpartum mothers in USU hospital and maternity clinics by observing feeding to infants whether only breastfeeding or providing additional food other than ASI. Data analysis was carried out with frequency distribution and presentation, independent t-test with a significance level of 5% (α = 0.05). The tool used is the ASI DMV made by the researcher as displayed in figure 1.

Figure 1: DC Motor Vibrilatory Stimulant

Results

The characteristics of the postpartum woman are described in Table 1 below.

Table 1: Demografic Data of Respondent Characteristic (n = 38) in Intervention Group

Mother Characteristic	Frequency (n)	Percentage (%)
Age		
Risk (< 20 and 35) Years	13	34
No Risk (20-35)Years	25	66
Obstetric History		
Primi (1)	9	24
2-3	23	61
> 3	6	15
Education		
Low (Elementary – Junior Hight School)	7	18
Middle (Senior High School)	22	58
High (university)	9	24
Work		
Housewife	17	45
Government Employee	8	21
Entrepreneur	13	34
Total	**38**	**100**

Based on Table 1, it shows that most postpartum mothers are in the age range of 20-35 years with 25 people or 66% of the total. Most of the mother parity is 2-3 people as many as 61 people. Mother's education level in a senior high school dominated the sample with 22 people as much as 58%, and most mothers are 17% housewife with no occupation.

Table 2: Demografic Data of Respondent Characteristic (n = 38) in Control Group

Mother Characteristic	Frequency (n)	Percentage (%)
Age		
Risk (< 20 and 35) Years	14	35
No Risk (20-35)Years	24	63
Obstetric History		
Primi (1)	9	24
2-3	22	58
> 3	7	18
Education		
Low (Elementary – Junior Hight School)	5	13
Middle (Senior High School)	24	63
High (university)	9	24
Work		
Housewife	18	47
Government Employee	9	24
Entrepreneur	11	29
Total	**38**	**100**

Based on Table 2, it indicates that most postpartum mothers are in the age range between 20-35 years with 24 people or 63% of the total. Most of the mother parity is 2-3 people as many as 58%of sample. Mother's education level in a senior high school dominated the sample with 63%, and most mothers are 18% housewife.

Table 3: Distribution of Food Giving to New-borns Intervention Groups and Control Groups

Feeding Infants	Intervention		Control	
	N	%	N	%
Breast Milk	30	78.9	16	42.1
Supplementary Infant Feeding	16	21.1	22	57.9
Total	**38**	**100**	**38**	**100**

Based on Table 3, postpartum mothers who only gave the breast milk were recorded as 78.9%, and 21.1% of them were giving additional food, while postpartum mothers in the control group who only gave breast milk were 42.1% and 57.9% of postpartum mothers provide additional food.

Table 4: Effectiveness of DC Motor Vibrilatory Stimulus on Giving Breast Milk in Intervention and Control Group

Variable	Mean	Sum .ranks	Z *P.*
Intervention	27.50	522.50	-5.187 0.000
Control	11.50	218.50	

Lastly, the effectiveness of DMV on breast milk production is described in table 4. Based on table 4, the result shows a p-value of 0.000, which means that the value of p <0.05 indicates the significant difference in infant feeding in the intervention group and control group.

Discussion

Breast milk (ASI) is a natural nutrient and the most suitable food for babies because the nutritional content in ASI is best suited for optimal growth and development of infants [11]. Breast milk is the only nutrient source that also contributes to the rapid and healthy growth of the baby's brain and nervous system, the maturation of the digestive system and the development of the immune system and immunity [12]. According to the World Health Organisation (WHO), exclusive breastfeeding is only given for 6 (six) months without any additional liquid either formula milk, water, orange juice, or other supplementary foods before reaching the age of six months [13]. The success of exclusive breastfeeding begins with the success of early breastfeeding. In general, the failure of breastfeeding mothers at the beginning of a baby's birth is caused by ASI that has not been released immediately [14].

To speed up the process of removing breast milk requires the process of breast care in postpartum mothers. Some of the treatments that have been done frequently include oxytocin massage, acupressure and acupuncture. But the process of breast care is certainly inseparable from the development of technology in the health sector so that there will be some of the latest innovations in the development of breast care. So every postpartum mother can do breast care independently. But not all postpartum mothers immediately excrete milk because the milk supply is a very complex interaction between mechanical stimuli [3], nerves and various hormones that affect oxytocin release. The release of the oxytocin hormone besides being influenced by baby suction is also influenced by receptors located in the ductal system when the ducts dilate or become soft; oxytocin is reflexively removed by the pituitary which acts to squeeze milk from the alveoli [2]. Hormones that affect the production of breast milk (ASI) include oxytocin. This hormone causes the contraction of epithelial cells around the alveoli, urging breast milk to enter the lactiferous duct. Stimulation of the nipple causes the release of oxytocin for 3-4 seconds into the bloodstream for every 5-15 minutes [15]. Prolactin is produced by the anterior pituitary which functions to stimulate the ASI gland to produce breast milk, prolactin release occurs in response to a direct stimulus to the nipple or areola which controls autocrine in lactogenesis. Prolactin will come out if there is a space of milk in the breast. Decreasing production and expenditure of breast milk in the first day after giving birth can be caused by a lack of stimulation of the prolactin and oxytocin hormones which greatly contribute to the smooth production and expenditure of breast milk [11].

Breast care should be performed immediately after the baby delivery process (1-2 days), and the mother must do it regularly. By providing stimulation to the muscles of the breast will help to stimulate the hormone prolactin to help to produce milk [5]. Lack of breast milk production is one of the causes of the mother deciding to give formula milk to her baby. UNICEF insists that babies who use formula milk have a chance of dying in the first month of birth, and the possibility of formula-fed infants is 25 times higher than the number of babies whose mother's breastfeeding exclusively [16].

Constraints in breastfeeding have been identified including the factors such as lack of information, apathy on the side of health care providers, inappropriate hospital practices such as providing water and supplements for babies without any medical needs and lack of follow-up care at the beginning of the postpartum period [3], [17]. The low maternal behaviour in breastfeeding is also influenced by various factors, namely socio-cultural community, psychological condition, the physical condition of the mother, lack of information from health workers, incessant promotion of formula or canned milk, the condition of newborns, and lack of knowledge about breastfeeding. Moreover, there is a complaint of breast milk that has not been produced and insufficient for the baby's needs. These reasons and problems cause mothers and families to make decisions from experiences and habits that are contrary to health, such as not breastfeeding and providing additional food to babies.

The results of the study generally show that DC Vibratory Motor (smooth DMV ASI) can accelerate the release of breast milk and affect the behaviour of

mothers in providing food to infants, namely the intervention group can generally provide only breast milk in infants when compared with the control group. Use or Implementation of Dc Vibratory Motor which consists of Arduino Nano components: As the brain of the tool, which is useful in controlling motor speed and setting the use time of the tool? Transistor: To provide more current to the motor so that the motor can be faster. Diodes: As a voltage rectifier. Potential set the motor speed by changing the resistance value. The laser is used to shooting light to the intended point. Led is to provide more lighting on therapy. Vibratory motor: As a vibrator. The tools used in this intervention will help stimulate the hormone prolactin and the hormone oxytocin in the removal of breast milk. Vibration propagation methods that vibrate ions in the breast are directed directly to activation of the prolactin and oxytocin hormones which results in a stimulus to the alveoli and drain the milk through the ductulus to the lactiferous duct and into the sinuses and leave the breast through the nipple.

The presence of vibrations causes the smooth muscles of the breast to contract and accelerate expenditure, due to increased oxytocin secretion. Increased secretion of oxytocin is caused by vibrations that stimulate the surrounding nervous system and continue stimulation to the anterior pituitary in the brain so that prolactin is secreted and continued into the anterior pituitary. Through this intervention, it is expected that postpartum mothers can perform breast care independently with practically and overcome problems in the process of breastfeeding. The use of this tool is expected to provide a solution for mothers who do not breastfeed their babies because ASI does not come out, or breastfeeding is insufficient. The provision of maximum and immediate breastfeeding in newborns can meet the nutritional and nutritional elements so that babies avoid pain and death which in turn can improve the level of infant health in Indonesia.

In conclusion, the DMV tool can increase and accelerate the release of breast milk so that it affects the postpartum mothers in providing food to infants only by using breast milk. This can be seen from the statistical value which shows the value of $p < 0.05$ ($p = 0.000$).

Acknowledgements

The authors gratefully acknowledge the Ministry of Research and Technology and Higher Education of Indonesia to support the research fund. The support is under the research grant via TALENTA USU of the Year 2018.

Ethical Aspects

Authors claim that the research followed the ethical aspect as regulated by University of Sumatera Utara, Indonesia. Also, there is no conflict of interest in this research.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Dec 20; 6(12):2310-2315.
https://doi.org/10.3889/oamjms.2018.270
eISSN: 1857-9655
Clinical Science

Three Dimensional (3D) Echocardiography as a Tool of Left Ventricular Assessment in Children with Dilated Cardiomyopathy: Comparison to Cardiac MRI

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Citation: Habeeb NM, Youssef OI, Elguindy WM, Ibrahim AS, Hussein WH. Three Dimensional (3D) Echocardiography as a Tool of Left Ventricular Assessment in Children with Dilated Cardiomyopathy: Comparison to Cardiac MRI. Open Access Maced J Med Sci. 2018 Dec 20; 6(12):2310-2315. https://doi.org/10.3889/oamjms.2018.270

Keywords: (3D) echocardiography; CMRI; DCM

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Received: 17-May-2018; **Revised:** 02-Oct-2018; **Accepted:** 15-Oct-2018; **Online first:** 25-Oct-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Left ventricular (LV) volumes and ejection fraction (EF) is Strong prognostic indicators for DCM. Cardiac MRI (CMRI) is a preferred technique for LV volumes and EF assessment due to high spatial resolution and complete volumetric datasets. Three-dimensional echocardiography is a promising new technique under investigations.

AIM: Evaluate 3D echocardiography as a tool in LV assessment in DCM children about CMRI.

PATIENTS AND METHODS: A group of 20 DCM children (LVdiastolic diameter < 2 Z score, LVEF < 35%) at Children s Hospital, Ain-Shams University (gp1) (mean age 6.6 years) were compared to 20 age and sex-matched children as controls (gp2). Patients were subjected to: clinical examination, conventional echocardiography, automated 3D LV quantification, 3D speckle tracking echocardiography (3D-STE) (VIVID E9 Vingmed, Norway) and CMRI (Philips Achieva Nova, 1.5 Tesla scanner) for LV end systolic volume (LVESV), LVend diastolic volume (LVEDV) that were indexed to body surface area, EF% and wall motion abnormalities assessment.

RESUTS: No statistically significant difference was found between automated 3D LV quantification echocardiography, 3D-STE, and CMRI in ESV/BSA and EDV/BSA assessment (p = 1, 0.99 respectively), between automated LV quantification echocardiography and CMRI in EF% assessment (p = 0.99) and between CMRI and 3D-STE in LV Global hypokinesia detection (P = 0.255). As for segmental hypokinesia CMRI was more sensitive [45% of patients vs. 40%, (P = 0,036), basal septal hypokinesia 85% vs. 75%, (p = 0.045), mid septal hypokinesia 80% vs. 65%, (p = 0.012) and lateral wall hypokinesia 75% vs. 65%, (p = 0.028)].

CONCLUSION: Automated 3D LV quantification echocardiography and 3D-STE are reliable tools in LV volumetric and systolic function assessment about CMRIas a standard method. 3D speckle echocardiography is comparable to CMRI in global wall hypokinesia detection but less sensitive in segmental wall hypokinesia which mandates further studies.

Introduction

Pediatric dilated cardiomyopathy (DCM) is a serious often life-threatening condition. Left ventricular (L V) volumes and LV ejection fraction (EF) provide fundamental measures of function and are Strong prognostic indicators for patients with DCM [1]. In DCM, LV ejection fraction is the strongest predictor of progression to heart failure, while LV volume and mass are independently correlated with mortality and morbidity; therefore accurate quantification of all these parameters is essential for adequate patient's evaluation and also to monitor the progression of disease and response to different therapeutic agents [2]. CMR can be considered the reference technique for the quantification of ventricular volumes and functional parameters and ventricular mass in patients with DCM [3].

Three dimensional (3D) and 3D speckle tracking are promising new techniques that are still under investigations. The aim of the current study was To evaluate 3D echocardiography as a tool of LV assessment in children with DCM about CMR

Patients and Methods

The current cross-sectional study was conducted on 20 children with idiopathic dilated cardiomyopathy (IDCM) (LVdiastolic diameter > 2 Z score, LVEF < 35%) [4], attending the Pediatric Cardiology Clinic, Children's Hospital, Ain-Shams University (10 males and 10 females), and their ages ranged from 1 month to 14 years with a mean age of 6.6 years (gp1) that were compared to 20 age and sex-matched children as controls (gp2). Patients with any known cause of myocardial disease, underlying congenital heart disease, hypertrophic cardiomyopathy or secondary DCM, renal insufficiency (plasma creatinine > 2mg/dl), hepatic, autoimmune disorders or malignancies were excluded from the study. Informed consent was taken from parents/caregivers. The study was approved by the ethical committee. Studied groups were subjected to:

- Thorough history taking: laying stress on symptoms of heart failure, NYHA heart failure classification

- Detailed clinical examination with special emphasis on cardiac examination.

- Echocardiographic imaging.

Initially routine diagnostic imaging was performed and included Motion mode (M mode), two-dimensional echo (2D), Pulsed wave (PW), Continous wave (CW), as well as Colour flow (CF) Doppler studies. The following parameters were assessed by M-mode of left ventricle in short axis parasternal views: Left Ventricular End Systolic Volume (LVESV (ml), Left Ventricular End Diastolic Volume (LVEDV (ml), Ejection Fraction (EF%) Echocardiographic examinations were performed in a standard manner with use of a commercially available cardiac ultrasound unit device model (VIVId E9 ultrasound system, General electric Vingmed, Horten, Norway).

First, six-chamber views, as well as three short-axis views at different levels of the left ventricle from base to apex, were automatically selected from the RT3DE pyramidal dataset in the first time frame of the dataset, i.e. end-diastole. Then the anatomically correct, non-foreshortened apical views were identified by finding the largest long-axis dimensions. In these two planes, LV endocardial boundaries were manually initialised, while including the papillary muscles in the LV cavity. Then, the 3D endocardial surface was automatically reconstructed and tracked in 3D throughout the cardiac cycle. Finally, the endocardial surface was manually adjusted when necessary in the above five planes until the best match was visually verified. For each consecutive time frame, voxel count inside the detected endocardial surface was used to calculate LV volume. End-diastolic volume and ESV were then obtained from the LV volume curves as the maximum and minimum values, respectively**,** as well as detecting values of longitudinal strain and expressed as a percentage of the original length. The patient is considered to have wall motion abnormality if any segment showed longitudinal strain < 11 [5].

Pyramidal RT3DE datasets were analysed using the 3D wall motion tracking software (VIVId E9 ultrasound system (General Electric, Vingmed, Horten, Norway)) by an investigator experienced with STE analysis that was blinded to the results of cMRI measurements.

A Philips Achieva Nova (1.5 teslas) scanner superconducting system with 30 mt/mint gradient with cardiac coil was used in Radiology department (MRI unit) Ain Shams University for assessment of Ejection Fraction (EF%, End Systolic Volume (ESV) End Diastolic Volume (EDV).

The procedures of the MR examination were explained to the patient above 7 years old including breath hold instructions. The patients were briefly interviewed about MR contraindications, whether the patient has a pacemaker or any other implanted devices, or other foreign materials inside the body (in particular cerebral aneurysmal clips). Children below 7 years sedated with chloral hydrate and their parents were informed by precautions.

Patients were studied in the supine position, head first. The patients were offered cotton blankets for warmth. Headphones with the MRI machine were used to reduce repetitive gradient noise and at the same time allow the patients to hear the breath-hold instructions.

The first electrode was placed approximately 1 cm left of the xiphoid. The second and third electrodes (were positioned in such a way that they were aligned at approximately 90 degrees to each other, where the first electrode forms the right angle. The distance between the electrodes should be approximately 15 cm. The fourth electrode was placed below the first electrode. It was used to determine the cardiac frequency as it should be close to the patient's heart rate. The QRS complex was then checked on MRI monitor, adjustment of the site of the leads was made accordingly. The patient's heart rate was also detected on MRI monitor.

The respiratory sensor was placed over the maximum area of respiratory movement (abdomen and thorax) under the coil. A strap was used to fix the sensor. The respiratory signal was then checked as the respiratory wave appeared on the monitor and was used to detect the patient's respiratory rhythm and synchronise breath hold instructions to the patient's abilities.

The SENSE (sensitivity encoding) cardiac coil (6 elements phased array coil, receive only) was used. It has a rigid lower part and a flexible upper part. The coil was positioned on the chest so that the midline of its upper part lied just below the sternoclavicular notch and the lower part of the coil

lied underneath the patient. It was carefully strapped onto the patient. The connection to the magnet was checked.

Planning vertical long axis image from the axial orthogonal image at the level of the left ventricle, planning the horizontal long axis view from the vertical long axis view. Planning the short axis view from the horizontal long axis view. Breath-hold balanced turbo field echo sequence (b-TFE) in short axis view from the mitral annulus to the apex with the following parameters: TR (repetition time)/TE (echo time): 4.4/2.5, FOV (Field of view): 300, Phases: 25, NSA (Number of signal averages): 1, Flip angle: 15, Scan time: 7-12 sec, Slice thickness: 8 mm, Number of slices: 7.

Analysis of the CMR (DICOM) images was performed using Brilliance 170 P workstation. Left ventricular ejection fraction and volumes were quantified automatically from the cine images after manual tracing of LV endocardial border in the short axis images during end systole and end diastole for each slice position.

Results

Sixty-five per cent of studied patients (65%) were males and (35%) were females with males to females 2:1.

Thirty per cent of studied patients (30%) had increased heart rate for age, 55% had low systolic blood pressure for age, and 35% had low diastolic blood pressure for age.

Table 1: Comparison between 3D echocardiography, cMRI and 3D speckle echocardiography regarding ESV (ml) and EDV (ml) values indexed by BSA (m^2) and EF (%) mean values

	3D echocardiography		cMRI		3D speckle		One Way ANOVA	
	Mean	SD	Mean	SD	Mean	SD	F	P-value
ESV (ml)/BSA (m^2)	58.58	23.89	58.65	24.11	58.58	23.88	0.000	1.000 (NS)
EDV (ml)/BSA (m^2)	92.69	27.44	93.41	27.33	92.84	27.29	0.004	0.996 (NS)
EF (%)	40.25	7.65	39.56	9.80	-	-	4.817	0.996 (NS)

One Way ANOVA comparing three groups, P-value > 0.05 is non-significant, SD: Standard deviation, ESV: End Systolic Volume, EDV: End Diastolic Volume, cMRI: cardiac Magnetic Resonant Imaging, BSA: Body Surface Area, NS: Non-Significant.

All patients were on Frusemide and Captopril therapy, 90% of them were on Digoxin and Spironolactone, 35% were on low dose aspirin, and 20% of them were on L-carnitine.

A statiscally significant increase was found in patients ESV/BSA and EDV/BSA and a statistically significant decrease was found in patients EF% assessed by 3D-LV quantification compared to controls (58.58 ± 23.89 vs. 24.16 ± 1.58, p = 0.000; 92.69 ± 27.44 vs. 61,24 ± 1.58, p = 0.001 and 40.25 ± 7.65 vs. 69.4 ± 4.55, p = 0.00 respectively).

Table 2: Comparison between cMRI and 3DSTE as regards cardiac wall motion abnormalities assessment

	CMRI		3D STE		Chi-square test	
	No.	%	No.	%	X^2	P-value
Global hypokinesia	16	80.0%	16	80.0%	2.727	0.255 NS
Apical hypokinesia	9	45.0%	8	40.0%	6.624	0.036 S
Septal wall dyskinesia	3	15.0%	2	10.0%	3.055	0.217 NS
Basal septal hypokinesia	17	85.0%	15	75.0%	6.190	0.045 S
Mid septal hypokinesia	16	80.0%	13	65.0%	8.750	0.012 S
Inferior wall hypokinesia	14	70.0%	12	60.0%	5.253	0.072 NS
Lateral wall hypokinesia	15	75.0%	13	65.0%	7.131	0.028 S
Inferior wall akinesia	2	10.0%	1	5.0%	2.105	0.349 NS

cMRI: Cardiac magnetic resonance imaging, 3DSTE: 3D speckle tracking echocardiography, P value > 0.05 is non-significant, P value < 0.05 is significant, NS: Non Significant, S: Significant.

A statistically significant increase was found in patients ESV/BSA and EDV/BSA assessed by 3D-STE compared to controls (58.58 ± 23.88 vs. 24.16 ± 15.8, p = 0.000 and 92.84 ± 27.29 vs. 61.24 ± 2.84, p = 0.001 respectively).

Also, a stastically significant increase was found in patients ESV/BSA and EDV/BSA and a statistically significant decrease was found in patients EF%assessedbyCMRI compared to controls (58.65 ± 24.11 vs. 26.02 ± 1.77, p = 0.000; 93.41 ± 27.33 vs. 63.03 ± 3.05, p = 0.002 and 39.56 ± 9.8 vs. 67.65 ± 3.07, p = 0.000 respectively).

Table 3: Intraobserver variability of 3DSTE regarding wall motion abnormalities

3DSTE	Observer. I		Observer. II		Chi-square test	
	No.	%	No.	%	X^2	P-value
Global hypokinesia	16	80.0%	13	65.0%	0.864	0.3526 NS
Apical hypokinesia	8	40.0%	6	30.0%	0.110	0.7403 NS
Septal wall dyskinesia	2	10.0%	1	5.0%	0.000	1.0000 NS
Basal septal hypokinesia	15	75.0%	13	65.0%	0.119	0.7301 NS
Mid septal hypokinesia	13	65.0%	11	55.0%	0.114	0.7469 NS
Inferior wall hypokinesia	12	60.0%	10	50.0%	0.101	0.7506 NS
Lateral wall hypokinesia	13	65.0%	11	55.0%	0.104	0.7469 NS
Inferior wall akinesia	1	5.0%	1	5.0%	0.545	0.4602 NS

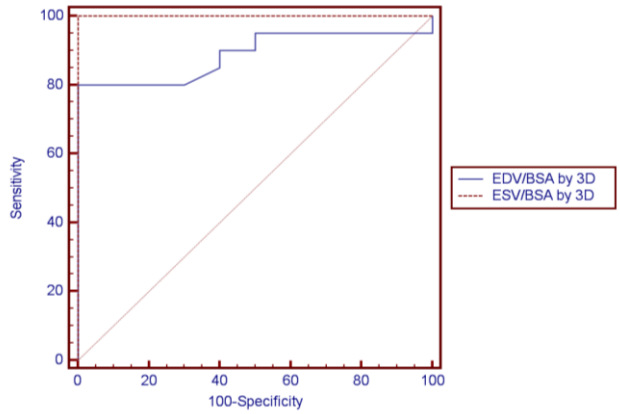
3DSTE: 3D speckle track echocardiography, P value > 0.05 is non significant, NS: Non Significant, Observ: Observation.

No statistically significant difference was found between automated 3D LV quantification echocardiography, 3D-speckle echocardiography, and cMRI in assessment of ESV/BSA (58.58 ± 23.89 ml/m^2), (58.58 ± 23.88 ml/m^2), (58.65 ± 24.11ml/m^2) respectively and EDV/BSA (92.69 ± 27.44 ml/m^2), (92.84 ± 27.29 ml/m^2), (93.41 ± 27.33 ml/m^2) respectively No statistically significant difference was found between automated LV quantification echocardiography and CMRI in EF% values (40.25 ± 7.65 vs. 39.56 ± 9.8, p = 0.99). All studied patients had global and segmental hypokinesia as assessed by 3D-STE and CMRI.

Table 4: Intraobserver variability of cMRI regarding wall motion abnormalities

cMRI	Observer. I		Observer. II		Chi-square test	
	No.	%	No.	%	X^2	P-value
Global hypokinesia	16	80.0%	14	70.0%	0.133	0.715 NS
Apical hypokinesia	9	45.0%	7	35.0%	0.104	0.746 NS
Septal wall dyskinesia	3	15.0%	1	5.0%	0.144	0.0578 NS
Basal septal hypokinesia	17	85.0%	15	75.0%	0.158	0.6926 NS
Mid septal hypokinesia	16	80.0%	13	65.0%	0.502	0.4788 NS
Inferior wall hypokinesia	14	70.0%	12	60.0%	0.110	0.7403 NS
Lateral wall hypokinesia	15	75.0%	13	65.0%	0.119	0.7301 NS
Inferior wall akinesia	2	10.0%	0	0.0%	0.526	0.4682 NS

cMRI: Cardiac magnetic resonance imaging, P-value > 0.05 is non significant, NS: Non Significant, Observer: Observation.

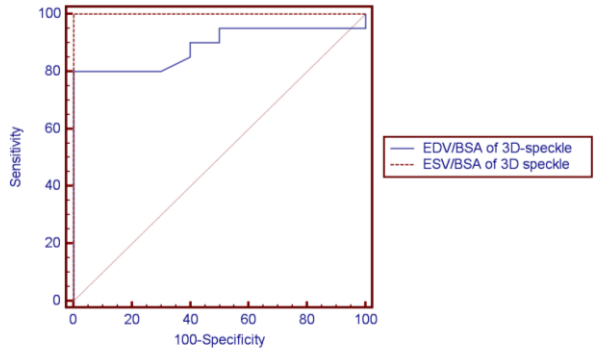


Parameters	Cut off point	AUC	Sensitivity	Specificity	+PV	-PV
EDV(ml)/BSA(m^2) by 3D echocardiography	>65.4 *	88.8	80.00	100.00	100.0	71.4
ESV(ml)/BSA(m^2) by 3D echocardiography	>26.6	100.00	100.00	100.00	100.00	100.0

ESV: End Systolic Volume, EDV: End Diastolic Volume, BSA: Body Surface Area,+ PV: Positive Predictive Value, -PV: Negative Predictive Value, AUC: Area Under the Curve.

Figure 1: ROC curve detect sensitivity and specificity of 3D echocardiography in the prediction of EDV and ESV indexed by BSA

Sensitivity of 3D and 3D-STE echocardiography in prediction of EDV (ml)/BSA (m^2) was 80% and 100% in prediction of ESV (ml) /BSA (m^2) (Figures 1 and 2). Sensitivity and specificity of 3D-echocardiography and cMRI were 100% in the assessment of EF% (Figure 3).



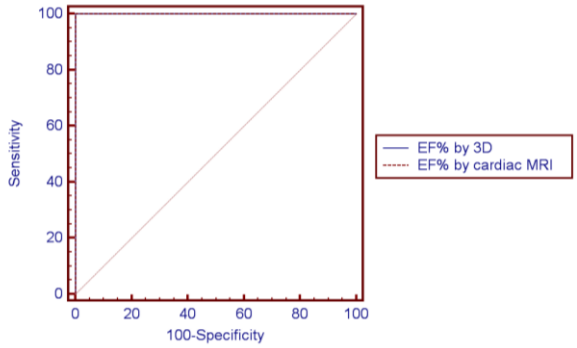
Parameters	Cut off point	AUC	Sensitivity	Specificity	+PV	-PV
EDV(ml)/BSA(m^2) by 3D-STE	> 65.4	88.8	80.0	100.0	100.0	71.4
ESV(ml)/BSA(m^2) by 3D-STE	> 26.6	100.0	100.0	100.0	100.0	100.0

ESV: End Systolic Volume, EDV: End Diastolic Volume, BSA: Body Surface Area, + PV: Positive Predictive Value, -PV: Negative Predictive Value, AUC: Area Under the Curve.

Figure 2: Sensitivity and specificity of 3D-STE in the prediction of EDV (ml) and ESV (ml) indexed by BSA (m^2)

No statistically significant intraobserver variability was found as regards ESV/BSA,EDV/BSA and assessed by 3D LV quantification (58.5 ± 2.3 vs. 58.2 ± 3.5, p = 0.75 and 92.6 ± 3.5 vs. 92.3 ± 3.2, p = 0.71 respectively).

No statistically significant difference was found between CMRI and 3D-Speckle tracking echocardiography in detection of LV Global hypokinesia (P = 0.255). CMRI was found to be a better tool in segmental wall hypokinesia detection than 3D-STE [(apical hypokinesia 45% vs. 40%, (P = 0,036), basal septal hypokinesia 85% vs. 75%, (p = 0.045), mid-septal hypokinesia 80% vs. 65%, (p = 0.012) and lateral wall hypokinesia 75% vs. 65%, (p = 0.028).



Parameters	Cut off point	AUC	Sensitivity	Specificity	PPV	NPV
EF% by 3D	≤49	100.0	100.0	100.0	100.0	100.0
EF by cardiac MRI	≤ 48.5	100.0	100.0	100.0	100.0	100.0

PV: Positive Predictive Value, -PV: Negative Predictive Value, AUC: Area Under the Curve, cMRI Cardiac magnetic resonance imaging.

Figure 3: ROC curve detect sensitivity and specificity of 3D echocardiography and cMRI in the prediction of EF

Also, no statistically significant intraobserver variability was found as regards ESV/BSA, EDV/BSA and EF% assessed by 3D-STE (58.45 ± 3.7, p = 0.923; 92.65 ± 3.4, p = 0.855) and no statistically significant intraobserver variability was found as regards EF% assessment by 3D LVquantification (40.25 ± 8.75 vs. 40.5 ± 7.95, p = 0.908) and by CMRI (39.56 ± 15 vs. 39.34 ± 15.8, p = 0.992).

Also, no statistically significant intraobserver variability was found as LV segmental wall motion abnormalities by 3D-STE and CMRI.

Discussion

The current study aimed to compare 3-D echocardiography versus cMRI in the assessment of left ventricular functions of children with dilated cardiomyopathy.

Thirty per cent of studied patients (30%) had increased heart rate for age, 55% had low systolic blood pressure for age, and 35% of them had low diastolic blood pressure for age which came in accordance with Marx et al., 2013 and can be attributed to decreased cardiac output and medications received [6]. All studied patients were on Frusemide, and Captopril therapy, 90% of them were on Digoxin and Spironolactone, 35% were on low dose aspirin, and 20% of them were on L-carnitine

which agreed with Daubeney et al., (2006) who reported that long-term medical therapy for congestive heart failure is the main treatment strategy in patients with DCM and include diuretics, digoxin, afterload reducing agents (usually ACEI), an aldosterone antagonist and beta-blockers, they also added that congestive heart failure is severe among children with DCM and although early mortality is high, the clinical status of long-term survivors is good with adequate management [7].

In the current study, no statistically significant difference was found between 3D-echocardiography and cMRI as regards EF% assessment (40.25 ± 25 vs 39.56 ± 9.80%, p = 0.996). This agreed with Jenkins et al., (2004) and (Hoffmann et al., 2006) and disagreed with Feng Wang et al., (2009) who reported an overestimation of LVEF by 3D-echocardiography compared to CMRI (30 ± 7%:19 ± 8%), and explained this overestimation by the delay of cardiac MRI gating so that the first frame was not always end-diastolic, and therefore EDVs of Cardiac MRI was sometimes underestimated [8], [9], [10]. Also, by the assumption that, in hypocontractile and enlarged ventricles, the difference in temporal resolution between 3D-echocardiography and cardiac MRI becomes less relevant than in contractile ventricles with a good LVEF or hypertrophy of the myocardial wall.

No statistically significant difference was found between 3D-echocardiography, 3D-speckle echocardiography, and cMRI as regards ESV/BSA [(58.58 ± 23.89 ml/m^2), (58.58 ± 23.88 ml/m^2), (58.65 ± 24.11 ml/m^2) respectively (P = 1)] and EDV/BSA[(92.69 ± 27.44 ml/m^2), (92.84 ± 27.29 ml/m^2), (93.41 ± 27.33 ml/m^2) respectively (P = 0.996)] which came in accordance with Gutierrez-Chico et al., (2005) and Hans-Joachim Nesser et al., (2009)and disagreed with Hakan Demir et al., (2007) who reported that the values of EDV and ESV were under estimated by cMRI (EDV 91.1 ± 38.0 ml, ESV 41.8 ± 26.9 ml) compared to ECHO (EDV 127.5 ± 42.2 ml, ESV 59.9 ± 37.6 ml) and attributed this difference in their study to exclusion of basal portions of left ventricle and high spatial resolution of (CMRI) [11], [12], [13]. Hakan Demir et al., (2007) exclusion of major vascular structures and valves from ventricular volume slices of CMRI were minimally repositioned to the midventricular region at the base of the heart. So, inclusion or exclusion of the most basal slice, which consists of parts of LV myocardium, outflow tract, and left atrium, could be the main reason for the difference between previous studies and the present study [13]. Also, Feng Wang et al., (2009) and Faber et al., (2001), reported underestimation of EDV and ESV measured by cMRI and explained that, trabeculation and papillary muscles are clearly visualized on cardiac MRI images, so they are usually excluded from the volume on analysis of cardiac MRI images and that cMRI allows inclusion of outflow tract tissue, which is not a part of LV volume acquisition [10], [14]. On the other hand, Sugeng et al., (2006) and Jenkins et al., (2007) reported that real-time 3D echocardiography underestimated MRI-derived end-diastolic LV volume (mean 168 ml) by 15 ml while 2D echocardiography underestimated MRI derived end-diastolic LV volume by 57 ml [8], [15].

No statistically significant difference was found regarding Global LV hypokinesia (P = 0.255), septal wall dyskinesia (P = 0.217), inferior wall hypokinesia (P = 0.072) and inferior wall akinesia (P = 0.349) measured by cMRI and 3D-STE. Hans-Joachim Nesser et al., (2009) [12] reported that the new 3D-STE technology is likely to become the method of choice for the assessment of regional LV function, replacing TDI. However, for this to happen, 3D-STE needs to be validated against an established reference technique. But because there is no non-invasive ‘gold standard’ technique that can be used in human subjects to validate regional ventricular function in three dimensions, it is essential to test the accuracy of 3D-STE using a global index, such as LV volume, against the current standard CMR reference. This is the first study designed to address this need.

In conclusion, automated 3D LV quantification echocardiography and 3D-STE are reliable tools in LV volumetric and systolic function assessment about CMRIas a standard method. 3D speckle echocardiography is comparable to CMRI in global wall hypokinesia detection but less sensitive in segmental wall hypokinesia assessment which mandates further studies.

More objective techniques are needed for assessment of segmental wall hypokinesia. More studies are needed on a larger number of patients to validate normal values of 3D speckle in children.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Dec 20; 6(12):2316-2322.
https://doi.org/10.3889/oamjms.2018.443
eISSN: 1857-9655
Clinical Science

Endovascular Treatment of Wide Neck Aneurysms

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Citation: Lazareska M, Aliji V, Stojovska-Jovanovska E, Businovska J, Mircevski V, Kostov M, Papazova M. Endovascular Treatment of Wide Neck Aneurysms. Open Access Maced J Med Sci. 2018 Dec 20; 6(12):2316-2322. https://doi.org/10.3889/oamjms.2018.443

Keywords: Intracranial aneurysm; Endovascular treatment; Wide neck; Intracranial stent; Flow diverter; Occlusion classification

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Received: 31-Oct-2018; **Revised:** 26-Nov-2018; **Accepted:** 27-Nov-2018; **Online first:** 10-Dec-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: An aneurysm is an abnormal focal dilatation of an artery. Most of the unruptured aneurysms are asymptomatic and discovered incidentally or some of them symptomatic with mass effect or nerve palsy, but rupture of aneurysm results in a potentially life-threatening subarachnoid haemorrhage. Aneurysms with wide necks are defined by neck diameters greater than 4 mm or dome-to-neck ratios less than 2 and are the most difficult to treat with the endovascular method.

AIM: This study aimed to analyse the endovascular treatment of intracranial aneurysms with a wide neck.

METHODS: The study population included 37 patients with 46 aneurysms referred to the University Clinic of Radiology in Skopje, the Republic of Macedonia for endovascular treatment during the period January 2013 to May 2018. This study included 24 females and 13 males, ranging in age from 25 to 74 years.

RESULTS: From total 46 treated aneurysms 13 were ruptured and 33 unruptured. Six patients were with multiple aneurysms. In these study complex aneurysms were treated with combined technique, 5 with balloon-assisted coiling, 25 with stent-assisted coiling, 6 stents, 2 with flow diverter assisted coiling, 6 FD and 2 with partial coil filling without assistance device.

CONCLUSION: Aneurysms with wide neck remain a challenge for endovascular treatment. But the development of new techniques and materials in the treatment of aneurysms makes endovascular treatment of intracranial aneurysms safe and feasible.

Introduction

An aneurysm is an abnormal focal dilatation of an artery (derived from the Greek word *aneurýnein*, to dilate) [1]. Most of the unruptured aneurysms are asymptomatic and discovered incidentally but rupture of aneurysm results in a potentially life-threatening subarachnoid haemorrhage (SAH) [2]. Unruptured aneurysms can be symptomatic with the mass effect of the brain or causing hydrocephalus, but usually symptoms of cranial nerves palsy. Originally, open direct surgery was the most common to treat aneurysms by placing a clip across the neck of an aneurysm to eliminate flow from the parent artery into the aneurysm sac [3]. Endovascular treatment methods offer an alternative method for the treatment of intracranial aneurysms.

Endovascular management of intracranial aneurysms using detachable platinum coils was introduced in 1990 by Guido Guglielmi, an Italian neurosurgeon. Despite advances, however, the treatment of wide-necked aneurysms (aneurysms with a fundus-to-neck ratio less than 2 remains problematic [4].

Endovascular treatment of intracranial aneurysms is associated with lower morbidity and mortality rates compared with traditional microsurgical clipping. However, despite advances in devices and techniques, aneurysms with wide necks, defined by neck diameters greater than 4 mm or dome-to-neck

ratios less than 2, are the most difficult to treat with the endovascular method. In wide-necked intracranial aneurysms, complete coil embolisation is often technically difficult owing to the risks of distal coil migration or coil impingement on the parent vessel [5].

Some techniques and devices can be used in the treatment of wide-necked aneurysms. These include balloon remodelling, use of three-dimensional (3D) coils, combined use of stents and coils, flow diverters, use of intrasaccular flow disruption (like WEB), simultaneous deposition of more than one coil in an aneurysm, intentional partial aneurysm embolisation, and combined extra- and intrasaccular treatment.

So far in the literature, none of the classifications includes the entire spectrum of current endovascular aneurysm treatment, irrespective to treatment technique.

This study aimed to analyse the options and efficiency of different modalities of endovascular treatment of aneurysms with a wide neck.

Material and Methods

The study population included 37 patients with 46 aneurysms, 6 patients were with multiple aneurysms, referred to the University Clinic for Radiology in Skopje, the Republic of Macedonia for endovascular treatment of aneurysms with wide neck during the period January 2013 to January 2018. The group of patients was comprised of 13 men and 24 women, aged 25 to 74 years.

From total treated 46 aneurysms, 16 were ruptured and 30 unruptured. In this study complex aneurysms were treated with combined techniques: 5 with balloon-assisted coiling, 25 with stent-assisted coiling, 6 with a stent, 2 with FD assisted coiling, 6 with FD and 2 with partial coil filling without assistance device.

This study was approved by the Ethics Committee of the Medical Faculty of Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia.

Before the procedure, all patients underwent computed tomography angiography (CTA) or digital subtraction angiography (DSA) for diagnostic evaluation. The morphologic characteristics of an aneurysm were carefully evaluated, including the size of the neck, height, width and length; the diameter and tortuosity of the parent artery; and major branches originating from the aneurysmal neck. The detailed strategies for the endovascular treatment of aneurysms depended on the angiographic findings.

All therapeutic procedures were performed through the femoral artery under general anaesthesia or conscious sedation.

Femoral access was obtained through a percutaneous femoral artery puncture, the introducer was placed, and the guiding catheter was inserted into the parent vessel. A microcatheter depends on technique one or two or balloon, and microcatheter was advanced over the micro-guidewire into the normal distal artery beyond an aneurysm, by 2 cm and one in the aneurismal sac.

Systemic heparin was administered in the following manner: a bolus of 3-5000 IU of heparin was administered intravenously at the beginning of the procedure of unruptured aneurysms, and after microcatheter placement into the aneurysms in cases of ruptured aneurysm. An additional 1000 IU bolus of heparin was administered in every hour.

Pre-procedural angiograms were then obtained in orthogonal planes. After the decision was made about the endovascular treatment approach, the aneurysm was treated either with a balloon or stent-assisted treatment. The patients that was planned for a stent or flow diverter were put on double antiplatelet therapy (Clopidogrel 75 mg x 1 and Aspirin protect 100 mg x 1) five days before in unruptured and loading dose one-day pre-procedural in risky of the previously ruptured aneurysm with min 3 x 75 mg Clopidogrel and 300 mg Aspirin. For ruptured if its possible first partial coil is filling with the sac and then in second procedure stenting.

Post-procedure care was done in the intensive care unit with close monitoring of all vital functions. The patients with stent or FD are kept on heparin infusion for 24 hours (intravenous 25 000 IU for 24 hours). After the procedures, these patients were put on therapy with Clopidogrel 75 mg x 1 at least 6 months (some authors recommend 3 months) and Aspirin 100 mg x 1 for a lifetime. Following the procedure, to prevent vasospasm, some patient was kept on nimodipine.

Patients were advised to come for follow-up visits at the end of 6 and 12 months, and yearly after that. The angiographic control was performed with three-dimensional time-of-flight magnetic resonance angiography (MRA) or contrast MRA and sometimes DSA.

Results

In this study, 37 patients with 46 wide neck aneurysms were treated during the 5-year study period. All of the aneurysms had a neck width of more than 4 mm or a dome-to-neck ratio less than 2. None of the ruptured aneurysms had been previously treated. The diameter of aneurysms was in the range

from 3 mm to more than 25 mm. According to the size of the aneurysms, they have divided into three groups: 9 aneurysms were small, 15 were medium, 13 aneurysms were large and 9 giants.

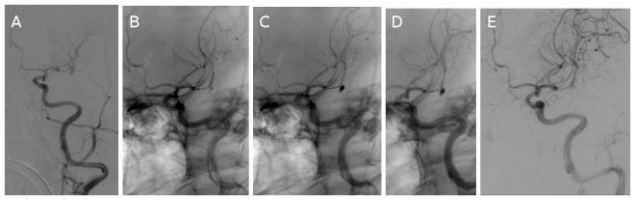

Figure 1: Unruptured M2 small aneurysm treated with balloon-assisted coiling (a, b, c); balloon deflation and protrusion of coil (d) and then stent placement, integrated branch reduced in calibre (e)

In our study wide-neck aneurysms were treated with: partial coil embolisation, balloon-assisted coiling, stent, stent-assisted coiling, flow diverter (FD), FD and coil.

For partial coil filling and balloon-assisted coiling, we used Raymond ray classification

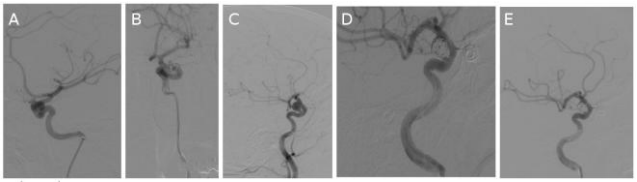

Figure 2: Left ICA (clinoid and ophthalmic segment) 2 aneurysms (a, b) and right ICA (cavernous and communicating segment) 2 aneurysms (c, d, e)-ruptured one on communicating segment all four treated with stent-assisted coiling on both ICA in one session

But together with wide neck aneurysms where we used flow modification devices, extra saccular (stents and FD) we used a modification of classification proposed from H.S. Cekirge and I. Saatci, for immediate DSA result in all treated aneurysms and 23 patient follow up.

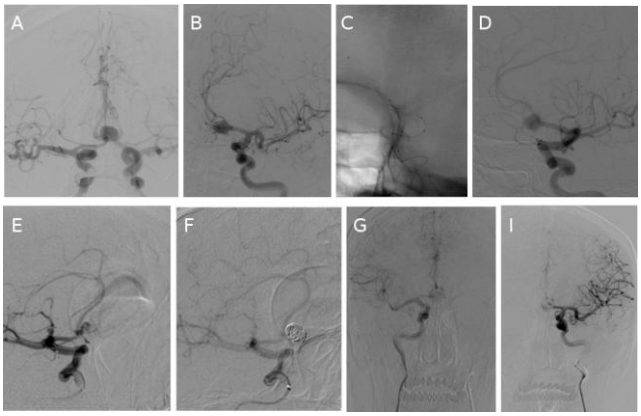

Figure 3: Unruptured Acom an aneurysm filling from both ICA (a); treated with the stent in left A1/A2 (b, c, d) and coiling from the right side (e, f); control angiograms from both ICA-complete occlusion g, i

Grading of occlusion for all types of treatment:

1. Complete occlusion of the aneurysm sac was found in 22 (47.82%).

2. Residual neck in 6 aneurysms (13.04%).

3. A residual aneurysm was observed in 5 (10.86%).

4. Aneurysmal filling immediate postoperative result-end of treatment DSA with flow modification treatment (from 39 aneurysms treated with flow modification).

   a. With contrast stagnation 9 (23.07%)

   b. Without contrast stagnation 4 (10.25%)

5. Stable remodelling with flow modification [from 39 aneurysms treated with FM only 23 (58.97%) were followed up on 6 and 12 months] – 5 aneurysms with residual neck, 4 aneurysms with a residual aneurysm, 7 aneurysms from 4a and 3 aneurysms from 4b.

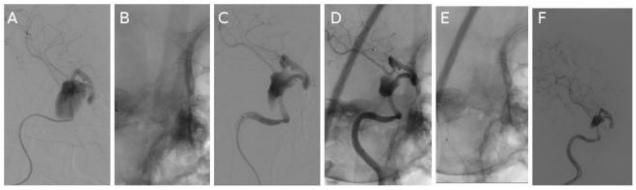

Figure 4: Giant unruptured aneurysm of right ICA (cavernous segment), partially thrombosed (a); treated with FD (b) and immediate contrast stagnation (c) and progressive thrombosis (d); balloon dilatation of the FD in proximal stenotic part (e); control angiogram after finished treatment (f)

Only two of all followed up aneurysms showed regrowth-recanalisation and need of retreatment.

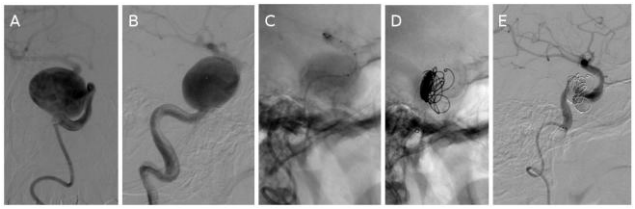

Figure 5: Giant unruptured right ICA aneurysm with cranial nerve palsy (a); treated first with stent because of tortuous anatomy of the parent vessel (b, c); placement of some coils in the sac, balloon dilatation of the stent on the stenosed part (d) and then placement in stent flow diverter(e) with total occlusion

In this study, there were ten cases (27.02%) of procedure-related complications in our patients. In two patients (5.40%) in-stent thrombosis was observed. In one patient in-stent thrombosis was resolved with IA and IV Aggrastat but with small distal embolus and small infarction zone and neurological deficit that was improved with rehabilitation.

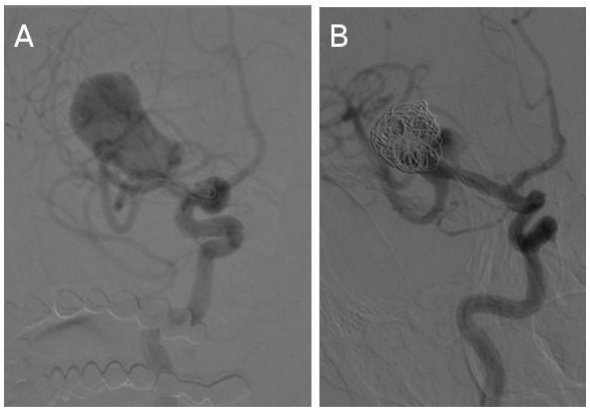

Figure 6: Giant right MCA a trifurcation aneurysm with mass effect (a); treated with stent-assisted coiling, the stent in M1/M2 inferior trunk and partial coil filling of the 2/3 of the sac (b)

The second patient presents thrombosis of the flow diverter immediately after placing the device, but there were sufficient cross filling and no neurological deficit. The thromboembolism was resolved without neurological deficits.

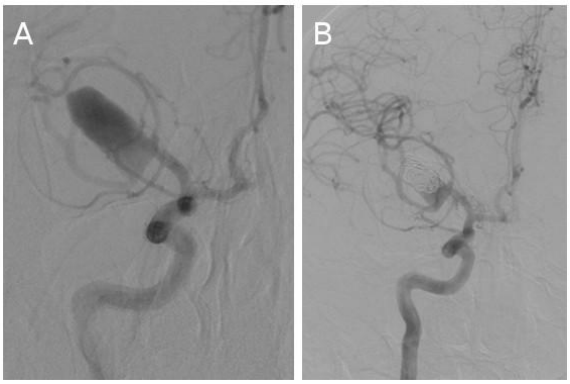

Figure 7: Ruptured right MCA a large aneurysm with wide neck and branches integrated (a); treated with intentional partial coiling, residual neck (b)

The spasm was observed in 7 (18.91%) of the patients, which was resolved spontaneous or with nimodipine. In this study in one patient (2.70%) spontaneous coil detaches successfully retrieved with the micro snare. Except for these complications, there was no evidence of other procedure-related complications such as coil protrusion during the procedure.

In this study, we didn't have death outcome.

Discussion

Aneurysms, both ruptured and unruptured, may be treated via craniotomy by surgical clip ligation and microsurgical clip(s) placed on the aneurysm neck to remove an aneurysm from the circulation and prevent possible rupture (or if an aneurysm had already ruptured, to stop bleeding and prevent re-rupture).

Based on the published literature, concerning safety events, there was a higher mortality rate reported with open surgery, i.e., 13.8% vs 10.7% for surgical clipping and traditional coiling, respectively. Patient age has been shown to be a strong indicator of poor surgical outcome [6]. It has also been shown that surgical clipping carries an increased risk of seizures both in short and long-term follow up where Molyneux et al., showed that 4.1% of open surgical patients (compared to 2.5% of the endovascular treated patients) had seizures associated with re-bleeding after receiving treatment for their aneurysm. Furthermore, as previously discussed in Section 2.2, open surgical clipping may be limited concerning accessibility to treat certain aneurysms based on their location in the neurovasculature (e.g., posterior circulation aneurysms in perforator rich regions) [7].

Endovascular treatment methods offer an alternative method for the treatment of intracranial aneurysms. Treatment of intracranial aneurysms is increasingly performed by endovascular means as an alternative to microsurgical clipping with lower morbidity and mortality rates in selected cases [8]. However, each an aneurysm should be assessed by an interdisciplinary approach, with the aim being to choose the best option for each patient [9].

Wide neck bifurcation aneurysms remain a challenge, both for surgical as well as endovascular techniques [10]. Treating wide-neck intracranial aneurysms is a difficult endeavour. From a surgical standpoint, they are technically demanding to dissect, especially when they reside in the posterior circulation or near the cranial skull base. Although initially considered by endovascular surgeons to be “poor open-surgery candidates,” patients with wide-neck aneurysms have posed problems for catheter-based therapy as well.

Soon experience showed 2 main challenges for aneurysm coiling: (1) some aneurysms were not easy to treat because of their shape (large and giant aneurysms, fusiform aneurysms, large neck aneurysms, aneurysms with unfavourable size relationship between aneurysm dome, neck, and parent artery) [11]. The risk of coil protrusion into the parent vessel, subtotal aneurysm occlusion and recanalisation of an aneurysm are still major limitations of image-guided, endovascular treatment of cerebral aneurysms [8].

This led to the development of new techniques and technologies, including balloon-assisted coiling (known as the remodelling technique), aneurysm coiling supported by stenting, and the more recent introduction of flow diversion/disruption [11].

One characteristic that makes an aneurysm ideal for endovascular therapy is a fundus-to-neck

ratio greater than 2. The greater this ratio, the greater the disparity between the orifice and the body, thus making coil deposition easier and coil stability within the lesion greater. As the ratio drops, it is more difficult to prevent coils from herniating out of the fundus, through the neck, and into the parent vessel. In some cases, “wide necked” can be considered a neck that exceeds the diameter of the widest diameter coil available. This diameter depends on the system used for embolisation [4].

Neck size, the size of the opening that connects the parent artery to the aneurysm body, is also an important descriptor for aneurysms and can dictate treatment options. Aneurysms with a neck > 4 mm in diameter or dome to neck ratio < 2 are typically classified as wide-neck [3].

Such aneurysms can be difficult to treat and are less amenable to coiling alone or surgical clipping in comparison to aneurysms with smaller necks [12].

Zubillaga et al. originally defined a “wide neck” as an absolute neck diameter of 4.0 mm. Debrun et al., defined “wide-neck” aneurysms as those with dome-to-neck ratios of < 2.0. These early definitions were created by success with endovascular coil therapy when the technique was in its infancy. Cloft et al. later noted that the technical advance of complex coil shapes allowed successful endovascular therapy of aneurysms with a dome-to-neck ratio of > 1.5 [13].

Results from early studies using single-dimensional coils alone demonstrated 72% to 80% complete occlusion when the ratio was greater than 2, and 53% when it was lower. Inability to keep coils within these lesions, the post-treatment presence of a residual aneurysm, and the occurrence of delayed aneurysm recanalisation led investigators to seek new ways to embolize these lesions safely and effectively [4].

Moret et al. first described the clinical use of the balloon-assisted technique in humans and described their results on 52 aneurysms in 50 patients, achieving complete occlusion in 77%, subtotal occlusion in 17%, and incomplete occlusion in 6% [14], [15].

In the 34 aneurysms treated by the remodelling technique with Hyper Form balloon, immediate angiographic results consisted of total occlusion in 31 cases (91.2%) and partial occlusion in three cases (8.8%). There were five procedure-related complications (14.7%), including two coil protrusions and three thromboembolisms; Except one patient, all were successfully resolved without permanent neurologic deficit. No new bleeding occurred during the follow-up. Twenty patients (59%) underwent angiographic follow-up from 2 to 33 months (mean 9.2 months) after treatment. Focal recanalisation with coil compaction of the neck portion was observed in 5 cases (25%). Only one case showed major recanalisation and underwent stent-assisted coil embolisation [15].

In general, treatment of wide-neck and wide-neck bifurcation aneurysms by using endovascular techniques is challenging. Embolisation of these aneurysms without the use of adjunctive devices is difficult because of the instability of the coil mass leading to the risk of coil protrusion into the parent artery. The risk of coil protrusion often makes attenuated packing of an aneurysm difficult or impossible to achieve, which can lead to lower rates of complete occlusion [16].

Follow-up imaging showed recanalisation in 25.0% (2/8) of initially completely occluded aneurysms and 50.0% (5/10) of neck-remnants or residual aneurysms resulting in an overall recanalisation rate of 38.5%. Recanalisation from neck remnants to residual aneurysms was observed in 28%. The follow-up in aneurysms after treatment with stent implantation with a mean of 22 months (range 2–79 months) is the longest observation period reported so far. Recanalization was found in 14.3% (1/7) of dome/neck ratio 92 aneurysms and in 66.7% (4/6) of dome/neck ratio > 2 aneurysms. Fiorella et al. reported 23% of recanalisation in 3–6 months’ follow-up with 52% showing progressive thrombosis, 25% showed no change [17]. Biondi et al. reported a mean follow-up of 9 months (range 3 to 24 months). Initially, occluded aneurysms remained occluded, and 36% of neck remnants and 33% of residual aneurysms progressed to complete occlusion. Recanalisation to residual aneurysms was observed in 28% of the neck remnants [18].

The study Cerebral Aneurysm re-rupture after Treatment [19] reported that the degree of aneurysm occlusion after treatment was strongly associated with risk of re-rupture, but aneurysm re-treatment may or may not carry a higher risk than the stable incomplete occlusion or recanalisation [7], [20]. Unfortunately, this mostly originates from coiled aneurysms and cannot be generalised to the entire population of cerebral aneurysms, especially for those aneurysms treated with new devices that modify flow from inside or outside of the aneurysm sac. Flow modifiers (FM) (dedicated extra saccular flow diverters, multiple stent-in-stent applications, and intra saccular flow disrupters) have been introduced as a new concept for treatment of IA [17], [21].

The Raymond-Roy Occlusion Classification, also known as the Montreal scale [22] has been the most widely used, and it classifies the results after aneurysm coiling, which can be applied immediately after the treatment as well as during the follow up (class I: complete obliteration; class II: residual neck; class III: residual aneurysm; class IIIa: contrast opacification within the coil interstices of a residual aneurysm; class IIIb: contrast opacification outside the coil interstices, along the residual aneurysm wall) [22, 23]. Modification proposed Mascitelli et al., a study

that found class IIIa aneurysms progress to complete occlusion more than class IIIb aneurysms and a validation study by Stapleton et al., [24], [25].

Introduction of the extra saccular flow diverters created a need for different classifications, not only to describe the initial results but also to anticipate the outcome, including the risk of infrequent but severe complications of postoperative rupture [19], [20].

A new classification is proposed for cerebral aneurysms treated with any endovascular technique, coiling with or without adjunctive devices, flow diversion, intra saccular flow modifiers, or any combination of the above. Raymond-Roy Occlusion Classification is expanded with novel subgroups such as class 1 represents complete occlusion and is subdivided if a branch is integrated too, or originated from, the aneurysm sac; class 2 represents neck filling; class 3 represents incomplete occlusion with an aneurysm filling as in the previous classification; and class 4 describes the immediate postoperative status after extra- or intra saccular flow modification treatment. A new concept, “stable remodelling,” is included as class 5, which represents filling in the neck region that stays unchanged or reduced, as shown with at least 2 consecutive control angiographies, at least 6 months apart, for not < 1 year, or the remodeled appearance of a dilated and/or tortuous vessel in continuation with the parent artery without sac filling [26], [27], [28].

In conclusion, wide-necked aneurysms remain difficult to treat and occlude permanently. In some cases, endovascular treatment is the only possible option for treatment of the wide neck aneurysms. According to our experience, despite some troubles during the treatment, endovascular treatment of intracranial aneurysms was revealed safe and feasible. The goal of endovascular treatment in intracranial aneurysms is preventing rupture meaning secured aneurysm with or without a perfect angiographic appearance. In complex aneurysms where “perfect” anatomic results can’t be achieved or have increased risks, then, “remodelling” is an acceptable option because provide stability.

For long-term result for successful treatment of wide-neck aneurysms, there is a need of more accurate classification of immediate DSA result and more studies with longer follow up due to the introduction of new flow modification devices extra and intra saccular.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Dec 20; 6(12):2323-2327.
https://doi.org/10.3889/oamjms.2018.490
eISSN: 1857-9655
Clinical Science

Survival and Side Effects in Non-Small Cell Lung Cancer Patients Treated With Combination of Chemotherapy and Conformal Radiotherapy

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Citation: Crvenkova S. Survival and Side Effects in Non-Small Cell Lung Cancer Patients Treated With Combination of Chemotherapy and Conformal Radiotherapy. Open Access Maced J Med Sci. 2018 Dec 20; 6(12):2323-2327. https://doi.org/10.3889/oamjms.2018.490

Keywords: Sequential and concurrent chemoradiotherapy; Conformal radiotherapy; Inoperable NSCLC

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Received: 14-Oct-2018; **Revised:** 21-Nov-2018; **Accepted:** 22-Nov-2018; **Online first:** 11-Dec-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Combined modality therapy is standard of care for patients with inoperable locally advanced non-small cell lung cancer (NSCLC), however, insufficient data exist regarding what chemoradiotherapy combination will be the gold standard.

AIM: The study aimed to compare the survival impact and side effects of concurrent versus sequential radiochemotherapy treatment in inoperable stage III non-small cell lung cancer (NSCLC).

METHODS: To evaluate the treatment results and prognostic variables, 85 NSCLC patients treated from October 2005 to November 2008 were randomly assigned to one of the two treatment arms. In the first arm (sequential arm), 45 patients received sequential chemotherapy with 4 cycles of carboplatin and etoposide followed by conformal 3-dimensional (3D) radiotherapy (RT). In the second arm (concurrent arm), 40 patients received concomitant chemotherapy with cisplatin and etoposide and conformal RT, followed by two cycles of consolidation chemotherapy with carboplatin and etoposide

RESULTS: The median survival was 13 months for the patients in the sequential arm and 19 months for those in the concurrent treatment arm ($p = 0.0039$). The disease-free survival (DFS) was 9 months in the sequential arm and 16 months in the concurrent treatment arm ($p = 0.0023$). Seven complete responses and 18 partial responses were obtained with sequential treatment. Twelve complete responses and 21 partial responses were obtained in concurrent arm. The differenced were statistically significant $p = 0.03$. Median survival for patients with complete response in concurrent treatment arm was 36 months versus 18 mounts for a sequential arm; partial response was 27 months versus 16 months and those with stable disease 11 months versus 9 months. Treatment-related toxicities were assessed according to the RTOG/EORTC criteria. Acute esophagitis and incidence of neutropenia were higher with the concurrent than with sequential treatment. Grade 3 esophagitis was characteristic only for concurrent treatment, and it was the reason for radiotherapy interruption, but no longer than 7 days. Secondary anaemia was more frequent in the sequential treatment arm.

CONCLUSION: The statistically significant differences in survival were suggested that the concurrent chemotherapy and conformal three-dimensional radiotherapy is the optimal strategy for patients with locally advanced NSCLC with acceptable toxicity rates.

Introduction

Lung cancer remains a worldwide epidemic. Approximately 1.2 million people die from lung cancer each year. NSCLC patients represent more than 80% of all lung cancers. Of the patients with NSCLC, 60-70% present with Stage III or IV disease. In the late 1980s, radiotherapy was the standard treatment for these patients [1]. Randomised trials and a 1995 overview subsequently showed that combination chemoradiotherapy was superior to radiotherapy alone [2]. Many chemotherapeutic agents active in NSCLC possess radiosensitising properties, thereby improving the probability of local control. Also, chemotherapy administered concurrently with thoracic radiation may act systemically and potentially eradicate distant micro-metastases. Several studies showed the feasibility of the cisplatin-etoposide combination plus radiotherapy for patients with stage III disease [3]. The primary endpoint on this study was the effect of sequential and concurrent chemoradiotherapy on overall survival.

Material and Methods

This study was prospective, randomised and started in the Institute of Radiotherapy and Oncology in Skopje, October 2005. Eligible 85 patients were aged between 18 and 70 years, had an Eastern Cooperative Oncology Group (ECOG) Score ≤ 1 and had ≤ 10 % weight loss in 3 months before inclusion. They have previously untreated histological or cytological proven NSCLC, unrespectable stage IIIA-N2 disease, or stage IIIB disease without pleural effusion. Stage IIIB disease was assigned either by N3 (contralateral mediastinal or supraclavicular nodes) or by T4 from the invasion of mediastinal structures. The following laboratory values were required: leucocytes ≥ 1.5 x 10^3/l, platelets ≥ 100 x 10/l, AST and ALT ≤ 2 x the upper limit of the referent rang. Ineligibility criteria were as follows: uncontrolled infection, or fever over 38ºC, unstable cardiovascular disease and previous malignancy.

Before enrollment, the patients gave their full medical histories and underwent a clinical examination with assessment of performance status (PS). Patients were randomly assigned to receive sequential or concurrent therapy. Patients were randomly assigned to receive sequential or concurrent therapy. In the sequential arm, 45 patients received four cycles of chemotherapy. They were administered first, consisting of carboplatin (AUC x 6) on day 1 and etoposide on days 1-3, repeated every 3 weeks. The radiotherapy began 4 weeks after the fourth cycle of chemotherapy administration. Chemotherapy and radiotherapy began simultaneously in concurrent arm consisted of 40 patients. The first cycle of chemotherapy, with cisplatin 30 mg/m² and etoposide 100 mg/m² was administered on days 1 to 3, and the second cycle of chemotherapy was administered the last 3 days of conformal radiotherapy, in concurrent arm. After 4 weeks of concurrent chemoradiotherapy schedule, two cycles of consolidation chemotherapy began, consisting of carboplatin (AUC x 6) and etoposide 100 mg/m² on day 1 to 3.

Conformal radiotherapy at both arms consisted of 60 Gy in 30 fractions of 2 Gy per fraction, for 5 days a week given throughout 6 weeks. A treatment planning CT was required to define the gross tumour volume (GTV). Each patient was positioned in an immobilisation device-wing board in the treatment position on a flat table. CT slices with 5 mm thickness were obtained starting from cricoid cartilage and extending inferiorly to the level of the L1 vertebral body. The GTV, clinical target volume (CTV), planning target volume (PTV) and normal organs were outlined on all CT slices. The normal tissues contoured included lungs (as the total lung volume), heart, spinal cord and oesophagus. The CTV included the entire GTV plus 0.7 cm, and the PTV included CTV plus another 0.7 cm adding margin. PTV44 was treated with parallel-opposed anterior-posterior fields and PTV60 was treated with any combination of fields depends on spinal cord constrain. If radiotherapy had to be delayed for more than 7 days, the patient was withdrawn from the study.

In the sequential arm, responses were assessed 8 weeks after the end of radiotherapy. In the concurrent arm, responses were assessed 8 weeks after the end of the consolidation chemotherapy. Imaging studies x-ray and/or computed tomography (CT) could be repeated at all times when clinically indicated. Complete and partial responses were based on RECIST criteria. Toxicity was graded according to RTOG/EORTC criteria. Follow-up visits were conducted every 2 months during the first year and after that every 3 months. Patients with evidence of progression at any time were removed from the study but continued to be evaluated for survival and toxicity. Survival and the interval to recurrence or progression were measured from the date of the first treatment session.

Results

One hundred and ten patients were identified from our database. Of these, 25 were excluded from analysis: 7 had metastatic disease, 7 had sudden deterioration of their general condition, 3 patients had pleural effusion, loss of data or loss of any contact in 3 patients, and 5 patients due to delivered tumour dose less than 60 Gy. Eighty-five patients were subsequently included for further analyses. The characteristics of 85 patients are listed in Table 1.

Table 1: Patient and disease characteristics

	Concurrent chemoradiotherapy arm (N = 40) N (%)	*Sequential chemoradiotherapy arm (N = 45) N (%)*	*p-value*
Age, years			
	0	1 (2)	0.13
	20 (50)	13 (29)	
	20 (50)	32 (71)	
Sex			
	35 (88)	40 (89)	0.98
	5 (12)	5 (11)	
Performance status			
0	26 (65)	23 (51)	0.19
1	14 (35)	22 (49)	
Weight loss (%)			
< 5	26 (65)	23 (51)	0.13
5-10	14 (35)	22 (49)	
Histology			
Squamous cell	22 (55)	34 (75)	
Adenocarcinoma	10 (25)	6 (13)	0.26
Large cell	3 (7)	2 (4)	
Unspecified	5 (1)	3 (6)	
N status			
N1			
N2	12 (30)	15 (33)	0.93
N3	25 (63)	27 (60)	
	3 (7)	3 (7)	
T status (cm)			
Tumor ≤ 5	13 (32)	21 (47)	0.38
Tumor > 5	27 (68)	24 (53)	
Hemoglobin (g/dl)			
<12	11 (27)	19 (42)	0.15
≥12	29 (73)	26 (58)	
Duration of symptoms (months)			
< 3	2 (5)	0	0.29
3-6	21 (53)	23 (51)	
> 6	17 (43)	22 (49)	

Survival was analysed until March 2010. The median OS was 13 months in the sequential arm

(95% CI 10.2-15.7), and 19 months in the concurrent treatment arm (95% CI 13.6-24.3), with statistically significant difference (log-rank test, p = 0.0039; Figure 1).

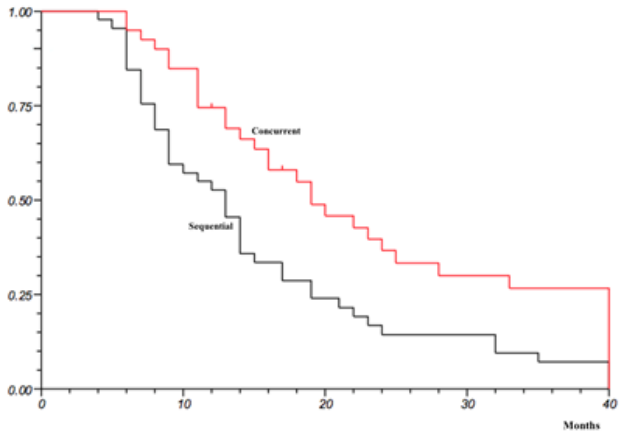

Figure 1: Overall survival according to the treatment arm (p = 0.0039)

The 1, 2 and 3-year OS rates were 74, 36 and 27% in the concurrent arm and 52, 14 and 7.1% in the sequential arm (p = 0.0039). DFS for the concurrent arm was 16 months (95% CI 12.7-19.2), and for the sequential arm, it was 9 months (95% CI 5.8-12.16). The difference was statistically significant (log-rank test, p = 0.0023; Figure 2).

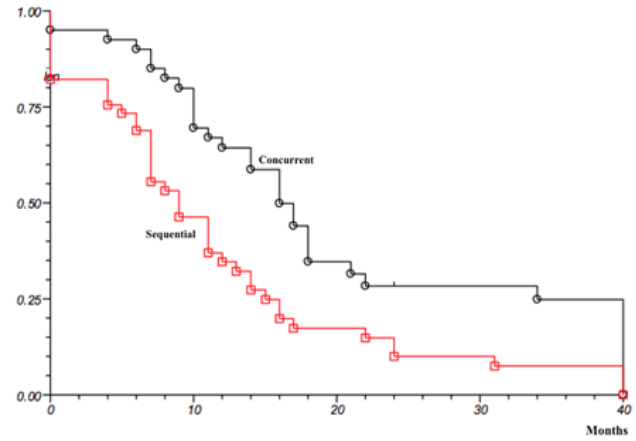

Figure 2: Disease-free survival according to the treatment arm (p = 0.0023)

Survival time was insignificant correlation with local tumor control (p < 0,001). Local tumour response was evaluated for each patient included in the study (Table 2). Seven patients with complete response and 18 with partial responses were obtained treated with sequential treatment. Twelve patients with complete response and 21 with partial response were obtained in the concurrent treatment arm. According to tumour responses, the difference between treatment arms was statistically significant p = 0.03. Median survival for patients with complete response in concurrent treatment arm was 36 months versus 18 months in sequential arm. Median survival for patients with partial response in the concurrent arm was 27 months versus 16 mounts in sequential arm and for those patients with stable disease median survival was 11 months in concurrent arm versus 9 months in the sequential treatment arm.

Table 2: Tumor response in concurrent and sequential treatment arm

Treatment arm	CR Complete response	PR Partial response	CR + PR Objective response	SD Stabile disease	NR No response	Patients
Concurrent arm	12 (30%)	21 (53%)	33 (83%)	3 (7%)	4 (10%)	40
Sequentional arm	7 (16%)	18 (40%)	25 (56%)	12 (27%)	8 (18%)	45
	19 (22%)	39 (46%)	58 (68%)	15 (18%)	12 (14%)	85 (100%)

p = 0.04573; SS = 3; x^2 = 8.01350.

Prognostic factors with a significant influence on survival were: initial performance status according to ECOG, initial weight loss, nodal involvement, tumour size and ages. Prognostic factors without any influence on survival were: gender, duration of symptoms, haemoglobin level and histological type.

Table 3: Treatment toxicity according to RTOG/EORTG criteria in sequential arm and concurrent arm

Treatment toxicity		Sequential arm				Concurrent arm			
		Grade 0	Grade 1	Grade 2	Grade 3	Grade 0	Grade 1	Grade 2	Grade 3
Early	Lungs	9 (20%)	24 (53%)	10 (22%)	2 (4%)	3 (8%)	24 (60%)	8 (20%)	5 (13%)
	Esophagus	17 (38%)	20 (44%)	8 (18%)	0	2 (5%)	20 (50%)	15 (37%)	0
	Hemoglobin	24 (53%)	14 (31%)	7 (15%)	0	35 (88%)	4 (10%)	1 (2%)	0
	Leucocytes	41 (91%)	2 (4.4%)	2 (4.4%)	0	13 (32%)	11 (27%)	14 (35%)	2 (5%)
Late	Lungs	7 (16%)	23 (51%)	9 (20%)	0	8 (20%)	16 (40%)	11 (27%)	5 (12%)
	Esophagus	36 (80%)	5 (11%)	4 (8%)	0	30 (75%)	3 (7%)	7 (17%)	0

Treatment-related toxicities according to RTOG/EORTC are listed in Table 3. Acute esophagitis and incidence of neutropenia were higher with the concurrent than with sequential treatment arm. Grade 3 esophagitis was characteristic only for concurrent treatment, and it was the reason for radiotherapy interruption but no longer than 7 days. Secondary anaemia was frequent in the sequential treatment arm. Due to these results, adverse effects of both chemoradiotherapy arms were mild and moderate.

Discussion

Randomised phase III trial comparing sequential and concurrent administration of chemotherapy and radiation therapy for NSCLC has been published so far [4]. In the study of Furuse et al., the West Japan Group, chemotherapy combined cisplatin, vindesine and mitomycin C. The total dose

of radiotherapy was 56 Gy, and in the concurrent arm, was administered in a split-course schedule, with a rest period of 10 days. Median survival was significantly better with concurrent therapy than with sequential therapy (16.5 and 13.3 months, respectively; p = 0.0398). The 2-, 3-, and 5-year survival rates were, respectively, 34.6%, 22.3% and 15.8% in concurrent arm, and 27.4%, 14.7% and 8.9% in the sequential arm. Radiation Therapy Oncology Group (RTOG) study 94-10 [5] compares sequential treatment with concurrent therapy in which the same dose of radiotherapy 63 Gy was administered during the two cycles of cisplatin-vinblastine therapy, and with concurrent treatment using a bi-fractionated and accelerated irradiation 69.6 Gy combined with two cycles of cisplatin-etoposide. The median survival rate in the concurrent treatment with cisplatin-vinblastine and standard radiotherapy was significantly better than that in the sequential arm (17 v 14.6 months; p = 0.046). The median survival rate in bi-fractionated irradiation was 15.2 months. The third study which supports the concomitant approach was from Zatloukal PV, 2004. Chemotherapy in both groups consisted of 4 cycles of cisplatin and vinorelbine every 4 weeks. Radiotherapy of 60 Gy was started in concurrent arm with the second cycle and in sequential arm 2 weeks after completion of the chemotherapy. Median survival time in the concurrent group was 16.8 months and in the sequential group was 12.9 months (p = 0.0216, log-rank test). Median time to progression was 11.9 in concurrent arm and 8.5 in sequential arm, respectively [6].

Our study compared sequential and concurrent chemoradiation therapy in locally advanced NSCLC. We found a benefit of concurrent therapy more than in previously listed trials, regarding overall and disease-free survival (19 vs 13; 16 vs 9 months), and the difference was significant with the log-rank test. When our study was designed, the cisplatin-etoposide combination was mostly used concurrently with radiotherapy [7]. Consolidation chemotherapy with two cycles of carboplatin-etoposide was administered in the concurrent arm to balance the dose of platinum-based chemotherapy in the two arms. This consolidation chemotherapy administered after concurrent chemoradiotherapy seems promising regarding survival, as shown in the Southwest Oncology Group (SWOG) S9504 and Locally Advanced Multimodality Protocol (LAMP) studies [8], [9]. In the SWOG S9504 study, consolidation docetaxel following concurrent chemoradiotherapy, shown a median survival of 26 months and median progression-free survival of 16 months. Our concurrent-consolidation arm was shown similar results (OS 19 months and DFS 16 months). In our study, the local relapse rate was lower in the concurrent arm than in the sequential arm. In the RTOG 94-10 [5] study, local failure rates at 2 years were significantly lower in the concurrent arm. Thus it seems that the superior survival observed with concurrent treatment is associated with better local control.

We didn't observe major toxicity in our study. The incidence of grade 3 esophagitis was lower than in RTOG 94-10 study and the possibility for incidence reduction in our study was performing conformal 3D RT. The same findings were shown by Socinski et al., [10], [11]. The North Central Cancer Treatment Group (NCCTG) reported a phase I trial escalating the dose of RT with 3-D planning between 70 and 78 Gy [12], [13]. They defined maximum-tolerated dose as 74 Gy and reported an impressive median survival time of 37 months. The dose-limiting toxicities were mainly pulmonary. These results suggest that the dose and technical aspects of RT delivery are important in the combined modality approach for stage III NSCLC.

In conclusion, the statistically significant differences in survival were suggested that the concurrent chemotherapy and conformal three-dimensional radiotherapy is the optimal strategy for patients with locally advanced NSCLC with acceptable toxicity rates. To date, it seems that more favourable outcomes may require than two cycles of full-dose systemic therapy, as well as chemotherapy concurrent with radiotherapy. In our study the dose-limiting toxicity, esophagitis was reduced by performing conformal radiotherapy

Conformal thoracic radiotherapy allows dose escalating and can probably improve survival and local control.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Dec 20; 6(12):2328-2332.
https://doi.org/10.3889/oamjms.2018.484
eISSN: 1857-9655
Clinical Science

Acute Myeloid Leukemia (AML) In Elderly: Cytogenetic Characteristics of Patients Treated At Hematology and Pediatric Oncology Center in Casablanca

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Citation: Bendari M, Khoubila N, Cherkaoui S, Hada N, Lamchahab M, Oukache B, Madani A, Rachid M, Qachouh M, Quessar A. Acute Myeloid Leukemia (AML) In Elderly: Cytogenetic Characteristics of Patients Treated At Hematology and Pediatric Oncology Center in Casablanca. Open Access Maced J Med Sci. 2018 Dec 20; 6(12):2328-2332. https://doi.org/10.3889/oamjms.2018.484

Keywords: Acute Myeloblastic leukaemia in older; Adults; Cytogenetics; Chromosome abnormalities

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Received: 26-Jun-2018; **Revised:** 02-Nov-2018; **Accepted:** 03-Nov-2018; **Online first:** 14-Dec-2018

Funding: This research did not receive any financial support

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

Abstract

AIM: The goals of this paper are: to report the incidence of AML in elderly, to describe cytogenetic characteristics of this population, to observe rare and novel cytogenetic abnormalities and lastly, to compare our finding with that previously reported in the literature.

METHODS: We conducted a retrospective analysis of 283 patients with acute myeloid leukaemia (AML) treated in our unit, we will report the incidence of AML in elderly, describe cytogenetic characteristics of this population, observe rare and novel cytogenetic abnormalities and compare our finding with that previously reported in the literature.

RESULTS: Among the 283 patients, 157 (54.4%) patients performed the karyotype, the cytogenetic analysis failed in 17 cases (11%). Prognostic group distribution was found to be favorable in 8 patients (6%) with 6 cases of t (8; 21) and 2 cases of inv (16), intermediate group in 94 patients (67%), including 58 cases (41,5%) with a normal karyotype, and an unfavorable group in 38 patients (27%) including complex karyotype (15%), -5 or del 5q (3%), -7 or del 7q (3.5%), t (9; 22) (2%). Some rare anomalies were observed.

CONCLUSION: However, the occurrence of a complex karyotype was more frequent than younger patients. In our unit, elderly benefit from supportive care, our study shows that it is a heterogeneous group and our treatment approach have to change especially for the patient from favourable risk group who can benefit from intensive chemotherapy. We have to improve our diagnosis with including molecular genetics that provides a documented substrate for a thoughtfully considered treatment plan.

Introduction

AML is an aggressive haematological malignancy, it's a rare disease, the incidence of AML increase with age, the median age at diagnosis is 67 years [1], the management of those patients is particularly difficult, both the nature of disease and the health of patient change with age, the take care of this fragile population is a veritable challenge for practician.

The cytogenetic profiles of elderly patients with AML are different from that of younger patients with more chromosomal abnormalities. The outcome in older adults is poor, high rates of good response translate into a 2-years survival of only about 15% to 20% [2], it's can be explained by a significant individual heterogeneity for those patients. Comorbid conditions, performance status, and decreased immune competence of elderly patients compromised the management of AML, and curative therapy as bone marrow transplantation cannot be proposed for those patients. On the other hand, AML in the elderly is not the same that younger people, a distinct gene expression profile noted for older compared with younger patients [3], [4].

A little is known about the cytogenetic profile of AML in Morocco, few studies are done, and no studies for elderly patients with AML are performed. In this article, we will try to describe the cytogenetic characteristics of patients having de novo acute

myeloid leukaemia AML aged more than 60 years treated in our unit from 2004 to 2016.

The goals of this paper are: to report the incidence of AML in elderly, to describe cytogenetic characteristics of this population, to observe rare and novel cytogenetic abnormalities and lastly, to compare our finding with that previously reported in the literature.

Patients and methods

The patients were identified by review of medical records at haematology and pediatric oncology centre of Casablanca, in Morocco during the period from January 2004 to July 2016. Informed consent was obtained from all individual participants included in the study. The patients had to have more than 60 years and should be followed for AML. Also, patients had to have a cytogenetic analysis at diagnosis. All samples were sent to a single reference laboratory who worked in collaboration with the university hospital.

Diagnosis of AML was confirmed by bone marrow aspiration and stained with May-Grunwald-Giemsa, and myeloperoxidase (MPO). The marrow blast count of 20% was required, and AML was classified into eight subtypes M0 to M7 according to the French American British (FAB) classification [5], [6].

Previously, the immunophenotyping was done in case of AML with minimal differentiation (AML-M0), acute megakaryoblastic leukaemia (AML-M7), erythroblastic leukaemia (AML-M6) and acute leukaemia of ambiguous lineage. To improve our diagnosis criteria, immunophenotyping has been done systematically for all young patients since 2011, for the elderly, few of patients benefited from immunophenotyping,

Cytogenetic analysis was done at diagnosis according to standard techniques with RHG banding. The bone marrow cells were cultured for 24 to 48 hours. Twenty cells were analysed, although examination of lower numbers of metaphases was also acceptable if an abnormal clone was detected. An abnormality was considered clonal when at least two metaphases had the same aberration in case of a structural abnormality or an extra chromosome. If there was monosomy, it had to be present in at least three metaphases. All the samples were sent at the time of diagnosis, to a single reference laboratory who worked in collaboration with the university hospital.

Chromosome identification and classification of chromosomal abnormalities were made according to the International System for Human Cytogenetic Nomenclature 2013 (ISCN) [7]. The cytogenetic findings were classified into three prognostic risk categories: favourable, intermediate and adverse, according to the classification proposed by Mrozek in 2006 [8]. The favorable group included patients with t (8; 21)(q22; q22), t (15; 17)(q24; q21) and inv (16) (p13·1q22)/t(16; 16)(p13·1; q22), whether alone or in combination with other abnormalities. The intermediate group included patients with normal karyotype and other aberrations excluded in the favourable or adverse group. The adverse one included those with complex karyotype defined with 3 or more abnormalities, inv (3) (q21q26)/t (3; 3) (q21; q26), t (6; 9) (p23; q34), t (6; 11) (q27; q23), t (11; 19) (q23; p13.1), del (5q) and monosomies 5 and 7.

Cytogenetic abnormalities such as t(8;21), t(15;17), inv(16)/t(16;16), 11q23,+8, t(9;11), -5/del(5q) and -7/del(7q) were further evaluated as sole or in combination with other anomalies. For the t(8;21) the characteristics and associated abnormalities were detailed.

To investigate the frequency of monosomal karyotype defined by the presence of at least 2 autosomal monosomies or single autosomal monosomy associated with at least one structural abnormality, we studied the distribution of autosomal chromosomal monosomies among patients with cytogenetic abnormalities other than core-binding factor.

Rare and novel abnormalities were also detailed. The research was done in the Atlas of Genetics and Cytogenetics in Oncology and Hematology [9] and Pubmed. All statistical analyses were evaluated using SPSS 16·0 software.

Results

From January 2004 to July 2016, 1483 patients aged more than 19 years old, with the novo AML were followed in our department. One thousand and two hundred (80%) were aged between 20 and 60 years old and 283 (20%) more than sixty years old. The total number of patients with de novo AML having more sixty than ears old entered the hospital per year varied between 12 patients on 2006 and 31 patients on 2011 with a median of 21 new patients per year. On the 283 patients, 150 (53%) were male, and 133 (47%) were female; the sex-ratio was at 1:12. The median age was 69 years (61-99) old and distribution of patients' ages per decade shows some difference of frequency: 151 (53.35%) patients were aged between 60 and 69 years, 100 (35.5%) between 70 and 79 years, 32 (11.3%) had more than 80 years old. Immunophenotyping was performed in 46% of cases. AML-M2 was the most frequent subtype with 157 (54.4%) patients. Patient's characteristics are showed in Table 1.

Table 1: characterisation of clinical, biological and immunologic presentation of AML, by age (WBC: white blood cell; FAB: French-American-British)

	60-69 years	70-79 years	Older than 80 years
No patients	151	100	32
Novo	151	93	32
Myélodysplasie. No	0	3	0
Sex. Male No (%)	76	55	11
Laboratory values, median (range) element/mm3			
WBC count	29953 (1000-184300)	77330 450-347000	22278 1100-229300
FAB classification, No (%)			
M1	41 (27.15)	21	5
M2	43 (28.48)	35	5
M3	4 (2.65)	0	0
M4	9 (5.96)	7	2
M5	2 (1.32)	4	3
M6	5 (3.31)	2	2
M7	0	2	0
M0	9 (5.96)	5	1
Unknown	38 (25.16)	24	14

Among the 283 patients, 157 (54.4%) patients performed the karyotype, the cytogenetic analysis failed in 17 cases (11%). The frequency of the most cytogenetic abnormalities detected at diagnosis among 157 cases of AML arising in older adults with cytogenetic study and their associated clinical, biologic and immunologic features are presented in table 2. Clonal abnormalities were observed in 82 (58.5%) of the 157 patients.

Prognostic group distribution was found to be favorable in 8 patients (6%) with 6 cases of t(8; 21) and 2 cases of inv (16), intermediate group in 94 patients (67%), including 58 cases (41.5%) with a normal karyotype, and an unfavorable group in 38 patients (27%) including complex karyotype (15%), -5 or del 5q (9%), -7 or del 7q (5%), t(9,22) (2%). In our analyze we found some rare anomalies, like t(6,12)(q12; p12) ,+3, +7, +16, -21, and t (5.16).

Table 2: Frequency and percentage of cytogenetic abnormalities (Percentage do not add to 100 because patients with more than one abnormality are counted more than once)

Abnormality	All patients (no:140) No	60-69 years	70-79 years	Older than 80 years
Normal	58	40	11	7
complexe	21	17	4	0
T(8,21)	6	6	0	0
Inv(16)	2	2	0	0
-5 /del(5q)	14	11	3	0
-7 /del (7q)	8	7	1	0
11q23	5	2	1	2
T(9,22)	3	2	1	0
Trisomy:				
+8		6	5	0
+21		3	0	0
+7		2	1	1
+19	24	0	1	0
+16		0	1	0
+18		1	0	0
+13		1	1	0
+3		0	1	0
Monosomy				
-8	5	0	2	0
-21		1	0	0
-Y		0	1	0
-X		1	0	0
Other abnormalities	11	6	5	0

Other abnormalities: del 12q ,del 20q, del 9q, del 8q, t(7,11)(p15 ;p15), t(9,11)(q23 ;q23), t(5,16)(q33,q22), t(3,5)(q26;q34),t(13,13)(q10 ;p10),t(13,14)(p11 ;q11), t(6,12)(q12 ;p12)

However, the occurrence of a complex karyotype was more frequent than younger patients. t(8; 21) and t(15; 17) were seen less than younger patients. No significant variation in frequency of particular abnormalities across the age range was noted. Frequency and percentage of cytogenetic abnormalities among cases are shown in Table 2.

In the favourable risk group t(8,21) was detected in 6 cases and it was accompanied by additional changes in 5 cases, the inversion of chromosome 6, it was found in 2 cases, it was presented as a sole abnormality. For the intermediate risk group, the majority of cases classified within the intermediate risk group had a normal karyotype (41.5%), the abnormalities that were detected were represented by trisomy 8 which was sole in 9 cases, and associated with trisomy 10 and trisomy 12 in one case. Trisomy 21 was observed in 3 cases, del 11q23 was noted in 4 cases as a sole abnormalities, and associated with other changes in 1 case. Trisomy 7, trisomy 13 and trisomy 3 were found in 4, 2, and 2cases respectively. Many single aberrations were detected like trisomy 18, trisomy 19, del 12q, del 9q, del 20q, del17q, monosomy 8, monosomy 20, loss of chromosome X and loss of chromosome Y.

Overall, 38 of 157 cases were assigned to the unfavourable risk group, in 15%, this was based on the presence of complex karyotype, monosomy 5/del5 was found in 12 cases sole, and in combination in 2 cases, monosomy 7/del 7 was sole in 6 cases, and combined in 3 cases.

Some new and rare abnormalities were noted like trisomy 3, trisomy 7, trisomy 16, monosomy 21. t(3,5)(q26;q34) as a sole anomaly was described in 68 years old patient; we also found t(5,16)(q23,q22) in 61 years old man. t(6,12) (q12;p12) was isolated in patient having 61 years old, other rare abnormalities were detected as t(13,14) (p11;q11), t(13,13) (q10;p10).

To further characterise the cytogenetic features of ML in older adults, we summarise the frequency of additional changes occurring in combination with primary chromosomal aberrations in Table 3.

Table 3: The frequency of additional changes occurring in combination with primary chromosomal aberrations

	Cytogenetic abnormalities									
	Total	T(8, 21)	Inv(16)	-7/del7	-5/del5	11q23	monosomies	+8	+21	Other abnormalities
Total		6	2	8	14	5				
Alone		1	2	4	2	4				
T(8,21)					1					
Inv(16)										
-7/del7					1					
-5/del5		1		1						
11q23					1					
Complexe				2	5	1				
+8		1								
+21										
-Y		1								
-X		1								
Other abnormalities		1			4					

Discussion

Acute myeloid leukaemia is a rare disease occurring in adults older than 55 years of age. It's affecting annually 3-4 persons per 100000 individuals [10]. The median age of patients with AML is around 70 years. AML is an aggressive haematological malignancy, with extremely poor prognosis with overall survival (OS) of less than 20% at 5 years [2].

The diagnosis of AML depends primarily on detection of leukemic blasts of myeloid lineage (more than 20%) in the bone marrow. The World Health Organization classifies AML into 4 major categories (each with 2 or more subtypes) using morphologic, immunophenotypic, genetic and clinical features. The main categories are (1) AML with recurrent genetic abnormalities, (2) AML with myélodysplasie-related features, (3) therapy-related AML and MDS, and (4) AML not otherwise specified. Genetic and molecular abnormalities highlight the heterogeneity of AML and identify subsets associated with better or worse prognosis.

In Morocco, cytogenetic analysis was done systematically since 2004 for all young patients, but not all elderly patients benefited from the assessment at diagnosis. The number of patients how had cytogenetic and immunophenotyping analysis decrease with increasing ages.

The cut-off of 60 years old is arbitrarily used to define "older" patients; it is unknown if with this age limit we can discriminate patients subgroups with different outcomes. In our unit, for adult patients with AML, treatment is proposed only for young patients, they are treated by a uniform protocol called AML-MA 03 which included two inductions (7 + 3), two consolidations and a maintenance treatment without any stratification, this protocol was proposed from 2003 to 2010. On 2011 another protocol, AML-MA 11 was developed with two risk groups stratification based on the age (more or less than thirty years old) and cytogenetic finding as favourable, core binding factor (CBF) leukaemias with t(8;21) or inv(16)/t(16;16) versus all the others groups, the favourable group was receiving intensive chemotherapy involving cytarabine at a range of doses. Patients with acute promyelocytic leukaemia were treated by the APL-2004 protocol.

Age was considered from many years one of the factors prognostic; it's associated with poor outcome. Because the worst survival in AML elderly patients, the lack of resources and beds availability, we concentrate all our efforts to treat patients aged between 20 and sixty years old by improving the diagnosis, risk stratification and supportive care. Patients aged more than sixty years old receive systematically palliative care, we propose a low dose of aracytine, hypomethylating agents, and best supportive care with oral cytostatic drugs like hydroxyurea. Patients also benefited from transfusions, antibiotics and analgesics.

In our study we found 283 patients aged more than 60 years old, with 53% of them aged between 60 and 69 years.

Some factors are implicated in the adverse outcome of elderly in comparison with younger individuals. In this age group, AML has a particularly dismal outcome with less than 5% of the patients being alive 5 years after the diagnosis, as compared to 40% in the young [11], [12]. Advanced age is often accompanied by frailty and comorbidities [13], with poorer tolerance of combination chemotherapy regimens leading to the use of less intensive treatment protocols. Come of AML in the elderly; it is necessary to distinguish subgroups of patients with the paramount curable disease, who can receive treatment and those with incurable disease who can benefit from supportive care [14].

Age is not the unique factor of poor outcome; physical condition is very important; it can vary considerably among older people of the same age. Polypharmacy also constitutes an important prognostic factor [15]. Furthermore, poor prognosis in this group is associated with increased frequency of adverse cytogenetic features. Higher frequencies of adverse cytogenetics and unfavourable molecular aberrations are more common among the ageing populations. Distinct gene expression profiles noted for older compared with younger patients explain the poor outcomes in older individuals [16], [17].

Some previous studies have identified diagnostic cytogenetic as a key determinant of outcome in AML. Recent studies have revealed that the disorder arises from a series of recurrent hematopoietic stem cell genetic alterations accumulated with age [18]. The incidence of chromosomal abnormalities in AML differs according to geographical regions in the world. In this article, we analyse the largest cohort of patients with AML in the elderly done in Morocco. This study aims to describe the profile of our patients and destining subgroups. We defined prognosis subgroup, only 8 patients (6%) were in a favorable risk group, the most frequency of the common cytogenetic abnormalities detected at diagnosis permit to classified patients in the intermediate risk group with 94 patients (67%), and unfavourable risk group include 38 patients (27%). In light of our result, our diagnosis approach has to change with including molecular studies in our routine, and some of our patients will benefit from intensive chemotherapy.

In our study, some rare abnormalities were detected as t(3,5)(q26;q34) as a sole anomaly on 68 years old patient, this translocation was described on two cases, a 48 years old female patient and an unknown male age both with M2 AML, we also found t(5,16)(q23,q22) on 61 years old man, it's a rare abnormality, only two cases were reported in

literature. t(6,12) (q12;p12) was isolated on man having 61 years old, it's very rare abnormality in AML, other rare abnormalities were detected as t(13,14)(p11;q11), t(13,13)(q10;p10), and trisomy 16 or trisomy 3 which are very rare in AML [10]. Our finding is very important; it can help to define novel genes and mutations involved in the leukemic process.

In fact, recent molecular studies including next-generation sequencing (NGC) testing of myeloid neoplasms (MNs) have shown that acquired mutational events that can involve FLT3, NPM1, CEBPA, DNMT3A, IDH1, IDH2, KIT, MLL-PTD, TET, RUNX1, ASXL1, and TP53 are frequent in the novo AML or MDS and can be used for risk stratification, especially in patients with normal karyotype [19], [20], [21], [22].

In conclusion, the outcome in elderly with AML continuously declines with progressively increasing age. Comorbid conditions, performance status, adverse cytogenetic and unfavourable molecular aberrations are among the most critical determinant factors. This paper furnishes clinically and biological information, this background information can be useful in our treatment approach especially for the patient from favourable risk group who can benefit from intensive chemotherapy. Early referral to palliative medicine and the use of this subspecialty as a supportive care service it's not always the best proposition for those patients. This work sheds light on some missing practice on our routine like molecular studies which can offer useful guidance during the treatment.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Dec 20; 6(12):2333-2336.
https://doi.org/10.3889/oamjms.2018.477
eISSN: 1857-9655
Clinical Science

Correlation of Leukocyte Subtypes, Neutrohyl Lymphocyte Ratio, and Functional Outcome in Brain Metastasis

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Citation: Ritarwan K, Nasution IK, Erwin I, Nerdy N. Correlation of Leukocyte Subtypes, Neutrohyl Lymphocyte Ratio, and Functional Outcome in Brain Metastasis. Open Access Maced J Med Sci. 2018 Dec 20; 6(12):2333-2336. https://doi.org/10.3889/oamjms.2018.477

Keywords: Brain Metastasis; Neutrophils-Lymphocyte Ratio; Karnofsky Performance Scale

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Received: 12-Oct-2018; **Revised:** 05-Nov-2018; **Accepted:** 07-Nov-2018; **Online first:** 15-Dec-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: As the most cause of death in patients with solid extracranial malignancy, brain metastasis (BM) nowadays being studied extensively especially on how to find a reliable laboratory marker that can correlate with its clinical outcome. Leukocyte subtypes, primarily neutrophils and lymphocytes and its ratio known as Neutrophils-Lymphocyte Ratio (NLR) have been known before its relationship with progressivity of BM from other solid tumours.

AIM: The objectives of this research to study the correlation of leukocyte subtypes, neutrophil-lymphocyte ratio & functional outcome in brain metastasis.

METHODS: The study subjects were recruited consecutively from the study population. Venous blood was taken 5 ml of venous blood samples done in the first day of admission on emergency department and neurology clinic of Neurology Department of Adam Malik General Hospital before any drug injections. Samples were kept in vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA) and sent to Department of Clinical Pathology laboratory of Adam Malik General Hospital, immediately centrifuged at 3100 rpm for 10 minutes in -20°C temperature and analysed using Sysmex XT-2000i. Functional outcome of the patient assessed using Karnofsky performance scale (KPS) in a cross-sectional manner with laboratory analysis.

RESULTS: We conduct a mean differences and correlational leukocytes and its subsets analysis of 72 BM patients resulting on significant positive correlation on lymphocyte percentage ($r = 0.383$, $p = 0.001$) and lymphocyte absolute ($r = 0.265$, $p = 0.024$), also significant negative correlation on neutrophils ($r = -0.240$, $p = 0.042$) and NLR ($r = -0.432$, $p < 0.001$) with Karnofsky Performance Scale (KPS).

CONCLUSION: Increased lymphocyte absolute and lymphocyte percentage correlated significantly ($p < 0.05$) with better KPS, while elevated neutrophils percentage and increased NLR show significant correlation with worse outcome of BM patients.

Introduction

Brain metastasis (BM) is one of the central nervous system consequences of primary extracranial malignancy that mainly happen on 20-40% in adult cancer patients. As one of the main cause of death for these population, its progression is determined by several condition such tumour cell migration (intravasation, dissemination and extravasation) and colonisation also microenvironment inflammation that correlates in the severity of the disease. The basic mechanism of BM begin with Paget's "seed and soil" hypothesis included three main principles: the heterogeneous population of a tumour with different characteristics, specific traits of metastasis and secondary tumoral microenvironment [1], [2], [3], [4].

The human immune system, in general, have several myeloid cells especially leukocyte subtypes which are responsible in cancer metastasis regulation, such neutrophils and monocytes; both considered immunocytes that could secrete vascular endothelial growth factor (VEGF) contributing to increased tumour size and development. While regulation of aspartic proteinase cathepsin E, which act as tumour suppressor activated by lymphocytes, macrophage and a dendritic cell which and apoptosis induction [5], [6].

Neutrophil-to-lymphocyte ratio (NLR) is the ratio of the absolute neutrophil count to the absolute lymphocyte count and used as one of a simple, rapid, cost-effective and indicative marker of inflammatory process and stress of human body as it can be obtained on complete blood count (CBC) analysis.

While normal immune system would prevent the dissemination of cancer cells and prevent proliferation, about 0.01% escaping circulating tumor cells (CTC) survival from primary sites resulting from shear stress, mechanical detachment even cell-mediated cytotoxicity which provoked epithelial to mesenchymal transition (EMT) regulated by tumor necrosis factor α (TNF- α) and nuclear factor κβ (NF-κβ). Individual immunocompetence capability could also be estimated by NLR and correlated with disease progression and functional outcome in malignancy measured by Karnofsky performance scale (KPS) [5]. On this basis, elevated NLR ratio could be consequences of either neutrophilia which promotes tumour granulocyte colony-stimulating factor (GM-CSF) and/or lymphopenia that can cause circulating tumour cell escape systemic immunosurveillance in the human body [5], [6].

Growing interest of NLR count on has been established today as several studies tried to elaborate between this ratio and progression of the disease that implicated by even slight process of inflammation, for examples malignancy in various primary organ (e.g.: small cell lung cancer, renal cell carcinoma, pancreatic cancer, primary liver ca, breast cancer, glioblastoma multiforme), arterial disease (e.g: acute coronary syndrome, cerebral infarction, Takayasu arteritis), degenerative disease (e.g: ankylosing spondylitis) also in some autoimmune disorders (e.g: psoriasis, systemic lupus erythematosus [SLE]) [7], [8], [9], [10], [11], [12], [13], [14], [15], [16], [17], [18], [19].

Methods

Ethical approval received from the Health Research Ethical Committee, Faculty of Medicine Universitas Sumatera Utara, Medan-Indonesia. The research was conducted from January 2016 to December 2016 at Adam Malik General Hospital recruiting 72 consecutive sample of patients diagnosed with BM. Confirmation of BM diagnosis was done by means if there are any clinical neurologic deficits and/or any suspicious prove of in any established modalities (laboratory tumour markers, chest radiography, ultrasonography, computed tomography (CT) scan, radionucleotide tracing and/or another diagnostic test) supporting central nervous system (CNS) involvement from other extracranial primary malignancy [20].

Exclusion criteria in this study including sepsis, septicaemia, bacteremia, septic shock and other systemic inflammatory response syndrome (SIRS)-like condition, leukemia and leukemoid reaction, prior dexamethasone, methylprednisolone and/or any intravenous steroid injection, autoimmune disease (e.g: SLE, scleroderma) or immunodeficient syndrome (e.g: HIV/AIDS) essentials heart,liver and/or kidney disorders, pneumonia, seizure at the beginning or during hospitalization, massive upper and/or lower GI bleeding which all of the above could modify, primarily suppress the baseline immunological condition of the patients [21], [22], [23].

Informed consent was asked from the patients (or their legal responders in any means the patients cannot give consent) and collection of 5 ml of venous blood samples done in the first day of admission on emergency department and/or neurology clinic of Neurology Department of Adam Malik General Hospital before any drug injections. Samples were kept in vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA) and sent to Department of Clinical Pathology laboratory of Adam Malik General Hospital, immediately centrifuged at 3100 rpm for 10 minutes in -20o C temperature and analysed using Sysmex XT-2000i. Functional outcome of the patient assessed using Karnofsky performance scale (KPS) in a cross-sectional manner with laboratory analysis.

Data were collected and calculated using IBM SPSS Statistic for Windows, Version 24.0 and test of normality was conducted using Kolmogorov-Smirnov on more than 50 study sample. Variables characteristics were shown in Table 1 while mean differences were compared between groups using one-way analysis of variant (ANOVA) (Table 2), and each variable was correlated with KPS using Pearson's correlation test on the nature of normal distribution of the samples (Table 3).

Result

We divided subjects into 3 categorised KPS groups (KPS 80-100 as symptomatic, KPS 50-70 as symptomatic with assistance and KPS 0-40 as symptomatic with bed confinement). Test of normality was conducted using Kolmogorov-Smirnov test on more than 50 study sample. Variables characteristics of the patients were shown in Table 1.

Table 1: Characteristics of BM Subjects

Variable Characteristics (n = 72)	Mean (Percentage)
Age	51.74 ± 11.17 years old
Minimum	22 years old
Maximum	74 years old
Sex	
Male	30 (41.7%)
Female	42 (58.3%)
Origins of Primary Tumor	
Pulmonary	37 (51.4%)
Breast	17 (23.6%)
Cervix-genito-urinary	10 (13.9%)
Others	4 (5.55%)
Unknown	4(5.55%)
KPS	
80-100	10 (13.89%)
50-70	34 (47.22%)
0-40	28 (38.89%)

Mean differences were also compared between groups using analysis of variant (ANOVA) (Table 2).

Table 2: Means Difference of Leukocyte Subtypes

Variables		Karnofsky Performance Scale (KPS) Groups			P
	Total Subjects	KPS 80-100 (n = 10)	KPS 50-70 (n = 34)	KPS 0-40 (n = 28)	
Mean Leukocytes (x10^3/mm^3)	11.93 ± 5.96	11.48 ± 5.69	11.14 ± 5.38	13.05 ± 6.70	0.446
Mean Neutrophil Percentage (%)	78.28 ± 9.74	73.21 ± 10.34	77.75 ± 8.86	80.74 ± 10.09	0.099
Mean Neutrophil Absolute (x 10^3/µL)	9.57 ± 5.29	8.71 ± 5.01	8.83 ± 4.62	10.79 ± 6.06	0.303
Mean Lymphocyte Percentage (%)	13.18 ± 7.68	17.03 ± 9.39	14.81± 7.89	9.84 ± 5.38	0.008*
Mean Lymphocyte Absolute (x 10^3/µL)	1.40 ± 0.74	1.66 ± 0.74	1.47 ± 0.67	1.23 ± 0.81	0.217
Mean Monocyte Percentage (%)	7.14 ± 3.21	7.04 ± 2.38	7.45 ± 3.42	6.81 ± 3.28	0.739
Mean Monocyte Absolute (x 10^3/µL)	0.90 ± 0.63	0.92 ± 0.66	0.94 ± 0.67	0.84 ± 0.60	0.827
Mean Basophil Percentage (%)	0.31 ± 0.36	0.40 ± 0.36	0.36 ± 0.42	0.21 ± 0.28	0.202
Mean Basophil Absolute (x 10^3/µL)	0.03 ± 0.03	0.04 ± 0.05	0.03 ± 0.03	0.03 ± 0.03	0.424
Mean Neutrophil-to-Lymphocyte Ratio (NLR)	8.23 ± 5.05	5.62 ± 2.94	6.87 ± 4.02	10.82 ± 5.72	0.001*

*significant p-value (< 0.05).

Each variable was then correlated with KPS using Pearson's correlation test on the nature of normal distribution of the samples as seen in Table 3.

Table 3: Pearson's Correlation of Leukocytes Subtypes and KPS

Leukocytes Subtype	Karnofsky Performance Scale	
	r (Pearson's Correlation)	p (Significance)
Leukocytes (x10^3/mm^3)	-0.569	0.625
Neutrophil Percentage (%)	-0.240	0.042*
Neutrophil Absolute (x 10^3/µL)	-0.106	0.375
Lymphocyte Percentage (%)	0.383	0.001*
Lymphocyte Absolute (x 10^3/µL)	0.265	0.024*
Monocyte Percentage (%)	0.100	0.405
Monocyte Absolute (x 10^3/µL)	0.121	0.311
Basophil Percentage (%)	0.188	0.115
Basophil Absolute (x 10^3/µL)	0.153	0.200
Neutrophil-to- Lymphocyte Ratio (NLR)	-0.432	< 0.001*

*significant p-value (< 0.05).

Discussion

On the mean differences and correlational analysis, we found significant differences on mean lymphocyte percentage between symptomatic group (KPS 80-100), symptomatic with assistance group (KPS 50-70) and symptomatic with bed confinement group (KPS 0-40), (17.03 ± 9.39% vs 14.81 ± 7.89% vs 9.84 ± 5.38 %, respectively) with Pearson correlation product moment of lymphocyte percentage and KPS show rather weak but significant positive correlation (r = 0.383; p = 0.001) [24].

Although we were not getting any ANOVA's significant differences on mean neutrophile percentage and meant lymphocyte absolute, there are weak significant negative correlation on neutrophile percentage and KPS (r = -0.240, p = 0.042) also on lymphocyte absolute and KPS show weak significant positive correlation on lymphocyte absolute and KPS (r = 0.265, p = 0.024). This view was in line with my review on multiple tumour progression correlated with tumour-infiltrating neutrophils, as elevated blood neutrophils proven to be a poor prognostic factor for functional outcome [25] also stated that circulating tumour-infiltrating-lymphocyte number elevation also has correlated with favourable clinical outcomes in subsets of human cancer [26].

On NLR variable, we found significant mean differences percentage between symptomatic group (KPS 80-100), symptomatic with assistance group (KPS 50-70) and symptomatic with bed confinement group (KPS 0-40) (5.62 ± 2.94 vs 6.87 ± 4.02 vs 10.82 ± 5.72, respectively) and also significant moderate negative correlation between NLR and KPS (r = -0.432, p < 0.001). Although applied on different population (Glioblastoma Multiforme/ GBM), NLR also has been studied by Alexiou et al., resulting on NLR > 4.7 was associated with decreased survival time (11 vs 18.7 months, p = 0.01) [7].

Templeton et al., allege that high NLR has an adverse effect on overall survival (OS) in many solid tumours [27], and this result was also agreeable with the previous study by Serdarevic et al., [28] on BM and non-BM population of non-small-cell lung cancer (NSCLC) which conclude higher NLR mean on BM group and more progressive condition (6.05 vs 4.6, p = 0.023) [28]. This was also confirmed by another prognostic study of NLR that elevated preoperative NLR is a predictor of worse survival after BM resection (OS 14 month for NLR < 5 and 5 months for NLR ≥ 5, p = 0.001) [27], [29].

In conclusion, increased lymphocyte absolute and lymphocyte percentage correlated significantly with better KPS, while elevated neutrophils percentage and increased NLR show significant correlation with worse outcome of BM patients.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Dec 20; 6(12):2337-2341.
https://doi.org/10.3889/oamjms.2018.367
eISSN: 1857-9655
Clinical Science

Adaptive Functioning and Psychosocial Problems in Children with Beta Thalassemia Major

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Citation: Elzaree FA, Shehata MA, El Wakeel MA, El-Alameey IR, AbuShady MM, Helal SI. Adaptive Functioning and Psychosocial problems in Children with Beta Thalassemia Major. Open Access Maced J Med Sci. 2018 Dec 20; 6(12):2337-2341. https://doi.org/10.3889/oamjms.2018.367

Keywords: β-Thalassemia major; Children; Adaptive Functioning; Psychosocial problems

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Received: 23-Oct-2018; **Revised:** 07-Nov-2018; **Accepted:** 08-Nov-2018; **Online first:** 16-Dec-2018

Funding: The study was a part of a project supported financially by National Research Centre Egypt (Grant No. 11010145)

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

Abstract

BACKGROUND: Beta thalassemia major is considered one of the serious health problems and the commonest hemoglobinopathy in Egypt that creates a burden not only on health system but also on the affected families and children who become vulnerable to emotional, social, psychological and behavioural problems.

AIM: This study was designed to assess the psychosocial burden and the adaptive functioning in children with beta-thalassemia major.

SUBJECTS AND METHODS: A group of 50 children with thalassemia major and 50 normal children matched for age and sex were included in a case-control study. Vineland Adaptive Functioning Scale was used to assess the adaptive functions; while the Pediatric Symptom Checklist (PSCL) was used to assess psychosocial morbidity.

RESULTS: A group of 50 children aged 5-17 years old with thalassemia major, their mean age was 11.05 ± 3.8, showed a statistically significant lower total adaptive behaviour score and communication subscale score. All the mean values of adaptive behaviour for cases and controls were within the average values. Results from the PSCL revealed no significant difference between mean scores of children with thalassemia and controls. A score of attention domain was markedly higher in children with thalassemia. Internalising behaviour was the most dominant as it was detected in 10% of the patient group.

CONCLUSION: Thalassemic patients had a relatively mild affection for adaptive and psychosocial functioning that can be explained by social and medical support they receive, which may increase their competence and psychological wellbeing.

Introduction

β-Thalassemia major is considered the commonest hemoglobinopathy in the Mediterranean area particularly Egypt with an estimated carrier rate of 9-10.2% [1]. Registered cases of homozygous β-thalassemia in big centres of Egypt in 2006 up to Sept 2007 (n = 9912) [2]. From about 10,000 registered β-thalassemia cases and more than 20,000 non-registered cases; 95%of them are β-thalassemia major, and 5% are thalassemia intermediate or haemoglobin H disease [3]. β-thalassemia is a chronic condition, which put the huge psychosocial burden on the patient and his family [4], [5]. It is also considered a major health problem for the Public Health System of any country due to high expenses of treatment involving regular blood transfusions, iron chelation, frequent hospitalization and general medical follow up; that creates a burden not only on the health system but also on the affected families and their children, who become more liable to emotional, social, psychological and behavioural problems [6], [7]. The chronic illness usually affects the progress of growth and development. The chronic illness, treatment requirements, frequent hospitalisation and surgery, when necessary, all increase worries about physical appearance, interfere with the process of gaining independence and healthy relationships with parents and friends. Also, developmental issues complicate the children and adolescents' capability of being

responsible for managing their illness [8]. The drawbacks of the disease in many aspects of life become strongly evident during the school age and adolescence when children ask for independence [9] [10]. It has been related with psychosocial aspect and a significant negative effect on areas of school functioning because of the likelihood of physical deformity, growth retardation and delayed puberty besides the difficulty of management (such as regular transfusion and time-consuming iron chelation treatment) [11], [12].

This study was designed to assess the psychosocial burden and the adaptive functioning in children with β-thalassemia major. It is hypothesised that these patients will have higher psychosocial problems that may affect the adaptive functioning.

Subjects and Methods

A case-control study was done on 50 transfusion dependent β-thalassemia children compared with 50 normal children as controls, matched for age and sex recruited from the outpatient haematology clinic of El-Demerdash pediatric hospital-Ain Shams University and Child Health Clinic and Pediatric Neurology Clinic in Centre of Medical Excellence in National Research centre. Vineland Adaptive Functioning Scale was used to assess the adaptive functions; while the Pediatric Symptom Checklist (PSCL) was used to assess psychosocial morbidity. Written informed consent was taken from all patients' parents before enrollment in the study and after full explanation of their role in the study. The consent was approved by The Ethical Committee of The National Research Center and Ain Shams University under the registration number (16358).

All children in the patient and control groups were subjected to the following measures:

1. Vineland Adaptive Behavior Scales: Children's behaviours and ability to function adequately in the environment are measured using the Vineland Adaptive Behavior Scales, Arabic version [13], [14]. It is frequently used to measure, social profile and social-emotional skills. This test includes four items: Communication, Socialization, Daily Living Skills and Motor Skills (used for children below 6 years). The Vineland scale uses a semi-structured interview technique and can be administered by a trained interviewer to the guardian. The items that guide the interviewer on the survey form are shown in developmental sequence. The interviewer begins with items that correspond to mental or chronological age and establishes a basal and ceiling score before concluding the interview. Every item is calculated to know whether the individual performs the activity described: 2 = yes, the behaviour is usually performed; 1 = sometimes or partially; and 0 = no, the behaviour is never performed. The mean total score, according to the Arabic version, was classified as low adaptive behaviour (≤ 69), below average (70-84), average (85-115), above average (116-130), and high adaptive behaviour (≥ 131).

2. The Pediatric Symptom Checklist-17 (PSC-17): is a psychosocial screen designed to facilitate the recognition of cognitive, emotional, and behavioural problems so that proper interventions can be started as early as possible. The PSC-17 is composed of 17 items that are rated as "Never," "Sometimes," or "Often" present. A value of 0 is assigned to "Never", 1 to "Sometimes," and 2 to "Often". The total score is calculated by adding together the score for each of the 17 items. Items that are left unanswered are omitted (i.e., score equals 0). If four or more items are left unanswered, the questionnaire is considered invalid. A PSC-17 score of 15 or higher suggests the presence of major behavioural or emotional problems. To identify which type of mental health problems are present, fix the 3-factor scores on the PSC: γ The PSC-17 Internalizing Subscale (Cutoff 5 or more items), the Attention Subscale (Cutoff 7 or more items), and the Externalizing Subscale (Cutoff 7 or more items) [15].

Data were analysed using Statistical Program for Social Science (SPSS) version 20.0.

Quantitative data were described as mean± standard deviation (SD). Qualitative data were described as frequency and percentage.

Non-parametric data was represented by median and range. Data were analysed to test statistically significant difference between groups.

1. For quantitative data (mean ± SD), student t-test was used to make a comparison between 2 groups.

2. For qualitative data (frequency and proportion), the chi-square test was used.

3. The correlation coefficient was done to test the association between variables.

P is significant if ≤ 0.05 at a confidence interval of 95%.

Results

The study included 50 thalassemic patients, 25 males and 25females, with age range 5-17 years, their mean age was 11.05 ± 3.8, 30% of cases had mongoloid facies and 46% had hemosiderosis. The control group included 50 subjects age and sex matched. Most of the subjects were from the middle

social class. Socio-demographic and clinical criteria of the participants are shown in Table 1.

Table 1: Demographic and Clinical Characteristics Data of thalassemic patients and Control Children

Parameter	Patients Frequency of Mean ± SD	Controls Frequency of Mean ± SD	P
Sex			
Male	25 (50%)	25 (50%)	1.00
Female	25 (50%)	25 (50%)	
Patient Education			
Uneducated	4 (8%)	2 (4%)	
Read and Write	8 (16%)	8 (16%)	0.69
Educated	38 (76%)	40 (80%)	
Social Standard			0.002*
Low class	17 (34%)	14 (28%)	
Middle Class	33 (66%)	25 (50%)	
High class	---	11 (22%)	
School Performance			
Poor	18 (36%)	14 (28%)	0.203
Average	22 (44%)	18 (36%)	
Above average	10 (20%)	18 (36%)	
Current schooling			
Yes	14 (28%)	50 (100%)	0.000*
No	36 (72%)	0	
Previous bone fracture			
No	26 (52%)	42 (84%)	0.001*
Yes	24 (48%)	8 (16%)	
Bone Aches			0.001*
No	10 (20%)	44 (88%)	
Yes	40 (80%)	6 (12%)	
Growth Retardation			
No	29 (58%)	44 (88%)	0.001*
Yes	21 (42%)	6 (12%)	
Chelation Therapy			
No	2 (4%)	---	---
Yes	48 (96%)		
Ferritin level	3612.4 ± 2410.2	142.4 ± 69.6	0.000*
Hemoglobin	7.79 ± 1.28	12.43 ± 1.05	0.000*

*Highly significant test p < 0.01.

Comparison of adaptive behaviour scores between cases and controls are shown in Table 2. There is a statistically significant difference between the two groups in the total adaptive behaviour score and the communication subscale score. All the mean values of adaptive behaviour for cases and controls were within the average degrees, 38% and 24% of thalassemic patients had below average scores in communication and daily living skills respectively, while only 8% had below average social skills.

Results from the PSCL revealed no significant difference between mean scores of children with thalassemia and controls (p = 0.06). A score of attention domain was significantly higher in children with thalassemia (p = 0.000). Internalising behaviour was the most prevalent as it was detected in 10% of the patient group.

Table 2: Comparison between cases and controls as regard to adaptive behaviour mean scores and PSCL variables

	Group	N	Mean	Std. Deviation	T-test	P
Communication	Patients	50	86.10	22.81	-3.878	-.000*
	Controls	50	100.28	12.17		
Daily life activity	Patients	50	96.60	14.34	-1.771	0.080
	Controls	50	100.82	8.85		
Social	Patients	50	103.12	13.75	0.529	0.598
	Controls	50	101.90	8.74		
Total Adaptive behavior Score	Patients	50	95.24	12.04	-2.693	0.008*
	Controls	50	101.02	9.25		
Attention PSCL	Patients	50	3.22	2.10	3.68	0.000*
	Controls	50	2.02	0.93		
Internalization PSCL	Patients	50	1.72	1.99	1.529	0.13
	Controls	50	1.26	0.77		
Externalization PSCL	Patients	50	1.58	1.77	-1.33	0.18
	Controls	50	1.98	0.96		
Total PSCL	Patients	50	6.54	4.41	1.88	0.06
	Controls	50	5.26	1.9		

*Highly significant test p<0.01; PSCL: Pediatric symptoms checklist.

Table 3 is showing the correlation between adaptive behaviour scores and some studied clinical variables. Total adaptive behaviour score showed a significant positive correlation with the age of disease onset, while the social score had a positive association with patient education, school performance and age of disease onset. Daily life activity score had a significant positive association with age, the frequency of bone fractures and growth retardation.

Table 3: Correlation between adaptive behaviour scores and clinical variables (showing Correlation Coefficient)

	Communication	Daily Life Activity	Social	Total
Age	-0.114	0.294*	-0.115	0.006
Sex	-0.017	0.141	0.024	0.057
Patient Education	0.103	0.150	0.287*	0.226
Social Standard	0.192	-0.017	0.177	0.185
School Performance	0.173	-0.083	0.342*	0.202
Age of Onset	0.193	0.211	0.280*	0.318 *
Duration of Illness	-0.171	0.172	-0.222	-0.122
Duration of Chelation Therapy	-0.186	0.165	-0.235	-0.140
Growth Retardation	-0.025	0.355*	-0.183	0.054
Frequency of Bone Fracture	0.138	0.332*	-0.050	0.202
The frequency of Blood Transfusion /year	-0.170	-0.115	0.219	-0.074
Ferritin level	-0.013	0.148	0.078	0.082

*Significant at p < 0.05. level.

Correlation between psychological variables and clinical variables are presented in Table 4. There was a significant positive association between internalising behaviour and the age, duration of illness and duration of chelation therapy.

Table 4: Correlation between psychological variables and clinical variables (showing Correlation Coefficient)

	Internalization	Externalization	Attention	Total
Age	0.401*	-0.057	0.131	0.217
Sex	-0.163	0.034	0.125	-0.005
Patient Education	0.208	-0.087	0.149	0.132
Social Standard	0.005	-0.172	-0.188	-0.153
School Performance	-0.087	0.057	-0.029	-0.036
Age of Onset	0.067	0.105	0.110	0.123
Duration of Illness	0.347*	-0.078	0.078	0.160
Bone Aches	0.005	0.080	0.029	0.050
Duration of Chelation Therapy	0.340*	-0.100	0.064	0.142
Growth Retardation	-0.189	-0.096	0.144	-0.059
Mongoloid Facies	0.205	0.057	0.140	0.179
Frequency of Blood Transfusion /year	-0.007	-0.073	-0.059	-0.061
Ferritin level	-0.102	-0.163	-0.092	-0.151

*Significant at p < 0.05. level.

Discussion

This study investigated adaptive functioning and psychosocial burdens in children having β-thalassemia. Adaptive functioning includes age-appropriate behaviours that individuals need to complete every-day tasks efficiently and independently. These behaviours can include self-care activities, social skills, functional communication,

functional academics, and the use of community facilities [16]. Children complaining of chronic disease have a greater demand when needing to complete daily life tasks, which includes managing their illness [8].

In this study, the Vineland Adaptive Behavior Scale was used to evaluate the adaptive functioning in thalassemic patients in comparison with age and sex-matched normal children. The communication skills were the most affected, as 38% of diseased children had below average score, there was a significant difference in the mean score of communication among cases and controls. Also, a statistically significant difference was found between patients and controls in the whole adaptive behaviour score. However, all the mean values of adaptive behaviour items for cases and controls were within the average degrees of the general population. In contrary to our findings, [17] reported that the thalassemic scores in the Vineland subscales were considered within the low-performance range. Moreover, they found a statistically significant difference between cases and controls regarding the domains of communication skills, daily activity skills and social skills. However, their results did not show a significant difference between cases and controls in the total adaptive behaviour scores.

The social interaction was the least affected domain in the current study, only 8% of cases indicated mild deficit, and it showed a significant association with patient education, school performance and age of onset of the disease. Similarly, socialisation was affected in18% of β-thalassemic children in a Syrian study; they explained their mild deficit in social interactions to be attributed to well-built family relations in the Arabic community [18]. In accordance, Hongally et al., [19] also reported that the patients believed that the disease did not affect their family or social relations. Also, Ali et al., [20] observed that thalassemic children had significantly higher scores in the social domain. A possible explanation for this could be that children with β-thalassemia receive more attention, making them feel better socially [21]. However, these findings were not similar to other studies [22], [23].

Also, neither scores of adaptive behaviour nor the PSCL correlated with ferritin levels in our study. In accordance, a study carried out by Cakaloz et al., [5] the mean score of the children behaviour checklist and the ferritin levels showed no correlation. They suggested that the social and psychological impacts of chronic illness contribute to the behavioural problems more than the ferritin level.

A recent study had supported the continuous clinical use of the PSC-17 as a screening tool for children's psychosocial functioning [24]. In a study carried out by Saini et al., [25] using PSCL, its whole mean score was observed to be higher in the thalassemia group in comparison with the controls. In contrary, our results from the PSCL revealed no significant difference between mean scores of β-thalassemia cases and controls except for the score of attention that was significantly higher in cases in comparison with controls ($p = 0.000$). In accordance, an Indonesian study in 2017 investigated attention and executive function in β-thalassemic patients, attention impairment was found in 26% of their sample children [26]. Attention is a primary cognitive function critical for perception, language, and memory, the mechanism of attention and executive impairment in beta-thalassemia children was thought to be the results of chronic hypoxia, which is known to be related chronic anaemia conditions [27]. On the contrary, previous researches indicated that β-thalassemic cases had more internalising and externalising problems as compared to healthy children [5], [28].

The results of our study found only 10% of β-thalassemic patients with internalising behaviours. In accordance, Di Palma et al., [29] explored the effect of β-thalassemia major on the psychosocial adjustment of adolescents; their data confirmed that teens and youth with thalassemia have psychosocial development problems in comparison to the same aged healthy controls. It was suggested that three main factors might play a beneficial role in the psychosocial adjustment of β-thalassemic patients. Firstly, a positive role could be achieved by the improvement in medical treatment. Secondly, the level of understanding of the problems of thalassemia in the general population is good, and this makes it better for subjects with this disease not be treated as abnormal. Thirdly, the optimistic attitude of the medical staff and their continuous and good relationship with the patients and their parents. These factors could have made the acceptance of the disease and the psychosocial adjustment of the cases and their families easier [29]. Similar to our data, Conatan et al., [30] reported that school problems were common in patients with thalassemia because of frequent hospitalisation, school absenteeism, and disease complications.

Psychological and social wellbeing is interrelated with competence and adaptation, thus improving positive mental health among children with a chronic disease means promoting adaptation in living with that illness. Several strategies are considered in improving mental health and adaptation including, encouraging ordinary life activities, increasing coping skills and encouraging use of social and family reinforcement [8].

In conclusion, in this study patient with β-thalassemia had a relatively mild affection of adaptive and psychosocial functioning, that can be explained by the strong effect of social and medical support they receive, which may increase their competence and psychological wellbeing.

So, the study empathises the need to

encourage psychosocial strategies in managing chronic illnesses.

Acknowledgements

The authors thank all the candidates who participated in the study and their parents.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Dec 20; 6(12):2342-2347.
https://doi.org/10.3889/oamjms.2018.451
eISSN: 1857-9655
Clinical Science

A Cross-Sectional Study Examining the Correlation between Nocturnal Melatonin Level and Sleep Quality in Patients Admitted To the Cardiac Care Unit

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Citation: Kamkar MZ, Rezvani Khorshidi S, Alinaghi Maddah SM, Emami Zeydi A, Modanloo M. A Cross-Sectional Study Examining the Correlation between Nocturnal Melatonin Level and Sleep Quality in Patients Admitted To the Cardiac Care Unit. Open Access Maced J Med Sci. 2018 Dec 20; 6(12):2342-2347. https://doi.org/10.3889/oamjms.2018.451

Keywords: Sleep Quality; Melatonin; Light; Cardiac Care Unit

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Received: 28-Sep-2018; **Revised:** 09-Nov-2018; **Accepted:** 10-Nov-2018; **Online first:** 17-Dec-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Quality of sleep, as a basic need, is an important factor for surviving patients in hospitals. Many factors may contribute to disturbing patients sleep, such as continuous ambient light, is required for healthcare providers to monitor patients. Ambient light can influence patients' quality of sleep due to melatonin secretion.

AIM: Study aimed to determine the correlation between nocturnal melatonin levels and sleep quality in patients admitted to the Cardiac Care Units (CCU).

MATERIAL AND METHODS: This cross-sectional study was done on inpatients of CCUs at Amir-Almomenin Hospital in Kordkoy city, a cardiac referral hospital in the northeastern of Iran in 2015. Sixty-eight inpatients were selected through convenience sampling. Before data gathering light level of CCUs was measured every one hour in 2 days, the quality of nocturnal sleep was investigated through Verran and Snyder-Halpern (VSH) Sleep Scale at the second night of admission urinary melatonin level was measured at the same night in all urine excreted between 22:00 pm and 07:00 am.

RESULTS: The mean and standard deviation (SD) score of sleep quality in three dimensions of sleep disturbance, sleep effectiveness and sleep supplementation were 336.6 ± 149.9, 269.0 ± 82.2, and 175.2 ± 30.7, respectively. Also, the mean and SD of nocturnal urinary melatonin levels was 323.02 ± 136.21 pg/ml. There was not a significant correlation between level of nocturnal melatonin and three domains of sleep quality; sleep disturbance ($r = 0.005$, $P = 0.968$), sleep effectiveness ($r = 0.090$, $P = 0.464$), and sleep supplementation ($r = -0.037$, $P = 0.763$).

CONCLUSION: According to the result, most CCUs patients suffer from sleep disturbance. However, there was no correlation between the level of melatonin and sleep quality. There is a need for recognising the reasons for sleep disturbances in Cardiac Care Units. It is imperative for care providers to be able to recognise the causes of sleep disturbances and to modify environmental factors such as ambient light to improve sleep quality in hospitalised patients.

Introduction

Coronary Artery Disease (CAD) is the most common type of cardiovascular disease which frequently causes hospital readmission [1]. It is a major cause of death and disability in developed countries [2]. Cardiovascular diseases cause nearly one-third of all deaths worldwide [3]. According to the Centers for Disease Control and Prevention (CDC), more than 370,000 people die from CAD each year in the United States. CAD has an impact on 110 million people and as a consequence 8.9 million deaths globally [4]. It is estimated that CAD will be the most common cause of deaths up to 2020 [5]. The incidence of Cardiovascular diseases is increasing in the developing country. According to the Ministry of

Health and Medical Education report, the number of CAD is increasing in Iran which is affected 15 million people and accounts for approximately 46% of deaths in patients over 35 years old. It is the first leading cause of mortality [2], [6], [7]. In some situation, patients required to be admitted in Cardiac Care Units (CCUs) to prevent complication which leads to improving quality of life. When patients stay in the hospital, some patient-related factors; such as pain, anxiety, and primary illness and environment factors; such as continuous ambient light exposure, equipment noise, alarm, and beepers may cause sleep disruption. Also, physician intervention and nursing care; including checking vital signs, diagnostic testing and other procedures, are the leading cause of disturbed sleep pattern of patients [5], [8], [9]. Although the research findings indicate that quality of sleep is a basic human need and one of the important factors for surviving patients, evidence shows that they suffer from sleep disturbance [10] [11]. More than fifty percent of hospitalized patients experience sleep disturbance during the early days of admission [12]. Sleep disturbances may increase epinephrine and norepinephrine secretion and considering that heart rate, respiratory rate, blood pressure and myocardial oxygen demand will be increased. As a result, heart ischemic would be expanded independently [13], therefore, sleep disruptions is associated with the symptoms of the cardiovascular disease and rate of mortality [14].

Research findings indicate that multiple factors may contribute to disturbing the sleep of patients in the hospital, and continuous ambient light is significant contributors to sleep disruption [11], [14], [15], [16]. Given the fact that continuous ambient light is required for health care providers to monitor patients, light can influence on patients' quality of sleep and circadian rhythm due to nocturnal secretion of melatonin [14], [15], [17].

Melatonin (N-Acetyl-5-methoxytryptamine) secretion as crucial biomarker follows a stable circadian rhythm in healthy individuals [15]. Research findings indicate that light-induced neural and endocrine signals that regulate behavioural and physiological circadian rhythms associated with melatonin secretion of patients in intensive care units, which is often accompanied by sleep disturbance [18]. Thereby, light can disturb circadian rhythm and suppress melatonin release, in consequence, sleep deprivation will be occurred and hinder the progress of patients' treatment and recovery. As a result, it can make more problems which increase the cost of health care services [19].

Serum concentrations of melatonin vary from 80 to 120 pg/ml. About 80 per cent of the melatonin is produced at night. The lowest level of melatonin is 10-20 pg/ml in daylight hours [14]. In healthy individuals, melatonin as a pivotal biomarker acts by a stable circadian rhythm. Findings of the study demonstrate that light-induced–melatonin secretion of patients in critical care units associated with sleep problems [17], [18]. Thereby, light can disturb circadian rhythm and suppress melatonin release, in consequence, sleep deprivation will be occurred and hinder the progress of patients' treatment and recovery. As a result, it can increase the cost of health care services [19]. Research finds, which assessed the pattern of melatonin secretion and its relation with sleep impairment in different groups of patients, has been discussed. Also, there is a controversy about the correlation between severity of disease and the amount of melatonin secretion [16], [17], [19].

By determining the affecting factors on melatonin secretion such as continuous light, the health care providers can do suitable interventions to improve patients' quality of sleep. This study was done to determine the correlation between nocturnal melatonin levels and sleep quality in patients admitted to the CCUs.

Methods

This cross-sectional study was conducted at CCUs of Amiralmomenin Hospital, a cardiac referral hospital in the northeastern of Iran, in 2015. Sixty-eight eligible patients with CAD were selected via convenience sampling which was admitted to the CCU, a day before the study. Inclusion criteria were age more than 18 years old, ability to complete the sleep assessment questionnaires, the absence of psychiatric disorders or illness that led to sleep loss or disruption during the last two months and non-attendance to work in a night shift with daytime sleeping.

For data gathering first, 2 days, a day of weekday and a day of the weekend, was chosen randomly. Then light levels were measured every hour of 2 days. Light levels were recorded in 25 patient's bedsides one meter high (next to the head of patients) and 2 nursing stations, using lux-meter TES-1339 made in Taiwan.

The consenting patients were given written informed consent. They were assured about the confidentiality of private information and they're volunteers for participation. Potential participants were asked fulfilling the questionnaire to capture information pertaining to patient demographics, pre-admission sleep pattern and sleep quality. On the second night of hospitalization in CCU, patients completed a checklist to record pre-admission sleep characteristics, and Verran and Snyder-Halpern (VSH) Sleep Scale. The level of nocturnal urinary melatonin (MT) was measured for the same night. Pre-admission sleep characteristics were assessed through a checklist which was developed to monitor sleep pattern and factors affecting sleep, and to

identify the causes of sleep disturbance in patients prior to hospitalization [20]. The VSH sleep scale was developed in order to assess the subjective sleep quality of hospitalized individuals – those without preexisting sleep difficulties. The 15-item VSH scale evaluates three main sleep domains; disturbance (including sleep latency and fragmentation), effectiveness (including sleep quality and length), and supplementation which are scored 0-700, 0-500, and 0-400 respectively. The validity and reliability of the Persian version of VSH sleep scale was evaluated by Mashayekhi [21]. To measure nocturnal urinary melatonin, urine was collected through the indwelling urine catheter for a total period of 9 hours starting at 22 in the night (from 10 p.m to 7 a.m in next day). Samples of 5 mL of urine were obtained from urine portion and transferring to a laboratory for analyzing. 6-hydroxymelatonin, as a metabolite of melatonin (N-Acetyl-5-methoxytryptamine), was excreted in urine. It was determined from urine samples by Enzyme-Linked Immunosorbent Assay (ELISA) using EASTBIOPHARM ELISA kit made in USA [22].

Data were analysed using Statistical Package for the Social Sciences (SPSS; version 16). Descriptive statistics were used to describe patients' demographic and to analyse the frequencies of pre-admission sleep characteristics. Normality of scores was assessed by Shapiro-Wilk and distribution was not normal; therefore, data were analysed using Kruskal Wallis test. Spearman correlation coefficients were calculated to assess the correlation between melatonin level and Quality of sleep. Significant level was considered 0.05.

Results

The findings showed that the majority of the patients were male (51.5%); the mean age was 56.2 ± 8.5 years (range from 25 to 65 years). Most of the patients had a diagnosis of Acute Coronary Syndrome (66.2%) and had a history of hospitalisation in Cardiac Care Units (51.5%). Fifty per cent of the patients were illiterate, and only 5.9% of them graduated from university, and 23.5% of them reported that they had no comorbid disease. An overview of the demographic characteristics of patients is given in Table 1.

Table 1: Socio-demographic characteristics of the patients (n = 68)

Variables		N	%
Gender	Male	35	51.5
	Female	33	48.5
History of hospitalisation	Yes	35	51.5
	No	33	48.5
Patients' diagnosis	ACS*	52	76.5
	UA**	16	23.5
History of comorbidity	Yes	52	76.5
	No	16	23.5

* Acute coronary syndrome; ** Unstable angina

The mean of light levels in a day of weekday and weekend was 244.4 and 261 Lux, respectively and in a night of weekday and weekend were 104.1 and 130 Lux, respectively (Table 2).

Table 2: The mean of light levels in a weekday and weekend in CCUs

Time of evaluation	Light Level (Lux)	
	Night	Day
Weekend	104.1	244.4
Weekday	130	261
Standard	100	300

The results revealed 45.6% of patients had well-ordered sleep pattern before admission. Majority of them reported that light and noise effect on their sleep during the night, only 1.5 per cent of patients reported that light and noise never impact on their nocturnal sleep and 86.8% of them were awakening for going to the toilet more than once in a night. Only 2.9% of patients pointed out that they woke up to take the drug. Most patients (94.1%) had less than 2 hours of daily sleep (Table 3).

Table 3: Frequency distributions of the patients' sleep pattern and sleep affecting factors

Items		N	%
Sleep pattern	Well ordered	31	45.6
	Partly disordered	18	26.5
	Disorderly	19	27.9
The effect of light on sleep	Never	1	1.5
	Rarely	14	20.6
	Sometime	34	50
	Always	19	27.9
The impact of noise on nocturnal sleep	Never	1	1.5
	Rarely	17	25
	Sometime	31	45.6
	Always	19	27.9
Waking up to take the drug	Never	52	76.5
	Rarely	14	20.6
	Mostly	2	2.9
To take a nap during the day	Never	13	19.1
	Once in a day	50	73.5
	Twice or more in a day	5	7.4
Duration of daily sleep	2 hours or less	64	94.1
	More than 2 hours	4	5.9
Awakening for going to the toilet	Never	9	13.2
	Once in a night	31	45.6
	Twice or more in a night	28	41.2

According to finding, the mean score of sleep disturbance was 336.6 ± 149.9; sleep effectiveness was 269.0 ± 82.2, sleep supplementation was 175.2 ± 30.7, and mean nocturnal urinary melatonin levels was 323.02 ± 136.21 pg/ml (Table 4).

Table 4: The means of patients' melatonin level and scores of sleep quality (n = 68)

Domains of sleep quality scale	Mean	Standard deviation
Sleep disturbance	336.6	149.9
Fragmentation	244.4	108.5
Latency	92.2	43.4
Sleep effectiveness	269.4	82.2
Quality	13.3	62.4
Length	13.7	27.2
Sleep supplementation	175.2	30.7
Melatonin level (pg/ml)	323.02	136.21

Results of the correlation between nocturnal melatonin level and sleep measurements showed there was not a significant correlation between the level of nocturnal melatonin and three domains of sleep quality($P > 0.464$) (Table 5).

Table 5: The correlation of sleep quality domains with nocturnal melatonin levels (n = 68)

	Supplementation Sleep	Sleep Effectiveness	Sleep Disturbance
R	0.005	0.090	-0.037
P-value	0.968	0.464	0.763

Discussion

The low mean score of sleep in domains of sleep disturbance, supplementary sleep and sleep efficiency indicates the low quality of sleep in the patients. Findings of several studies have shown that inpatients do not have sufficient sleep quantity and quality. According to the finding study that carried out by Redekeret et al., during the first five post-operative days after open heart surgery, although no significant change was observed in the duration of sleep, the quality of sleep reduced due to repeated sleep interruptions during the first three days [23]. Research finding on patients with heart disease showed that 51% of the patients had difficulty falling asleep, 44% had trouble sleeping, 40% had difficulty in the last phases of sleep, and 39% were waking up too early [24]. The findings are consistent with the results of several studies conducted on the quality of sleep in ICU patients over the past three decades [25], [26]. Study of Mashayekhi et al. on CCU patients demonstrated the short nighttime sleep-duration of the patients and the poor quality of sleep regarding efficiency and disturbance [27]. In intervention studies on the quality of sleep in ICU patients, the subject had poor sleep quality before the intervention, and controlling some environmental factors could improve the quality of sleep in the patients [2], [15], [28]. Findings of the sleep pattern showed that 27.9% of the patients had sleep problems before hospitalisation, and the majority of them reported noises, light and having to go to the bathroom as the main causes of sleep problem. A review study showed that noise, pain, discomfort, the severity of disease, nursing and medical interventions, and medications are the factors disrupting the sleep pattern of patients [11]. Parker reported that sleep problems are more prevalent in heart failure patients than in those without the disease, and factors such as respiratory problems, age, medications, anxiety and depression play a significant role in this regard [29].

In this study, the majority of patients pointed out that noise and light influenced their sleep. Freeman investigated the effect of environmental factors on sleep disorders in patients undergoing ventilation in ICUs. Findings of 24- and 48-hour polysomnography indicated that sleep-wake cycles of patients were disrupted, and the maximum amount of sleep was between 6:00 and 22:00, which is normally awake time [30]. This could be attributed to environmental factors such as continuous night light. The findings of this study showed that the light intensity in ICUs was less than the standard limit during the daytime and higher than the standard limit at nighttime. Consistent with these findings, the study of Golmohammadi reported that the average intensity of artificial, general and local illumination during daytime was less than the recommended limit in hospitals of Hamadan [31]. Hu measured the light intensity in an ICU every hour for 24 hours under laboratory conditions. Although the average nightlight was 100 lux (near the standard), the light intensity varied from 5 to 2238 lux at different times of the day [15], [32]. Some studies have demonstrated the positive effect of increased light exposure on ICU patients, while other studies did not find a relationship between exposure to light and clinical outcomes in patients [33]. According to some studies, using eye masks overnight for prevention of overexposure to light improves the quality of sleep in patients [15], [26], [34]. Given that the standard light level is 300 lux during the day and 100 lux during the night, these contradictory results may be because none of these studies has considered the difference in the level of light during day and night.

Melatonin is secreted earlier at night when people are exposed to light in the morning. Low light levels throughout the day can delay the secretion of melatonin at night, while exposure to bright light in the evening can inhibit production of melatonin at night [35]. Therefore, the amount of light in ICUs can affect the secretion of melatonin in patients. In this study, the mean level of melatonin at night was 323.22 pg/ml during the second night of admission. However, there was no statistically significant relationship between melatonin level and patients' quality of sleep. Most interventional studies have investigated the effects of light exposure on patients' quality of sleep and melatonin, the relationship between melatonin levels and quality of sleep was not evaluated, while the amount of light is associated with melatonin level, circadian rhythm and sleep patterns. Melatonin affects the tendency to fall asleep and the duration and quality of sleep [35]. According to the findings of Bourne et al. consumption of melatonin increased the sleep time of patients admitted to the ICUs by one hour during nighttime [28]. Kakooei et al. monitored the level of melatonin in nurses for 24 hours (every three hours). They reported that the minimum and maximum 24-hour melatonin levels were 14.91 pg/ml at 4 a.m and 139.41 pg/ml at 4 p.m. In nurses with constant day shift, these values were 1.02 and 177.40 pg/ml, respectively. This indicates the association between melatonin and nighttime sleep [36]. Study of Shamir et al. measured the urinary melatonin levels in 19 patients with schizophrenia every 3 hours. Their findings indicated that the level of urinary melatonin was lower in patients with sleep problems [37], which is inconsistent with the findings of the present study. This difference between the results could be because the mentioned study measured melatonin levels at different times of a day, while we measured the mean

level of melatonin at nighttime. Patients in the CCU have disease-related problems such as pain, respiratory problems and side effects of drugs that can disrupt their sleep pattern. Also, the subjects in the mentioned study were over 50 years of age and often had sleep problems [38].

The limitation of this study was using a questionnaire to measure the quality of sleep since we were unable to use the standard objective method of polysomnography for measuring the quantity and quality of sleep due to the difficulty for use on CCU patients. Also, since half of the subjects in the study were illiterate, they might have completed the questionnaire inaccurately. It is recommended to use both subjective and objective methods in future studies.

According to the result, most CCUs patients suffer from sleep disturbance. However, there was no correlation between the level of melatonin and sleep quality. There is a need for recognising the reasons for sleep disturbances in Cardiac Care Units. It is imperative for health care providers to be able to recognize the causes of sleep disturbances and to modify environmental factors such as ambient light to improve sleep quality in hospitalised patients.

Acknowledgements

This article was extracted from an MSc thesis of Critical Care Nursing in Golestan University of Medical Sciences. The study has been approved by the Ethics Committee of the Deputy of Research at Golestan University of Medical Sciences, Iran (project no. 394592122510). The authors wish to thank all patients for their kind cooperation. We extend special thanks to the nursing staff of Amiralmomenin Hospital for their efforts in data collection.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Dec 20; 6(12):2348-2353.
https://doi.org/10.3889/oamjms.2018.442
eISSN: 1857-9655
Clinical Science

The Role of Magnetic Resonance Spectroscopy in Evaluating the Rate of Brain Metabolic Variations in Chemical Veterans with Respiratory Problem In Comparison To Control Group

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Citation: Mahdawy SA, Shekarchi B, Zaman M. The Role of Magnetic Resonance Spectroscopy in Evaluating the Rate of Brain Metabolic Variations in Chemical Veterans with Respiratory Problem In Comparison To Control Group. Open Access Maced J Med Sci. 2018 Dec 20; 6(12):2348-2353. https://doi.org/10.3889/oamjms.2018.442

Keywords: MR Spectroscopy; Creatinine; N-acetylaspartate; Choline; Brain injuries of chemically injured veterans

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Received: 13-Oct-2018; **Revised:** 19-Nov-2018; **Accepted:** 20-Nov-2018; **Online first:** 16-Dec-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: During the eight years of the imposed war, Iraq used various chemical agents such as sulfur mustard and nerve agents (mainly tabun and sometimes soman) on Iran's soldiers. Using information obtained from specialist sequences and analysing information obtained from magnetic resonance imaging (MRI) in a susceptibility weighted imaging (SWI) sequence and magnetic resonance spectroscopy (MRS) provides valuable information on continuation of treatment and identifying functional disorders.

AIM: The objective of this research was to evaluate the rate of metabolic variations in chemically injured veterans based on chemical neuromarkers using the chemical sequence MRS, which would help patients and physicians in terms of time, economics, and selection of appropriate therapeutic methods, so if the can physician can get complete information about the metabolic properties of the brain through paraclinical (especially MRI) tools before treatment, he might change his treatment program to reduce the complications caused by it.

METHODOLOGY: In this research, 40 chemically injured veterans with brain dysfunction admitted to the screening centre for MRI with specialized MRS sequence participated. Accordingly, we examined the rate of brain metabolic variations about the level of neuromarkers and evaluated the relationship between the level of neuromarkers and brain damages.

RESULTS: The results of this research revealed that while the demographic characteristics such as age of the two groups of chemically injured veterans and control was similar, only the median of the NAA/Cr (N-acetylaspartate to creatine ratio) ratio in PONS of chemically injured patients was significantly lower than that of the control group, and this ratio was similar in other parts of the brain in two groups. The results also showed that the ratio of NAA to total choline and Cr was similar in all parts of the brain in two groups.

CONCLUSION: Based on the research results, using the MR (Magnetic Resonance) spectroscopy device and determination of the value and ratio of markers such as creatinine and N-acetylaspartate and choline, the brain injuries of chemically injured veterans can be examined. By conducting further studies and larger sample size, the brain damages in veterans can be diagnosed early, which would be a great contribution in their treatment.

Introduction

The First World War has been known as the birth of the modern chemical war. In this war, more than 480,000 tons of chemical agents were used and led to approximately one million injured people [1], [2]. After World War I, the largest and most extensive chemical attacks throughout history occurred in the Iran-Iraq war. During the eight years of this war, Iraq used various chemical agents against the Iranian forces on various fronts of the West and the South. Official reports (including the reports of UN experts) proved the using two important types of chemical agents, including sulfur mustard and the nerve agent (mainly Tabun and sometimes Soman) by Iraq in the war. In addition to these two agents, there are other chemical agents, including blood agents (cyanide, etc.), and choking agents (phosgene) and some other rare agents, but the use of these agents in the Iran-Iraq war was not proven [1], [2], [3], [4]. Mustard gas usually does not cause significant symptoms in a few minutes or even a few hours of its releasing, while the onset of poisoning symptoms are seen at initial moments when nerve agents are used (2 and 5).

Inhalation of mustard gas usually causes inflammation in the respiratory tract, and it's entering to larynx also causes a violent sound. Gradually, inflammation of the respiratory tract and airways is intensified (depending on the severity of the infection

and the duration of exposure to the gas) and coughing, shortness of breath, wheezing, and symptoms similar to pneumonitis are created. The next infection might worsen the pulmonary lesions. The initial mortality caused by mustard gas is usually due to lung injury and respiratory failure [6], [7]. Medical examinations on those injured by mustard gas in the First World War and studies conducted on those injured by mustard gas in the Iran-Iraq war show that this chemical agent has long-term complications, which some of them are not very important and easily treatable, and some others are more important, and it is necessary to obtain more information on complications of each of them in order to find proper methods to treat it. At present, there are effective methods to treat the pulmonary and brain problems of chemically injured victims. The important point is that no research has been conducted on brain dysfunction in these patients, and chemists use magnetic resonance to examine the structure of molecules with very accurate measurement of peaks in the spectrum and the area of each peak is equal to the relative number of protons in that place. In the chemical environment of the human body, the number of water and fat proton is several thousand times greater than the number of protons in other molecules, so that we cannot make a distinction between these metabolites.

Spectroscopy is a compound in a living environment, using gradients for selective stimulation of a small volume of tissue, then recording the FID (Free induction decay) and creating a spectrum of that voxel instead of creating an image. This technique has been problematic technically and yielded uncertain results for many years, but it has become very accurate and sensitive, and many people consider it an essential part of the brain MR test. Phosphor spectroscopy is another developed application of spectroscopy in the living environment, used mainly for muscle metabolism [8]. Magnetic resonance imaging is a method developing rapidly. High sensitivity and contrast in soft tissues and its inherent safety for patients due to the lack of using ionising radiation are the main advantages of this method over other methods of imaging. A powerful non-invasive tool for characterising spatial variations in metabolic profiles for patients with brain disorders is magnetic resonance spectroscopy (MRS). Metabolic parameters obtained using this technique has shown to predict treatment response, disease progression, and transformation to a more malignant phenotype. The availability of ultra-high-field MR systems has the potential to improve the characterisation of metabolites. In reality, magnetic resonance spectroscopy (MRS) is a magnetic resonance-based imaging modality that allows non-invasive sampling of metabolic changes in normal and abnormal brain parenchyma. MRS is particularly useful in the differentiation of developmental or non-neoplastic disorders from neoplastic processes. MRS is also useful during routine imaging follow-up after radiation treatment or during antiangiogenic treatment and for predicting outcomes and treatment response [9].

The objective of this research was to evaluate the rate of metabolic variations in chemically injured veterans based on chemical neuromarkers using the chemical sequence MRS, which would help patients and physicians in terms of time, economics, and selection of appropriate therapeutic methods, so if the can physician can get complete information about the metabolic properties of the brain through paraclinical (especially MRI) tools before treatment, he might change his treatment program to reduce the complications caused by it. No research has been conducted so far on the brain damages of chemically injured veterans and the evaluation of neuromarkers function, such as N-acetylaspartate, choline, creatinine, lactate, and the role of these markers in examining the cause of seizure, weakness, brain impairment, and other injuries. In fact, by using specialised MRI sequences, we aim to establish a significant relationship between the brain damage of chemically injured patients and the rate of variations in neuromarkers and contribute to the treatment of these patients.

Material and Methods

This research is a fundamental-analytical study conducted on chemically injured veterans whose respiratory problems were confirmed as well as asthmatic patients who are candidates for the continuation of the treatment with coordination of a specialist physician for MRI imaging using the MRS protocol. A total of 40 chemically injured veterans admitted to imaging centre of Arian Hospital in Tehran due to respiratory problems were selected as research sample. Research inclusion criteria included lack of contraindication for MRI and having the willingness to participate in a research project after study and signing the written consent. Moreover, 40 patients with asthma were selected as the control group, and they were matched with case group regarding age and gender. Patients' data including age, gender, and other demographic information were recorded before entering the study. Patients were controlled regarding lack of contraindications for MRI tests.

Contraindications for MRI tests were as follows:

1. Patients with a heart pacemaker.

2. Patients who have metallic objects in their eyes or have a cerebral aneurysm clipping, as the magnetic field may interfere with the metal.

3. Patients with severe claustrophobia which may not be able to tolerate an MRI scan.

4. Patients with metal devices in their spinal cord (such as bolts and nuts) can have an MRI scan, but the scan resolution is often impaired by the metal object, and the spinal cord is not well displayed.

The imaging was performed with the 1.5 GHz Tesla Device (Magnetom Model). The patient was placed in a supine position on the bed and the patient's head was placed in a 16-channel coil special for brain imaging and some of the routine sequences of the brain imaging, including TSE-T2w axial images with parameters of TR/T: 5600/98 msec, slice thickness: 5mm and FLAIR Axial with parameters of TR/T: 8500/103 msec, slice thickness: 5 mm. Then, the MRS images with single & multi-voxel sequence were performed according to five parameters: 230 * 182 * 107 TR/TE: 2000/144 msec, NSA: 128. The images were evaluated by one radiologist and one MRI imaging physicians. Then, the severity of the markers obtained through the software was analysed after plotting the charts. Then, the results were given to the clinician to determine the proper treatment for the patients.

After collecting data, information was extracted from the files and analysed using SPSS 25 software. Mean, median, standard deviation, minimum and maximum, and range was calculated. In this research, T-test was used to analyse the data and to examine the normal distribution of data, KS (Kolmogorov–Smirnov) test was used. For rank data or data with non-normal distribution, non-parametric tests such as Kruskal-Wallis were used. Charts were plotted by using GraphPad Prism software. In all cases, $p < 0.05$ was considered significant. The data are presented as mean ± SD.

Results

In this research, 40 chemically injured veterans with respiratory problems and 40 patients with asthma as control were evaluated. The mean age of them was 54.6 years, with a standard deviation of 8.6. Examining and comparing the mean age of the patient group and the control group showed that the mean age was not significantly different in the two groups ($p > 0.05$). Injury percentage of the case group had a mean of 51.3% with a standard deviation of 16.88%. These patients were injured between the years 1983 and 1987, and the highest prevalence of injury was as follows: lung (66.7%), lung with eyes and skin (20%) and lung and gastrointestinal tract (6.7%). The harmful chemical agents were unknown in these patients. They were mustard gas in 20%, mustard gas and nerve gas in 20%, and blood and choking agents in 6.7%. The data distribution about the NAA/Cr ratio in patients studied by KGV test was examined. Results show that the NAA/Cr ratio in the studied population does not follow the normal curve. Thus, to compare this ratio in two groups, non-parametric tests were used. Based on the results obtained, the median of NAA/Cr ratio in Pons in the two groups is significantly different. As shown in Fig. 1, the mean of this ratio in chemically injured patients is significantly lower than that in the control group (p-value = 0.03) (Fig. 1).

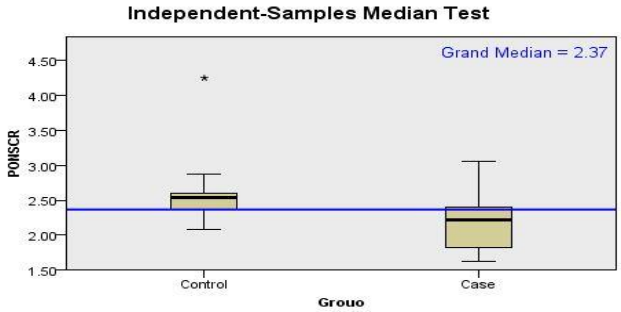

Figure 1: The median of Pons NAAR/CR ratio in two groups

Examining the distribution of NAA/CR ratio in basal ganglia of the left and right sides of brain as well as their mean of normal curve showed that this ratio does not follow the normal curve in the basal ganglia of the right side, but the curve function is normal in the left side of the brain and the mean of the two sides of the curve. Non-parametric test on the comparison of the median of NAA/CR ratio in the basal ganglia of the right side of the brain in both patient and control groups showed that the mean of this ratio was not significantly different in the two groups (p-value >0.99).

Also, the comparison of the mean NAA/CR ratio in basal ganglia in the two groups of patients and control by Students T-test showed that the mean of this ratio was not significantly different in two groups (p-value = 0.142). Finally, the comparison of the mean NAA/CR ratio of right and left brain ganglia in both groups of patients and control by Students T-test showed that the mean of this ratio was not significantly different in two groups (p-value = 0.336) (Fig. 2).

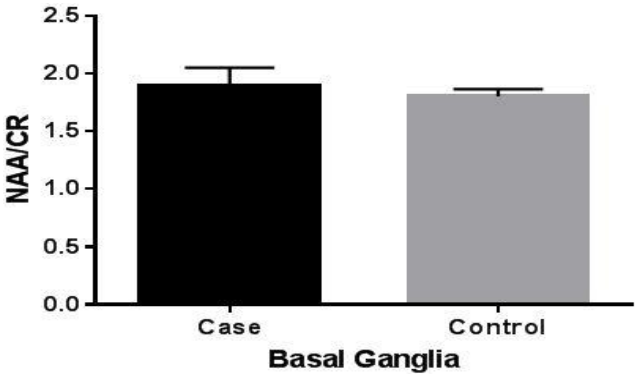

Figure 2: Mean and deviation from of NAA/CR ratio of basal ganglia of right and left sides of the brain in case and control groups

Comparison of mean NAA ratio to total Choline and Cr in the Pons region of the chemically injured patients and the control group by Students T-test showed that the mean of this ratio was not significantly different in the two groups (p-value = 0.117) (Fig. 3).

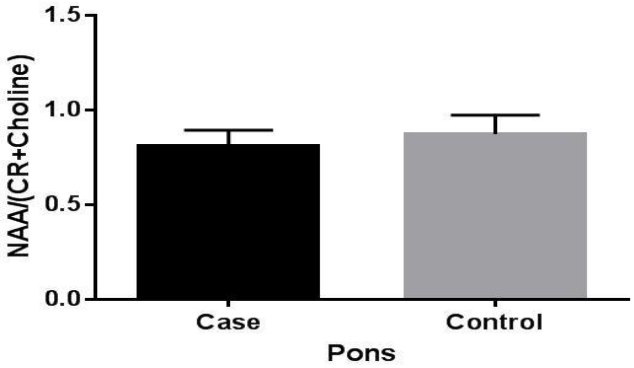

Figure 3: Mean and SD of the ratio of NAA to Total Choline and Cr in the Pons region of the case and control groups

The analysis of data related to ratio of NAA to Choline and Cr in the basal ganglia of left and right sides of brain and their mean in chemically injured and control groups showed that this ratio has a normal distribution in each of the two halves, but the ratio of mean of two halves is not normally distributed. Comparing the mean ratio of NAA to total choline and Cr in the basal ganglia of the left half of brain in the chemically injured and control groups by Students T-test showed that the mean of this ratio was not significantly different in the two groups (p-value = 0.702).

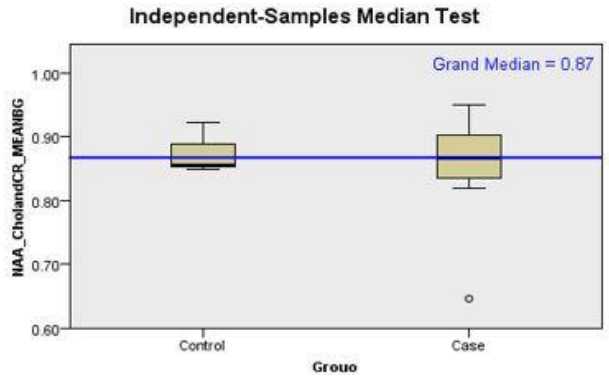

Figure 4: Median of mean ratio of NAA to total choline and Cr in the basal ganglia of left and right sides of the brain in the chemically injured group and control group

Comparing the mean ratio of NAA to total choline and Cr in the basal ganglia of the right side of the brain in the chemically injured patients and control group by Students T-test showed that the mean of this ratio was not significantly different in two groups (p-value = 0.753). Comparing the mean ratio of NAA to total choline and Cr in the basal ganglia of left and right sides of the brain in chemically injured patients group and control group by non-parametric test showed that the mean of this ratio did not differ significantly between two groups (p-value > 0.99). The results obtained from the comparison of the median of the ratio of NAA to total choline and Cr in the basal ganglia of left and right sides of the brain in chemically injured patients group and the control group by the non-parametric test are shown in (Fig. 4).

Discussion

Disorders are occurring in the human neurological system cause changes in the concentration of some of the metabolites, depending on their type and severity [10]. Studies have shown that brain cells, based on their type, have unique metabolites at least in vitro, which these patterns can be distinguished by using the MRS [11]. The MRS method is used to measure the level of various metabolites in tissues such as choline, creatinine, citrate and lactate. This method measures the level of various chemical agents in the tissues by showing waves with different peaks which each peak is related to a specific chemical agent [12]. By quantitative measuring of these chemicals in tissues, the level of cellular activity and the presence of malignant cells in the tissues can be detected. MRS has been used so far in diagnosing and assessing the diseases such as brain tumours, stroke, oxygen deficiency, epilepsy, and multiple sclerosis. However, there is little accurate and comprehensive research in this regard [13]. Previous studies, indicating the application of ratio of these metabolites in diagnosing of neurological diseases, have shown that masses of high-grade malignancy (Grade 3 anaplastic glioma and Grade 4 multiform glioblastoma), compared to low-grade masses, have higher choline and lower NAA. Increased Cho is related to proliferation and cell density. Sarin and mustard gases were used by the Iraqi during their war against Iran and even its Kurdish regions [14]. To examine these effects on the nerve system, new imaging techniques can be used. Effective methods have been identified in the treatment of pulmonary and brain problems of chemically injured veterans, and complementary research would reveal the outcome of these treatments.

The important point is that no research has been conducted on brain function disorders in these patients so far. The objective of this study was to evaluate the brain damages in chemically injured veterans using magnetic resonance imaging (MRI) and to evaluate the concentrations of choline, creatinine (Cr) and N-acetylaspartate (NAA). While estimating the pure concentration of choline is possible, it is sensitive to many of the errors, which their assuming is necessary. The ratios of NAA/cho

and cr/cho are used for very accurate examination. Hence, the ratios of N-acetylaspartate to creatinine and the ratio of N-acetylaspartate to total choline and creatinine were examined [15]. Measurement of brain biomarkers by MR spectroscopy shows that neuronal activity significantly decreased in the brain stem of war veterans compared to that of healthy control subjects. In the investigation conducted, the highest prevalence of injury was observed in the lung alone, lung along with eyes and skin, and lung along with the gastrointestinal tract, respectively. Measurement of NAA/Cr in MR spectroscopy showed a significant reduction in the veteran group compared to the control group in the brain stem.

In the measurement of NAA/Cr in the right and left basal ganglia with MR spectroscopy, results showed no significant reduction in this marker in the right and left basal ganglia. The other marker measured in this study was the ratio of NAA to total choline and Cr in Pons. Results showed no significant difference between the veterans group and the healthy control group. No significant reduction was seen in the ratio of NAA to total choline and Cr in the left and right basal ganglia in the control and case groups. In a study conducted on 34 thousand Iranians injured by mustard (13 to 20 years after exposure), 57.7% of them had pulmonary dysfunction, and 34%, 4.5% and 1% had weak, moderate and severe complications, respectively [16]. Studies conducted by Hollingworth et al., in 2006 showed that MRS can accurately distinguish between high glioma and low-grade glioma. However, the results of glioma grading using MRS are very extensive. This diversity can be due to different methods and metabolites. Among the different grades of the tumour, the Cr/Cho and NAA/Cho overlap is significant [11], [17].

In a study conducted by Robert et al., in 2000, Gulf war veterans were examined. In this study, 22 veterans of the Persian Gulf War were selected as case group, and 18 veterans who were matched with case group subjects regarding age and education level were selected as a control group. In this study, the Gulf War syndromes with the level of brain metabolites were examined. The Gulf War Syndrome was introduced in 1997 by Haley. It consists of three levels. In this study, the ratio of NAA to creatinine was examined. It showed neuronal mass activity. In this study, the level of N-acetylaspartate to creatinine, in contrast to our results, decreased significantly both in basal ganglia and brain stem in all three Gulf War syndromes [18]. However, in this study, the level of NAA / Cr decreased significantly only in the brainstem (and not in the basal ganglia) in chemically injured veterans with respiratory problems. However, this level in the right and left basal ganglia showed no significant difference with that of the control group. Physiological studies show that the respiratory centre located in the medulla spine plays a major role in the endogenous regulation of respiratory rhythm, chemo-regulation and mechano-regulation in an integrated way [19]. As a result, a significant reduction in the ratio of NAA/Cr in the brain stem of veterans with respiratory disorders suggests the important role of this region in central respiration regulation.

Based on the research results, the brain damages of chemically injured veterans can be examined by using MR Stereoscopy device and determining the value and ratio of markers such as creatinine and N-acetylaspartate and choline. Also, conducting further studies with larger sample size can be helpful in early diagnosis of brain damages in veterans, and it would be a great contribution in the treatment of these patients.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Dec 20; 6(12):2354-2358.
https://doi.org/10.3889/oamjms.2018.381
eISSN: 1857-9655
Clinical Science

Impact of Lower-Limb Endurance Training on Dyspnea and Lung Functions in Patients with COPD

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Citation: Tarigan AP, Pandia P, Mutiara E, Pradana A, Rhinsilva E, Efriyandi E. Impact of Lower-Limb Endurance Training on Dyspnea and Lung Functions in Patients with COPD. Open Access Maced J Med Sci. 2018 Dec 20; 6(12):2354-2358.
https://doi.org/10.3889/oamjms.2018.381

Keywords: COPD; Dyspnea; Pulmonary function; Lower-limb endurance training

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Received: 10-Oct-2018; **Revised:** 08-Nov-2018; **Accepted:** 09-Nov-2018; **Online first:** 17-Dec-2018

Funding: This study was funded by the Directorate General of Higher Education, Ministry of Education and Culture, Republic of Indonesia (No.70/UN5.2.3.1/PPM/KP-DRPM/2017)

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

Abstract

BACKGROUND: Patients with chronic obstructive pulmonary disease (COPD) exhibit persistent dyspnea in daily activities and irreversible airflow obstruction. These will finally lead to an inability to carry on daily activities and markedly decrease their quality of life. Endurance training was considered as therapy modality to alleviate several symptoms experienced by COPD patients.

AIM: This study aims to identify the impact of lower-limb exercise on dyspnea and spirometry test results in COPD patients.

METHODS: We performed a quasi-experimental study in July 2017 on 20 stable COPD patients divided both in group C and D according to GOLD 2017 criteria. Patients were given an individualised dose of stationary cycling twice a week for one month in which every session lasted 5-20 minutes gradually. Before and after rehabilitation program, pulmonary function tests were measured by spirometry to obtain per cent predicted of Forced Expiratory Volume in 1 second (FEV1), Forced Volume Capacity (FVC), Peak Expiratory Flow (PEF) and Forced Expiratory Flow at 25-75% of the pulmonary volume (FEF25-75), and dyspnea was measured by the mMRC index. Statistical analysis was performed by Wilcoxon and T-dependent test.

RESULTS: Baseline value of FVC (49.6 ± 21.6%) increased significantly to 59.65 ± 16.53% after one month of endurance training program ($p = 0.01$). Surprisingly, there was also a significant increase in FEV1 value from 46.9 ± 21.7 to 52.9 ± 20.7% ($p < 0.005$). The increase of FVC and FEV1 in group C was slightly higher than in group D although not statistically significant ($p = 0.29$; $p = 0.25$ respectively). However, no difference was observed in PEF and FEF25-75 value ($p > 0.05$). Patients' dyspnea scale also showed significant improvement ($p < 0.001$) from mMRC median scale 2 (range 1-3) to 1 (range 0-2) in both groups C and D. There was no exacerbation found during rehabilitation program.

CONCLUSION: Twice a week lower-limb endurance training for one-month improved dyspnea and pulmonary function test results in COPD patients safely and effectively.

Introduction

Chronic obstructive pulmonary disease (COPD) is a major cause of mortality worldwide. COPD is projected to be the 3rd leading cause of death by 2020. More than 3 million people died of COPD in 2012 accounting for 6% of all deaths globally. The COPD burden is projected to increase in coming decades because of continued exposure to COPD risk factors and ageing of the population [1].

Patients with COPD exhibit persistent dyspnea in daily activities and irreversible airflow obstruction. These will finally lead to an inability to carry on daily activities and markedly decrease their quality of life.

The mechanisms contributing to dyspnea must be approached in an integrative manner. Respiratory muscle function and its relationship to metabolic and cardiopulmonary variables during exercise identify some of the factors that limit exercise performance in patients with COPD. The identification of other factors that contribute to variability in dyspnea during exercise could result in improvement in a patient's exercise capacity [2]. In 2016, Hodonska et al., [3] found a decreased level of exercise tolerance in patients with severe COPD. The study also

concluded that exercise, assessed by the average of the 6 minutes walk distance (6MWD), could cause greater desaturation in patients with COPD compared with the healthy person [3].

Total daily activities in patients with COPD are largely related to legs activity which is reduced compared with controls of similar age [4]. Muscle dysfunction is especially relevant in COPD because it is related to important clinical outcomes such as mortality, quality of life and exercise intolerance, independently of lung function impairment. Thus, improving muscle function is considered an important therapeutic goal in COPD management [5].

Management strategies should not only be limited to pharmacologic approach but also complemented by appropriate non-pharmacologic interventions. Built around exercise training, the rehabilitation program is a multidisciplinary, evidence-based comprehensive approach to address the patient comprehensively, not only the pulmonary component of the disease [6]. A rehabilitation program is indicated to all patients with relevant symptoms and/or high risk for exacerbation. The rehabilitation program on COPD aims to reduce symptoms, increase exercise tolerance and improve the quality of life of patients with COPD. The rehabilitation program consists of 3 components; physical exercise, psychosocial and breathing exercises. Physical exercises including cardiovascular training and muscle training are the main pillars of pulmonary rehabilitation and are considered as the best strategies for improving exercise tolerance and muscle function in patients with COPD [5].

Studies of skeletal muscle function in COPD have demonstrated that upper limb muscles were less affected than lower limb muscles. Thus, the reduction in quadriceps strength averaged 30% when compared with healthy subjects [7]. Endurance training was considered as therapy modality to alleviate several symptoms experienced by patients with COPD [1].

This study aims to identify the impact of lower-limb exercise on dyspnea and spirometry test results in patients with COPD.

Methods

This was a quasi-experimental study in stable COPD patients from COPD daily clinic at Adam Malik General Hospital. The diagnosis of COPD was established from the history and physical examination and then confirmed by spirometry examination. We performed this study in July 2017 on 20 stable COPD patients divided both in group C and D according to GOLD 2017 criteria. The inclusion criteria were patients with smoking history, aged from 40-80 years old and had not been involved in any exercise program one-month prior intervention. Subjects were excluded if having exacerbation history within the last one month. Patients received optimal medical therapy and were clinically stable at the time they came to the rehabilitation program. Patients were given an individualised dose of stationary cycling, counted based on Metabolic Equivalents (METs) with the formula METs = VO_2 max/3.5. The VO_2 max was calculated using Formula Nury® specifically designed for Indonesian population by converting the 6-minute walking distance, heart rate, and anthropometry value 10 [8]. Twenty patients were referred for endurance training two times a week for four weeks. The subjects underwent lower limb exercise with stationary cycling in which every session lasted 5-20 minutes gradually increased. The heart rate was monitored, and there was a physiotherapist during exercise for safety reasons. No other form of training was provided during the study period. Chest physiotherapy was performed every time before exercising (infrared, stretching, clapping).

Before and after endurance training, pulmonary function tests were measured by spirometry (Vitalograph, Alpha Model, United Kingdom) to obtain percent predicted of Forced Expiratory Volume in 1 second (FEV1), Forced Volume Capacity (FVC), Peak Expiratory Flow (PEF) and Forced Expiratory Flow at 25-75% of the pulmonary volume (FEF25-75), and dyspnea was measured by mMRC index.

Statistical analysis was performed by Wilcoxon and dependent T-test. Ethics approval and informed consent were obtained.

Result

A total of 20 patients with COPD who met the criteria were involved in this study. The overall subjects of this study were men with a history of smoking (ex-smoker), as described in Table 1.

Table 1: Demographic Characteristics of Research Subjects

Characteristics		n	%
Age			
(years old)	40-49	1	5.0
	50-59	2	10.0
	60-69	12	60.0
	70-79	5	25.0
Occupation			
	Retired government officer	11	55.0
	Entrepreneur	8	40.0
	Farmer	1	5.0
Body Mass Index			
	Underweight	3	15.0
	Normoweight	6	30.0
	Overweight	1	5.0
	Obese	10	50.0
Brinkman Index			
	Mild	3	15.0
	Moderate	2	10.0
	Severe	15	75.0

The majority subject of this study was in the age range 60-69 years (60%). Most of the subjects (55%) were an ex-government officer. From body mass index, majority subject was obese (50%), and based on the level of cigarette consumption, it was found that 75% of subjects were patients with severe consumption of cigarettes. The characteristics of research subjects based on the degree of severity of COPD are listed in Table 2.

Table 2: The degree of severity of COPD

COPD characteristics		n	%
Group COPD			
	Group A	0	0
	Group B	0	0
	Group C	6	30.0
	Group D	14	70.0
GOLD severity			
	GOLD I	3	15.0
	GOLD II	3	15.0
	GOLD III	11	55.0
	GOLD IV	3	15.0
CAT Score			
	CAT < 10	5	25.0
	CAT ≥ 10	15	75.0
mMRC Score			
	mMRC 0 – 1	9	45.0
	mMRC ≥ 2	11	55.0
Comorbid			
	Comorbid	9	45.0
	Cardiovascular	9	45.0
	Endocrine	2	10.0

Characteristics of the subjects based on the severity of COPD group, the degree of expiratory airflow obstruction according to GOLD criteria, CAT questionnaire scores reflecting the impact of COPD disease on daily life, mMRC score reflecting the degree of breathlessness, comorbid disease and Brinkman index reflecting consumption level cigarette.

Of the 20 subjects, most of the subjects were within group D (70%). Based on the degree of airflow obstruction according to the forced-expiratory volume in one second (FEV1) obtained from the spirometry examination, we found that more than half of subjects (55%) were within GOLD III. While according to the CAT questionnaire, it was found that 15 patients (75%) had scored more than or equal to 10, reflecting the magnitude of the negative impact that COPD causes on the patient's daily activities.

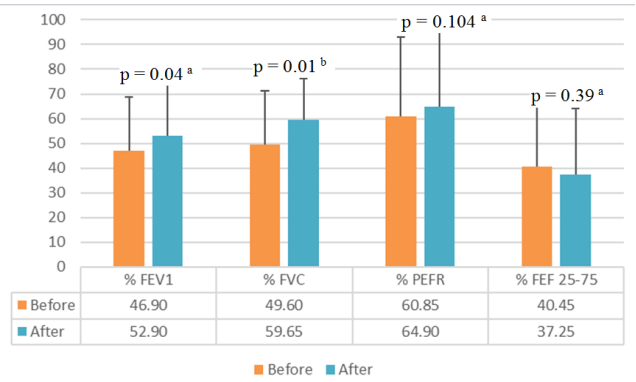

Figure 1: Impact of Lower Limb Endurance Training on Pulmonary Function Test; a) Wilcoxon test; b) Paired T-test

The baseline value of FVC was 49.6 ± 21.6%, and this number increased significantly to 59.65 ± 16.53% after one month of the endurance training program ($p = 0.01$). Surprisingly, there was also a significant increase in FEV1 value from 46.9 ± 21.7 to 52.9 ± 20.7% ($p < 0.005$). However, no difference was observed in PEF and FEF25-75 value ($p > 0.05$) (Figure 1).

Patients dyspnea index also showed significant improvement ($p < 0.001$) from mMRC median scale 2 (range 1-3) to 1 (range 0-2) in both group C and D (Figure 2). There was no exacerbation found during the rehabilitation program.

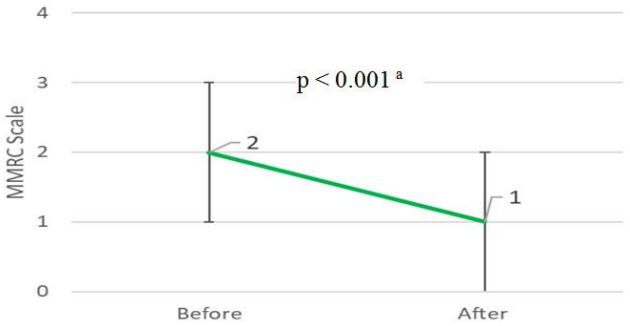

Figure 2: Impact of Lower Limb Endurance Training on mMRC Dyspnea Scale. a) Wilcoxon test

We also evaluated whether the degree of COPD severity affected the increase of pulmonary function test after the rehabilitation program. It was found that the increase of FVC and FEV1 in group C was slightly higher than in group D although not statistically significant ($p = 0.29$; $p = 0.25$ respectively) (Table 3.)

Table 3: Effect of COPD grouping on the increase of Pulmonary function test

Variable	Mean ± Standard Deviation		p-value
	COPD Group C	COPD Group D	
Delta FEV1	8.8 ± 10.9	4.8 ± 6.9	0.32 [a]
Delta FVC	4 ± 7.2	12.6 ± 18.5	0.28 [a]
Delta PEFR	7.8 ± 9.8	2.4 ± 17.8	0.49 [a]
Delta FEF25-75	4.5 ± 16.4	-6.5 ± 16.0	0.18 [a]

Independent T-test.

It was clear now that pulmonary rehabilitation could give some benefits for patients with COPD regardless of their disease severity. Thus, pulmonary rehabilitation can be concluded as a safe and effective program for patients with COPD.

Discussion

All subjects in this study were male. This provides an advantage regarding data analysis because it will be able to reduce the biases that may arise due to gender. This finding is similar to conditions in the field which states that men are the

largest number of patients with COPD. The high incidence of COPD in a male is related to the fact that the prevalence rate of smokers is 16 times higher in males (65.9%) than female (4.2%) [9]. Men are more active outdoor, so they will have a higher risk of environmental biomass exposure, such as pollution in the workplace and on the road.

The average age of patients with COPD who were the subject of this study was 64.8 ± 8.21 years. As many as 60% of patients are in the age range 60-69 years. The effects of age on the incidence of COPD can be explained through the concept of pulmonary function decline phenomenon. Pulmonary function will increase from birth and peak at 21 years of age.

Distribution of body mass index found that 50% of patients were classified into obesity. Better measurement for monitoring the nutritional status of patients with COPD is a fat-free mass that reflects muscle mass, rather than fat mass. Obesity in patients with COPD is also influenced by the effects of reduced mobilisation due to deconditioning effect [10].

Characteristics of patients based on the level of cigarette consumption indicate that 75% of patients have a severe Brinkman index. High cigarette consumption will induce TNFα release by alveolar macrophages followed by increased production of metalloproteinase matrices (MMP). This MMP will initiate the destruction of smokers' breathing descent. Thus, the greater the Brinkman index, reflecting the longer and more cigarette consumption levels, the greater the destructive effects that occur. However, not all smokers develop into clinical COPD. This is due to the involvement of genetic polymorphism factors in the pathogenesis of COPD.

The distribution of patients based on grouping system shows that 30% of patients belonging to group C, and 70% sufferers belonging to group D. There are no patients with COPD belonging to group A or B in this study. Spirometry procedure is more widely available in large hospitals, so patients with group A and B COPD in the population are often undiagnosed. In this study, only 15% of patients belonging to GOLD I. This is due to patients with a minimal reduction in FEV1 usually does not experience significant complaints, so they do not look for treatment. Also, there is often a misdiagnosis of the patient while they diagnosed with chronic bronchitis [12].

Pulmonary rehabilitation has been recommended to be a standard of management in patients with stable COPD. The American College of Sports Medicine recommends exercise for 20-60 minutes with the target heart rate is about 40% to 85% of the maximum rate. High-intensity whole-body exercise programmes, which suitable for improving the fitness in normal subjects, are often not tolerated by patients with COPD because of reduced ventilatory reserve and incapacitating breathlessness. Nevertheless, improvements in mobility have been reported following a variety of pulmonary rehabilitation programmes. One explanation of such improvements may be the effects of regular exercise in countering the "vicious cycle" of deconditioning [13], which contribute to exercise intolerance through leg fatigue for example. Endurance training in the form of cycling is the preferred modality for patients with COPD because it gives greater weight to the thigh muscles, and results in less oxygen desaturation [1].

The lower limb survival training program should be integrated with medical therapy as a comprehensive management modality and performed regularly for patients with stable COPD to achieve improved quality of life. Endurance training was proved to increase cardiac output in order to meet the increased oxygen demand in muscles. Muscles are trained to work under aerobic circumstances, and along with these, there will be increased ventilation [1]. The effects of a lower-limb endurance training program on variables are reflecting thoracoabdominal motion. The endurance training program influenced the thoracoabdominal motion positively leading to a significant decrease in asynchrony during exercise in patients assessed. In this study, endurance training used a static bicycle with the intensity based on the individual assessment by medical rehabilitation practitioner.

There is strong evidence that either dyspnea or leg effort limit exercise performance. Leg fatigue appears to be more important in those with less COPD. This is consistent that both respiratory and peripheral muscles play an important role in limiting muscular performance. In conditions of moderate intensity of submaximal exercise, when cardiac output is abnormally low and ventilatory work is high, the effect of respiratory muscle load on maximal exercise performance might be due to the associated reduction in leg blood flow which increases both leg effort and intensity. During prolonged submaximal exercise with a constant load, both the perceived effort of breathing and the perceived effort of exercising the skeletal muscles gradually increase with time, eventually reaching the subject’s tolerable limit.

The present study showed a significant increase in lung functions post exercise intervention. Patients with COPD often have an altered breathing pattern and experience shortness of breath, particularly when they act. This exercise changes the breathing pattern, improves muscle strength and endurance the respiratory muscles that contribute increase the ventilation and lung functions in patients with COPD.

Some of the limitations of this study include the number of sessions, and the duration of exercise is shorter when compared with other studies. The endurance training program conducted in this study was 2 sessions per week which lasted for 4 weeks. Longer sessions and duration of exercise are required for patients with COPD, 8-12 weeks and various forms

of exercise to assess the long-term effects of endurance training and to formulate training methods that deliver the best outcomes for patients with COPD, particularly those in COPD group D. However, this study emphasizes the value of pulmonary rehabilitation, thus further study with larger number of patients may be required.

In conclusion, twice week lower-limb endurance training for one-month improves dyspnea scale and pulmonary function test results of patients with COPD safely and effectively.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Dec 20; 6(12):2359-2362.
https://doi.org/10.3889/oamjms.2018.513
eISSN: 1857-9655
Clinical Science

Adverse Reactions to Intravenous Immunoglobulins - Our Experience

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Citation: Kareva L, Mironska K, Stavric K, Hasani A. Adverse Reactions to Intravenous Immunoglobulins-Our Experience. Open Access Maced J Med Sci. 2018 Dec 20; 6(12):2359-2362. https://doi.org/10.3889/oamjms.2018.513

Keywords: Intravenous immunoglobulins; adverse reactions

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Received: 09-Nov-2018; **Revised:** 04-Dec-2018; **Accepted:** 05-Dec-2018; **Online first:** 17-Dec-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Adverse reactions to intravenous immunoglobulins (IVIG) are divided by organ system involved, or by timing of onset–immediate which occur during infusion usually rate-related, true IgE-mediated anaphylaxis and delayed reaction which occur hours to days after the infusion.

AIM: To describe the adverse events of patients given IVIG infusions.

METHODS: Total number of patients receiving IVIG was 41 with 25 males (60.97%) and 16 females (39.02%), age 2 months-35 years. A total number of infusions was 1350.

RESULTS: Total number of adverse reactions 15, 14 patients with immediate-type and 1 with delayed type. Total percentage of adverse reactions in a given sample was 1.1% of all IVIG infusions. Fever was the most common immediate type of reaction occurring in 11 patients (78.57%) followed by acrocyanosis 10 patients (71.42%), skin rash 9 patients (64.28%) and headache 8 patients (57.14). Delayed-type of reactions (like fever, headache and vomiting) was present in one patient. Majority of the adverse effects occurred at the infusion rate higher than 1, 5 ml/kg/hour, which is still within recommended speed.

CONCLUSION: About 1.1% of IVG infusions where with adverse events. Most common manifestations where: fever, acrocyanosis, skin rash and headache, which occurred 1-6 hours from the beginning of the infusion. The occurrence of adverse reactions to IVIG was related to the infusion rates in a fashion that faster infusion rate gives more reactions. Adverse reactions were managed by reduction of the infusion rate and administration of medications such as paracetamol, antihistamines and steroids.

Introduction

Intravenous immunoglobulins (IVIG) are the preparation of highly purified IgG derived from large pools of human plasma via ethanol fractionation. Preparations are stabilised using substances such as human albumin, glycine, polyethene glycol, or sugars such as sucrose, maltose or glucose. Intravenous immunoglobulins are mainly used as replacement therapy for immunodeficiency, and immunomodulatory therapy in autoimmune and inflammatory conditions [1], [2], [3]. These preparations are manufactured by different companies and are at the disposal of the clinician. As a result of preparation processes reactions may occur to either the immunoglobulin, aggregates the preparation or the stabilising agent [5].

Possible adverse effects of IVIG may be divided by organ system or by timing of onset, i.e., immediate or delayed. Immediate reactions occur during the infusion, and include rate-related reactions, true immunoglobulin E (IgE)-mediated anaphylaxis (in immunoglobulin A (IgA)-deficient patients), and reactions related to concurrent infection [4], [7], [8], [9], [10], [11], [12]. Delayed reactions generally occur hours to days after the infusion [13], These can be a headache, aseptic meningitis, acute kidney injury, hemolysis, venous thrombosis, myocardial infarction,

transient ischemic attacks, and stroke. Some of these, particularly thrombotic events, may also occur during infusions [14]. These adverse events may be due to the relative "impurity" of the commercial preparations, or the undesirable effects of its active component immunoglobulin G (IgG). The most common adverse effects occur soon after infusions and can include a headache, flushing, chills, myalgia, wheezing, tachycardia, lower back pain, nausea, and hypotension [4], [7], [8], [9], [10], [11], [12]. Intravenous immunoglobulin is a biological product, and potentially important product-manufacturing differences may exist. It is perhaps for this reason that adverse effects appear to vary considerably among different IVIG preparations available in the market [5], [6].

This study aims to determine the incidence and type of adverse events following the infusion of different IVIG preparations. Understanding the common side effects and the IVIG preparations are important in caring for patients receiving IVIG.

Methods

This is a retrospective study. Records of patients given IVIG at the ward and the outpatient department from January 2006 to January 2016 at Immunology department of Pediatric Hospital-Skopje were reviewed. Demographic data such as age, sex, indication for IVIG treatment were taken from the charts of the patients. Total of 41 patients receives IVIG preparations, 25 males (60.97%), 16 females (39.02%), age 2 months -35 years, with a total number of infusions 1350. Indication for IVIG treatment and dosage where as follows: 18 patients with primary immunodeficiency 400 mg/kg/monthly, 5 patients with Kawasaki syndrome 400 mg/kg/5doses/daily, 10 patients with sepsis 400 mg/kg/dose/1-2 times divided by 2 days, 3 patients with atopic dermatitis and transient infantile hypogammaglobulinemia 400 mg/kg/monthly/6monts, 2 patients with transient infantile hypogammaglobulinemia and recurrent infections 400 mg/kg/monthly/6months and 5 patients with ITP 400 mg/kg/5doses/daily. The rate of IVIG infusions was 0.5 ml/kg/hour and increased every 15-30 min, based on the patient's tolerance up to 3 ml/kg/hour. IVIG preparation used was Kiovig- Baxter (glycin as a stabiliser) IgVena-Kedrion (sucrose as a stabiliser) Octogam-Octapharma (maltose as a stabiliser).

Once patients with adverse reactions have been identified, the following data were taken and reviewed: a specific type of adverse reaction to IVIG; the time interval between onset of adverse reaction and beginning of IVIG infusion and appearance of adverse reactions; IVIG preparation, dose and infusion rate; and medical management did during the adverse event. Statistics were used to analyse and interpret the data.

Results

A total number of patients receiving IVIG was 41 [25 males (60.97%), 16 females (39.02)] with age 2 months to 35 years. A total number of infusions was 1350. Total numbers of adverse reactions were 15, 14 patients with immediate-type and 1 with delayed type. Total percentage of adverse reactions in a given sample was 1.1% of all IVIG infusions. Immediate-type of reactions were: Fever was the most common immediate type of reaction occurring in 11 patients (78.57%) followed by acrocyanosis 10 (71.42%) and skin rash 9 (64.28%), followed by headache 8 (57.14%)shortness of breath 6 (42.85%), perioral cyanosis 6 (42.85), hypotension 5 (35.71) and chest pain 3 (21.42%), (Table 1).

Table1: Immediate adverse reactions to IVIG

Symptoms	Number of patients (%)
Fever	11 (78.57)
Acrocyanosis	10 (71.42)
Skin rash	9 (64.28)
A headache	8 (57.14)
Perioral cyanosis	6 (42.82)
Shortness of breath	6 (42.82)
Hypotension	5 (35.71)
Chest pain	3 (21.42)

Delayed types of reaction were a headache, fever and vomiting in one patient (6.6%), (Table 2).

Table 2: Delayed adverse reactions to IVIG

Symptoms	No of patients
Vomiting	1
Fever	1
A headache	1

Time of onset from the beginning of the infusion: 0-30 min 7 patients, 30 min to 1 hour 5 patients, 1 hour to 6 hours 2 patients, 24 hours 1 patient, (Table 3).

Table 3: IVIG infusions and time of onset of adverse reactions

Time from the beginning of the infusion	Frequency
0-30 min	7
30-60 min	5
60 min- 6hours	2
24 hours	1

Symptoms were affected by fast infusion rates, the faster the infusion rate, the more adverse reactions occur. In our study, most of the adverse effects occurred at the infusion rate higher than 1, 5 ml/kg/hour, which is still within the recommended speed. There was notable difference in frequency of adverse reactions depending on the brand of IVIG used for infusion (Figure 1).

Reactions were managed by reducing the

rate of infusion and giving of medications such as paracetamol, antihistamines and steroids.

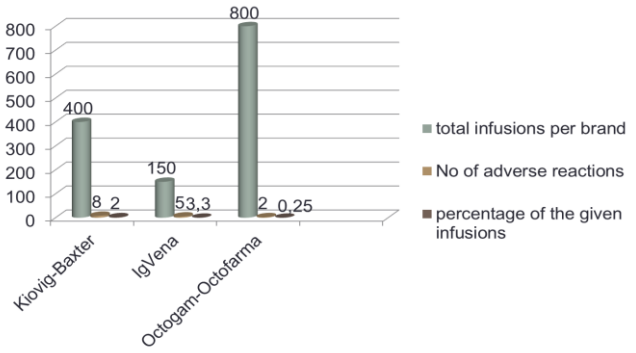

Figure 1: Adverse reactions of IVIG per brand

Discussion

The pathogenesis of adverse reactions of IVIG is still unknown. IVIG preparations differ in their composition and properties, and these can contribute to its efficacy and tolerability [5], [6]. Major determinants are immunoglobulins, particularly IgA content, sodium content, sugar content and osmolality [6]. The most common adverse reactions of IVIG in our study are the immediate type, occur in the first 30-60 min of administration. This includes fever, chills, headache, skin rash and even some vasomotor and cardiovascular manifestations marked by changes in blood pressure, tachycardia and cyanosis. These reactions are most common [4], [7], [8], [9], [10], [11], [12] and probably are results from aggregated immunoglobulin molecules which cause the complement system to be activated, antigen-antibody reactions, possible contaminants or even stabilizers that may have been used during the manufacturing process. Symptoms were affected by fast infusion rates, faster the infusion rate more adverse reactions occur. In our study, most of the adverse effects occurred at the infusion rate higher than 1, 5 ml/kg/hour, which is still within the recommended speed. Delayed types of reaction were a headache, fever and vomiting in one patient. This finding is not in correlation with the finding of other authors [13], where the majority of the reaction were delayed type.

Sugars such as sorbitol, maltose, sucrose and glucose are added to some IVIG preparations as stabilisers which will prevent aggregate formation. However, there seems to be an association between these sugars and the development of acute renal failure or renal insufficiency in treated patients [6]. Sugar-stabilised solutions, such as IgVena and Octagam tend to have higher osmolality compared to the sugar-free preparations like Kiovig which is glycine stabilised. The hyperosmolar solutions may cause fluid shifts when given intravenously, and this may result in hemodynamic changes leading to infusion-related adverse effects [6]. The sodium content of IVIG determines the osmolality of the infused solution, which in turn can affect tolerability and occurrence of adverse effects. Patients with hypertension and renal impairment may be affected by a high-sodium content solution. The IgA content of a solution is significant for patients with IgA deficiency. These patients are more likely to develop severe and sometimes fatal anaphylactic reactions [1]. The content of IgA varies among different IVIG preparations. However, using a preparation that is low in IgA does not guarantee an adverse reaction-free infusion. In our study, we were not able to determine reactions due to anti-IgA antibodies present in the patients because we don't have laboratory tests for anti-IgA antibodies.

These adverse reactions can be managed by slowing down the infusion rate and giving of medications such as antihistamines, paracetamol and corticosteroids, or switching the IVIG to different preparation.

In conclusion, the ability to tolerate the effects of IVIG infusion without experiencing adverse effects varies from one person to another and from one IVIG preparation to another. The rate of infusion influences the occurrence of adverse reactions, as well as osmolality, sugar and IgA content of a preparation. In our study, 1.1% of patients given IVIG infusions experienced adverse events, which is less than the percentage of the reactions described in the literature. We speculate that the low rate of infusion which is preferable at our clinic is the main reason for less occurrence of adverse reactions, as well as the type of the immunoglobulins we used. In our study, most of the adverse effects occurred at the higher infusion rate of the IVIG preparation, but still in the recommended speed from the manufacturer. IVIG preparations differ in their composition and properties, and these can contribute to its efficacy and tolerability with some types showing a greater rate of adverse reactions than the others. Fever was the most common manifestation, followed by skin rash and chills. Symptoms occurred within 1 to 6 h from onset of infusion, were affected by fast infusion rates, and managed by reducing the rate of infusion and giving of medications such as paracetamol, antihistamines and steroids.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Dec 20; 6(12):2363-2368.
https://doi.org/10.3889/oamjms.2018.491
eISSN: 1857-9655
Clinical Science

Ondansetron Is an Effective Alternative to Decrease the Incidence of Postspinal Hypotension in Healthy Subjects Undergoing Infra-Umbilical Surgeries Compared To Combined Volume Loading and Vasoconstrictors: Randomized Controlled Trial

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Citation: Mohamed SA, Hussam AM, Abdallah SA, Sarhan KA, Shaban AM. Ondansetron Is an Effective Alternative to Decrease the Incidence of Postspinal Hypotension in Healthy Subjects Undergoing Infra-Umbilical Surgeries Compared To Combined Volume Loading and Vasoconstrictors: Randomized Controlled Trial. Open Access Maced J Med Sci. 2018 Dec 20; 6(12):2363-2368.
https://doi.org/10.3889/oamjms.2018.491

Keywords: Fluid preload; Ephedrine; Ondansetron; Postspinal hypotension

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Received: 04-Oct-2018; **Revised:** 21-Nov-2018; **Accepted:** 22-Nov-2018; **Online first:** 19-Dec-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: One of the important and predicted physiological effects of spinal anaesthesia is hypotension. A range of strategies including mechanical interventions, intravenous fluids and vasoconstrictor drugs have been used to minimise or prevent spinal anaesthesia-induced hypotension. Observational studies suggest that ondansetron reduces the incidence of post-spinal hypotension (PSH) and support the use of combined fluid preloading and vasoconstrictors for this purpose (but with limited doses) to avoid side effects as fluid overload and tachycardia respectively.

AIM: As no RCT had ever compared the use of Ondansetron alone with combined vasoconstrictors and fluid preload, so, this randomised controlled trial has evaluated the efficacy of the use of ondansetron alone compared to the combined use of fluid preload and vasoconstrictors to decrease the incidence of spinal hypotension.

METHODS: Ninety patients of ASA grade I between the age of 18 and 45 years scheduled to undergo elective surgical procedures on the lower extremity or lower abdomen under spinal anaesthesia were included in the study. The patients were randomly allocated into two groups of 45 each. Group I patients (ondansetron group) received 4 mg ondansetron in 5 ml normal saline (IV) 15 minutes before induction of spinal anaesthesia. Group II patients (combination group) received preloading with 7.5 ml/kg/min of Ringer's lactate over 10 minute period preceding the spinal block followed by intravenous bolus of 2.5 mg ephedrine in the first and second minute and 2.5 mg ephedrine every 5 minutes for the next 20 minutes after the injection of spinal anesthetic drug. Non-invasive measurement of mean arterial pressures, heart rate, reactive hypertension, nausea and vomiting were documented.

RESULTS: The incidence of hypotension following the subarachnoid block in Group I (ondansetron group) was 17.6% versus group II (combination group) was 13.3%, while difference among the groups is statistically insignificant (P = 0.082). Group IV fluids alone could reverse hypotension in 57.1% of patients in group I 33.3% in group II. 42.9% of patients in group I and 67.7% in group II could not be managed with IV fluids alone and had to be treated with 5 mg boluses of ephedrine for reversal of hypotension. The difference in the mean number of fluid boluses and a dose of ephedrine used between both groups was statistically insignificant (P = 0.11 and P = 0.21). HR showed a significant increase in group II and a statistically insignificant change in group I with a statistically significant difference in the heart rate (HR) between both groups (P < 0.05). Reactive hypertension, nausea and vomiting between both groups were statistically insignificant.

CONCLUSION: The preemptive use of Ondansetron alone versus combined vasoconstrictors with fluid preload significantly reduces the incidence of post-spinal hypotension (PSH) with no significant difference between both regimens. Furthermore, they also reduced consumption of the used vasoconstrictors and fluids to correct hypotension.

Introduction

Spinal anaesthesia was introduced by the German surgeon Karl August Bier in 1898 [1]. Nowadays it is one of the most commonly used techniques for lower limb and lower abdominal procedures, including cesarean section. Unfortunately one of its important adverse effects is hypotension [2] which occurs mainly as result of sympathectomy resulting from the neuroaxial blockade [3] and venous pooling of blood in the legs, resulting in decreased

venous return and cardiac output [4]. The incidence of hypotension was reported to be 92% in the control group during cesarean section with spinal anaesthesia [5] while in the nonobstetric patient was 33% [6]. In high-risk patients such as the elderly and those with underlying organ dysfunction, even a mild decrease in blood pressure must be avoided [7].

To prevent postspinal hypotension, mechanical techniques, volume preloading and loading and vasopressor drugs have been tried in several studies with variable results [8]. Most studies are centered around the effects of preloading [9], [10], [11] or vasopressors [12], [13], [14]. However, a large volume of fluids would be dangerous in elderly patients [8] and parturients [15] whom risky for pulmonary oedema. Ephedrine has alpha and beta actions [8] and is used as a vasoconstrictor in the treatment of spinal hypotension. Ephedrine causes tachycardia and hypertension [16] and should be used cautiously in ischemic heart patients [12].

Ondansetron is a serotonin 5-HT3 receptor selective antagonist. Several studies revealed that it could prevent postspinal hypotension in pregnant and non-pregnant women [17], [18]. The mode of action is thought to be by prevention of the Bezold-Jarisch reflex (BJR). This reflex is cardiac inhibitory which produces a decrease in heart rate, blood pressure and cardiovascular collapse by type C fibres whose terminals lie in the heart [19]. Stimulating the peripheral serotonin receptors elicits the BJR [20].

As no one had ever compared the use of Ondansetron alone and combined vasoconstrictors with fluid preload, so, this randomised controlled trial evaluated in a single-blinded manner the efficacy of the use of ondansetron alone compared to the combined use of preloading and vasoconstrictors to decrease the incidence of spinal hypotension.

Methods

After approval of the ethical research committee, a prospective randomised single-blinded clinical trial was conducted in Kasr Alainy hospital theatres. Written informed consent was obtained from each patient. Ninety patients of the American Society of Anesthesiologists (ASA) grade I between the age of 18 and 45 years scheduled to undergo elective surgical procedures on the lower extremity or lower abdomen under spinal anaesthesia were included in the study. Patients with cardiovascular or respiratory disorders, abnormal cardiac anatomy, hypertension, pregnancy, diabetes, electrolyte imbalance, patients with hemoglobin concentration less than 10 gm%, weight more than 80 kg, height < 150 cm, fasting for less than 6 hours, those on medication which have direct cardiac effects such as beta blockers, coagulopathy, hypersensitivity to the used drugs and patients who take antidepressants in the form of serotonin antagonists were not included in the study. In the preparation room, history was taken from all patients with documentation of the age, weight, height, American Society of Anesthesiologists score (ASA) and preoperative laboratory investigations as complete blood picture, coagulation profile, liver and renal functions and an 18 gauge cannula was secured with the entryway. The patients were randomly allocated into two groups of 45 each. Group I patients (ondansetron group) received 4 mg ondansetron in 5 ml normal saline intravenously (IV) 15 minutes before induction of spinal anaesthesia. Group II patients (combination group) received (IV) preloading with 7.5 ml/kg/min of Ringer's lactate over 10 minute period preceding the spinal block followed by intravenous bolus of 2.5 mg ephedrine in the first and second minute and 2.5 mg ephedrine every 5 minutes for the next 20 minutes after the injection of spinal anesthetic drug.

Then, the patient was transferred to the operating room and baseline vital signs were recorded 10 minutes before conduction of anaesthesia including non-invasive measurement of mean arterial pressures, heart rate, electrocardiogram (ECG), oxygen saturation.

No premedication was given, and the subarachnoid puncture was performed using a 25 gauge spinal needle at L3-4 interspace with patients in sitting position, 2.5-3 ml of hyperbaric bupivacaine 0.5% injected intrathecally according to height (< or = 160 cm - > 160 cm) and the patients returned to the supine position. The level of loss to pinprick sensation was assessed, and surgery was started when sensory loss of T10 was achieved.

Supplemental oxygen 5 L/min was given through face mask, and an infusion of lactated Ringer's solution at the rate of 2 ml/kg/hr was administered during anaesthesia, and the rate was not altered during the study period. Subsequently, the recording was done at 5, 10, 15, 20, 25, and 30 minutes after the subarachnoid injection of the anaesthetic drug. However, minute to minute monitoring was done to assess any hemodynamic changes and institution of corrective therapy. Non-invasive blood pressure (NIBP) around the upper arm and brachial artery pressure were recorded in the form of mean arterial pressure (MAP). Hypotension was defined as a decrease of MAP more than 20% of the baseline or less than 70 mmHg. During an episode of hypotension, an additional bolus of 2 ml/kg of lactated Ringer’s solution was given. A maximum of three boluses was given. However, if supplementation of IV fluids failed to reverse hypotension, a bolus dose of ephedrine 5 mg was given intravenously then 2 ml/kg solution followed by 5 mg ephedrine are repeated if necessary. Pulse oximeter and ECG were used to record the oxygen saturation and heart rate respectively. The patients were monitored for any

reactive hypertension (MAP more than 20% of the baseline values), nausea and vomiting.

The sample size was calculated using the G-power software. Power analysis was done on the incidence of post-spinal hypotension (PSH) after spinal block as this is the primary outcome of our study. Previous studies reported an incidence of PSH in the nonobstetric patient as 33% [6]. The sample size was calculated to detect a 50% decrease in the incidence of PSH. Taking a study power of 80% and a P value less than 0.05 a minimum number of 45 patients were required for each group after exclusion of dropouts. Continuous data were presented as means (standard deviations) and medians (quartiles) and analysed using an unpaired t-test or Wilcoxon rank test as appropriate. Categorical data were presented as frequency (%) and analysed using Chi-square test. Repeated measures were analysed using two-way Analysis of variance (ANOVA). A p-value less than 0.05 were considered statistically significant.

Results

A total number of 145 patients were assessed for eligibility to be enrolled in the study while 52 were excluded as 44 didn't meet criteria and 8 declined to participate.the rest were randomized into group I with 92 patients and group II with 91 patients from group I and II, 3 patients were excluded due to failed spinal anaesthesia. Forty-five patients from each group received the allocated intervention and continued to be analysed with no further exclusions.

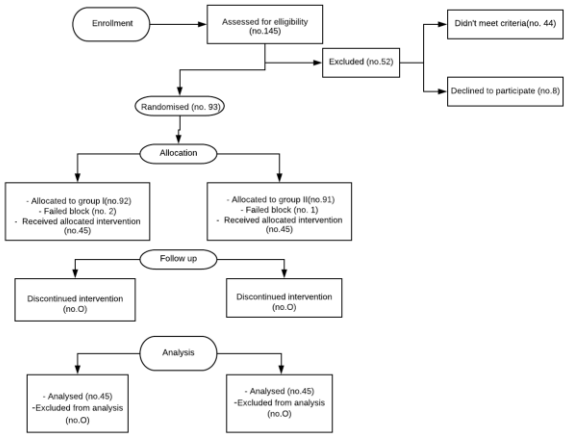

Figure 1: Flow chart for patient enrolment

The demographic data of the patients as shown in Table 1 showed that a number of 12 male and 18 female patients for group I with average age of 30.3 years, weight of 74.4 Kg and height of 171 cm while a number of 10 male and 20 female patients for group II with average age of 28.2 years, weight of 76 Kg and height of 173.6 cm are included in the study.

Table 1: Demographic data

	Group I (n = 45)	Group II (n = 45)
Age (yrs)	30.3 ± 10	28.2 ± 11.7
Sex (M/F)	12/18	10/20
Weight (kg)	74.4 ± 11.1	76 ± 7.8
Height (cm)	171± 9.3	173.6 ± 9.4

Values are mean ± SD.

Figure 2 shows that There was a significant decrease in mean arterial pressure (MAP) from baseline in group 1 at 10, 15, minutes while there was a significant fall in the MAP in group II from the baseline value at 15 and 20 minutes of the study with P < 0.05 while the rest of 30 minutes of the study MAP was insignificant in both groups to baseline value.

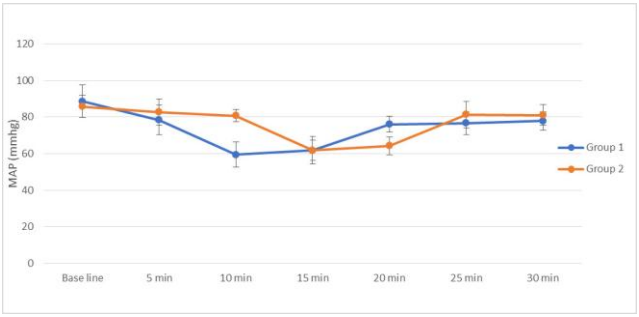

Figure 2: Mean arterial blood pressure. (Data are means, error bars are standard deviations. where the blue line is for the group I and a red line for group II)

Table 2 shows that seven patients in group I and six in group II had hypotension following the subarachnoid block and the difference among the groups is statistically insignificant. IV fluids alone could reverse hypotension in four patients in group I, two in group II. Three patients in group I and four patients in group II could not be managed with IV fluids alone and had to be treated with 5 mg boluses of ephedrine for reversal of hypotension. The difference in the mean number of fluid boluses and a dose of ephedrine used between both groups was statistically insignificant.

Table 2: Hypotension and its management

	Group I (n = 45)	Group II (n = 45)	p-value
No. of hypotensive patients	7 (15.6%)	6 (13.3)	0.082
(%) of patients managed by IV fluids bolus alone	4 (57.1%)	2 (33.3)	0.055
Mean No. of boluses of IV fluids	5	4	0.11
(%) of patients requiring bolus ephedrine	3 (42.9%)	4 (66.7)	0.14
Mean dose of bolus ephedrine	10	10	0.21

In Figure 3 the heart rate (HR) showed a significant increase in group II, throughout at 10 minutes till 30 minutes of the study and statistically insignificance change in group I and there was a statistically significant difference in HR between both groups with P < 0.05.

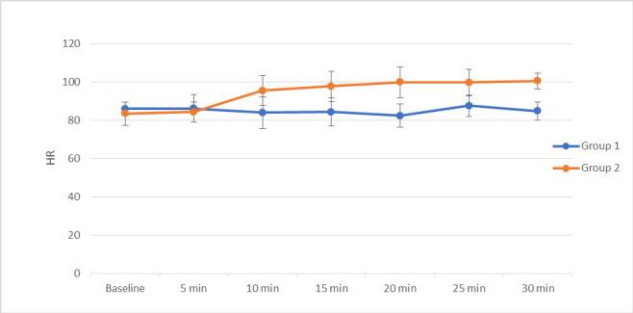

Figure 3: HR trends in both groups. (Data are means, error bars are standard deviations. where the blue line is for the group I and a red line for group II)

Table 3 showed that in group I, 2 patients had nausea while in group II, 3 patients had nausea but none had an episode of vomiting or reactive hypertension in either group and the difference among the groups is not statistically significant.

Table 3: Incidence of reactive hypertension, nausea and vomiting

	Group I (n = 45)	Group II (n = 45)	p-value
Hypertension	0	0	-
Nausea	2 (4.4%)	3 (6.6%)	0.075
Vomiting	0	0	-

Discussion

This randomised controlled trial (RCT) demonstrated that the preemptive use of both combined fluid preload and vasoconstrictors and use of Ondansetron alone significantly decreased the incidence of post-spinal hypotension (PSH) from 33% to 13.3% and 15.6 respectively. However no significant difference was shown between both regimens in reducing the incidence of PSH and also they reduced consumption of the used vasoconstrictors and fluids to correct hypotension while the difference in the mean number of fluid boluses and dose of ephedrine used between both groups was statistically insignificant which concludes that Ondansetron can be used as a sole agent in decreasing the incidence of post-spinal hypotension.

PSH is caused most probably due to reducing vascular tone and these results in decreases venous return and systemic vascular resistance [21]. Thus, measures used for prevention of PSH are directed to increase vascular tone and venous return which can be done by using vasoconstrictors, fluid administration, and positioning regimens [21], [22], [23], [24]. In many trials, fluid loading has been investigated to prevent PSH, but the results were not in its favour. With this in mind, investigators have turned their attention to vasoconstrictors protocols to prevent postspinal hypotension [25]. Conventionally, ephedrine was used as the first-choice agent to maintain blood pressure [26]. Its stimulating action on alpha and beta-adrenergic receptors causes positive inotropic and chronotropic effects on the heart [27].

Malhotra HB compared the use of Preload alone, vasoconstrictors alone and a combined preload and vasoconstrictor with half volume and dose used in the previous 2 groups to prevent PSH. They found that a combination of preload and vasoconstrictors had maximum effect in preventing spinal hypotension, followed by the sole use of vasoconstrictor, while preload alone had the least protection against postspinal hypotension [28].

A Lee et al. used Prophylactic ephedrine prevents hypotension during spinal anaesthesia for Cesarean delivery in 12 RCTs over 571 women where significantly fewer women experienced hypotension with ephedrine, compared with control [29].

Kang YG et al. used Prophylactic intravenous ephedrine infusion during spinal anaesthesia for cesarean section and found that In patients given the infusion, systolic blood pressure did not change significantly from the baseline systolic blood pressure following spinal anaesthesia ($p > 0.1$) [30].

The mechanism of ondansetron in preventing PSH was mediated by inhibition of Bezold-Jarisch reflex (BJR). This reflex is mediated through vagal afferents. When activated, it causes hypotension and bradycardia. Triggering of chemoreceptors sensitive to serotonin in the intracardiac wall can occur by a reduction in blood volume. It may lead to increased vagal nerve activity, followed by bradycardia and vasodilatation [19]. In the ondansetron group, several studies have tested its use for prophylaxis against Postspinal hypotension (PSH).

The current study results were consistent with those of Sahoo T et al., who studied the effect of ondansetron in patients undergoing lower segment cesarean section (LSCS) [18], Wang M et al., which compared different doses of ondansetron for prophylaxis against PSH. They compared placebo with 2, 4, 6 and 8 mg of ondansetron. They found that 4 mg of ondansetron was the best dose [31].

Similar to this study, Trabelsi W et al., who used a dose of 4 mg of ondansetron with 10 ml/kg of crystalloid versus placebo. They found that hypotension, bradycardia and vasopressor consumption was less to occur in those received prophylactic ondansetron [32]. The study results were consistent with those of Gao L et al., who compared the effects of prophylactic ondansetron on PSH in a meta-analysis and found that it reduced its incidence as well as vasopressor consumption in both obstetric and non-obstetric patients. Also, it also reduced related adverse outcomes such as bradycardia, nausea and vomiting [33].

However, Ortiz-Gómez JR et al. found that ondansetron was not effective in the prevention of

drop in blood pressure in patients undergoing spinal anaesthesia for cesarean section [34], but they used bupivacaine combined with fentanyl while in our study we didn't use fentanyl. Omyma Sh. et al. also supports our findings, they compared the effect of ephedrine versus ondansetron in the prevention of PSH in patients undergoing cesarean section (C.S) and found that results of both groups are nearly comparable and both had significantly fewer vasoconstrictors need and lower incidence of nausea [35].

Ondansetron has the advantage of more stable HR as Julius S. et al., studied the cardiovascular effect of rapid IV infusion of ondansetron in patients under general anesthesia and their results were consistent with our results as regards the change in heart rate as there were no clinically or statistically significant changes in heart rate during the five-minute period following administration of ondansetron [36].

Limitations: As no one had ever compared the use of Ondansetron alone versus combined vasoconstrictors with fluid preload, so we feel that further investigation and studies with larger groups are required to confirm our results, so as to eliminate the problem of hypotension associated with subarachnoid anaesthesia and as we used ephedrine as a vasoconstrictor so, repeated administration diminishes its vasoconstrictive effect and its slow onset of action and relatively long duration make accurate titration of blood pressure difficult, so another vasoconstrictor may be needed in future studies. Also, we didn't compare their effects on vulnerable groups as elderly and parturients.

In conclusion, the preemptive use of Ondansetron alone and combined vasoconstrictors with fluid preload significantly reduce the incidence of PSH with no significant difference between both regimens. Furthermore, they also reduced vasoconstrictors and fluids consumption.

Acknowledgements

We are grateful to the residents of our department for their valuable help in carrying out this study. We are thankful to Dr. Ahmed Shash for assistance and guidance.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Dec 20; 6(12):2369-2372.
https://doi.org/10.3889/oamjms.2018.511
eISSN: 1857-9655
Case Report

Comparative Analysis of the "Scholastic" Recommendations of the AJCC From 2011 for the Surgical Treatment of Cutaneous Melanoma with the Newly Suggested Guidelines for OSMS From the Bulgarian Society For Dermatologic Surgery!

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Citation: Tchernev G, Temelkova I. Comparative Analysis of the "Scholastic" Recommendations of the AJCC From 2011 for the Surgical Treatment of Cutaneous Melanoma with the Newly Suggested Guidelines For OSMS From the Bulgarian Society for Dermatologic Surgery Open Access Maced J Med Sci. 2018 Dec 20; 6(12):2369-2372.
https://doi.org/10.3889/oamjms.2018.511

Keywords: One step melanoma surgery; Survival benefits; AJCC recommendations; Two-step melanoma surgery model; Old school; Surgical innovation

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Received: 19-Nov-2018; **Revised:** 02-Dec-2018; **Accepted:** 03-Dec-2018; **Online first:** 18-Dec-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: The American Joint Committee on Cancer (AJCC) offers a two-stage, often insufficient or so-called variable model of cutaneous melanoma treatment. This model starts with primary excision and an initial operational safety margin of 0.5 cm in all directions, followed by a re-excision with an additional field of operational security, determined by histologically established tumor thickness (with or without removal of SLN). We present a brand new method of melanoma surgery, the so-called One Step melanoma surgery (OSMS), in which cutaneous melanomas (regardless of their thickness) could be removed by single surgical intervention.

CASE REPORT: We describe a case of a patient with cutaneous melanoma, with postoperatively established Breslow's tumor thickness of 6 mm, operated on the AJCC model within two surgical sessions. The usual primary excision was performed with a surgical safety margin of 0.5 cm in all directions, followed by a secondary excision with an additional surgical security field of 1.2 cm in all directions (due to the patient's wish for the optimal cosmetic result, agreed and approved by the dermatosurgeon performing the manipulation).

CONCLUSION: The two-stage method for the treatment of melanomas is often insufficient due to: 1) the inability (in this case) secondary excision in the face area to be conducted with an additional recommended operational security field of 1.5 cm in all directions; and 2) the patient's wish for a better cosmetic result, which should be achieved with less surgical security field, resulting in a compromise solution for re-excision with an additional surgical field of 1.2 cm in all directions.

Introduction

At this stage, melanoma surgery is based on the recommendations of the American Joint Committee of Cancer (AJCC) [1], [2]. One step melanoma surgery (OSMS) is a new and innovative method that offers a completely different model for the treatment of patients with malignant melanoma [3]. Its advantage is that on the one hand we have clear and precisely defined therapeutic steps that do not lead to hesitation and ambiguity, and on the other are performed in a single surgical session [3], [4], [5].

We present a case of treatment of a patient with malignant melanoma based on the recommendations of AJCC. It has been attempted to fully comply with these recommendations despite the anatomical features of the surgical field.

The advantages and disadvantages of the AJCC's melanoma surgery models are discussed, as well as the advantages of the one-step melanoma surgery-OSMS, first described in the world according to recommendations of the Bulgarian Society for Dermatologic Surgery - BULSDS.

Case Report

We present a 69-year-old man in good general condition. The patient was hospitalized for surgical removal of a pigment lesion located in the forehead area. The lesion has a 2-year duration within which it gradually increases in size (Figures 1a, 1b).

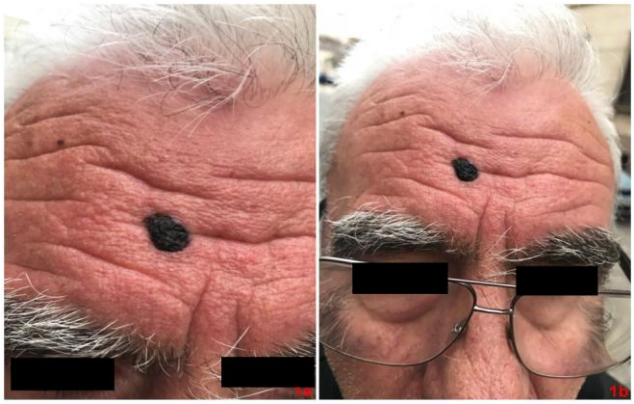

Figure 1: a and b) Clinical view of the melanocytic lesion in the forehead area

During the dermatological examination in the forehead area, the presence of a dark brown to the black melanocytic lesion, a nodular component, and an eroded surface was found (Figure 2a).

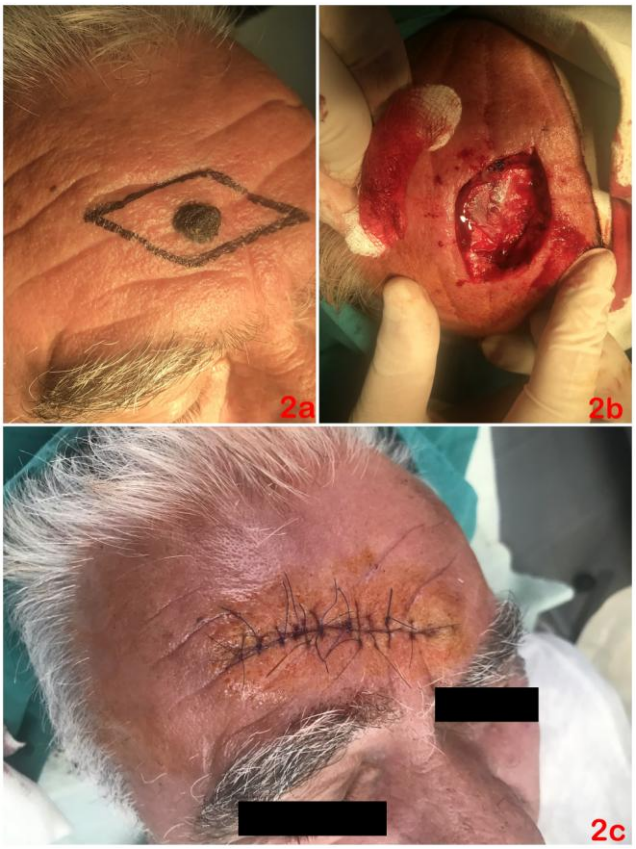

Figure 2: a) Outlining the 0,5 cm operational security boundaries in all directions, preoperative finding; b) Intraoperative finding of the lesion removed by elliptical excision; c) Postoperative clinical picture of surgical defect closed by single interrupted sutures

Clinically and dermatoscopically, these findings met the requirements for a malignant melanocytic lesion. The lesion was removed according to the recommendations of the current guidelines, with a primary excision with a surgical security field of 0.5 cm in all directions (Figure 2b). The resulting surgical defect was recovered by a single interrupted sutures (Figure 2c).

Histological verification has shown that it is a malignant melanoma nodular type with ulceration and size of 12/8 mm, Clark's IV level, Breslow's thickness 6mm, no perivasal and perineural invasion, no satellite deposits. Resection lines standing at 5 mm, 12 mm and 5 mm respectively. Staging showed that it is a stage IIC malignant melanoma (T4bN0M0). At the time of primary excision, pathologically enlarged cervical, pre- and retro-auricular, axillary and inguinal lymph nodes were not detected.

Twenty days later, the patient was hospitalized again to conduct a re-excision in the forehead area. Due to the specificities of the anatomical area and the impossibility to close the surgical defect with the necessary tight adaptation of the wound edges, the re-excision was performed with an operational security field of about 1.2 cm (Figure 3a, 3b, 3c, 3d and 3e) in all directions instead of the recommended, according to AJCC, field of 1.5 cm. Therefore, the final field of surgical safety after the two excisions was 1.7 cm instead of 2 cm. The surgical defect was again closed with single interrupted sutures (Figure 3f). The subsequent histological verification found the presence of skin with a foreign body type granuloma and fibrotic changes in the deep dermis.

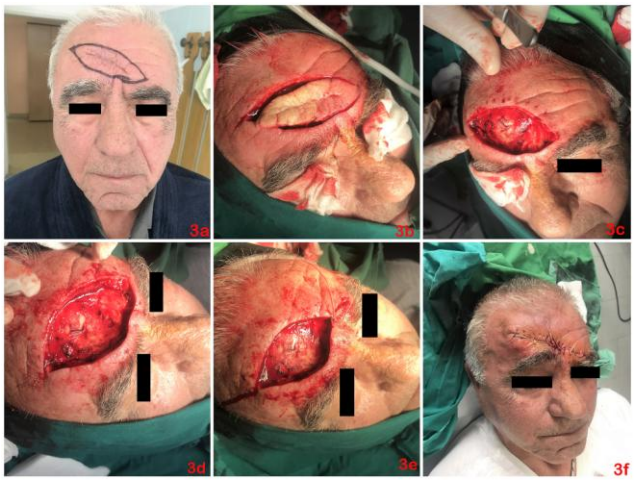

Figure 3: a) Outlining the 1,2 cm operational security boundaries in all directions, preoperative finding before the re-excision; b), c), d) and e) Intraoperative finding during re-excision; f) Postoperative clinical picture of surgical defects closed by single interrupted sutures after re-excision

Immediately before re-excision was conducted a consultation with oncology and within the secondary excision, removal of the sentinel lymph node (SLN) was not recommended. The conduct of

Pet scan on the whole body and presentation at local specialized oncology center was planned.

Discussion

Globally, the application of melanoma surgery is guided by the recommendations of the American Joint Committee of Cancer (AJCC) [1], [2]. What these guidelines postulates, is for a two-stage surgical approach where cutaneous melanoma treatment always starts with a primary excision with a fixed operational security margin of 0.5 cm in all directions [1], [2]. The subsequent postoperative histological measurement of tumor thickness passes to the second stage, namely re-excision [1], [2]. For a more detailed presentation of the differences in AJCC and OSMS recommendations, we have systematized data in tabular form (Table 1 and 2). What unites the two treatment models is that excisions and, respectively, in the two-stage model- the re-excisions, are dependent on the Breslow tumor thickness [1], [2], [3], [4], [5], [6], [7].

Table 1: AJCC recommendation [2]

Breslow thickness	Recommended surgical margins
Melanoma in situ	0.5 cm (primary excision with 0.5 cm in all directions, followed by secondary excision)
< 1 mm	0.5 cm primary excision (followed by secondary excision with 0.5 cm in all directions)
1.01 - 2.0 mm	0.5 cm primary excision (followed by secondary excision with 0.5 cm-1.5 cm/with SLND)
2 mm - 4 mm	0.5 cm primary excision (followed by secondary excision with 1.5 cm in all directions/with SLND)
> 4 mm	0.5 cm primary excision (followed by secondary excision with 1.5 cm in all directions/ without SLND if nodes not enlarged)

It is unclear why, in the AECC recommendations, the field of operational safety for melanomas with a thickness between 1 and 2 mm varies between 1 and 2 cm [1], [3]. Compared to them, European guidelines for the treatment of malignant melanoma have a clearly defined field of operational safety of 2 cm for melanomas with a thickness up to 2 mm [8].

After an initial excision of 0.5 cm for all cutaneous melanomas, according to the AJCC, the subsequent re-excision of histological data for melanoma in situ (MIS) and MM < 1 mm (for example) should be with an additional operational security field of 0.5 in all directions (Table 1) [1]. Instead of them, OSMS offers in cases of clinical and dermatoscopic data for thin melanomas-MIS and MM < 1 mm, to be performed a single excision directly with a safety margin of 1 cm in all directions (Table 2) [3]. In cases where clinical and dermatoscopic data are indicative of melanomas less than 1 mm thick, conducting a two-step operation is not optimal, and that is precisely the advantage of OSMS-diagnosis and treatment within one surgical session [3].

Table 2: One step melanoma surgery (OSMS): Tchernev G et al. recommendations [3]

Breslow thickness	Recommended surgical margins
Melanoma in situ	1.0 cm (clinical/dermatoscopical evaluation obligate/if possibility for echographical examination-from benefit)
< 1 mm	1.0 cm (clinical/dermatoscopical evaluation obligate/if possibility for echographical examination-from benefit)
1.01 - 2.0 mm	1.0 cm (with SLND), (echographical tumour thickness measurement preoperatively recommended)
2 mm- 4 mm	2.0 cm (with SLND) echographical tumor thickness measurement preoperatively recommended
> 4 mm	2.0 cm a) no enlarged lymph nodes- 2 cm resection is sufficient, b) in the presence of enlarged lymph nodes - to be removed together with the reexcison of the primary tumorous tissue!

For MM with a thickness above 1 mm (or for which dermatoscopy and clinics are not categorical/indicative in favor of thin melanoma), the AJCC recommendations are again to be started in all cases with a primary excision of 0.5 cm in all directions [2]. Compared to the AJCC guidelines from 2011 [2], when the postoperative histologically measured thickness indicates a malignant melanoma between 1.01 and 2.0 mm, the limits of the subsequent re-excision should be between 1 and 2 cm, with recommendations to be accompanied by SLND (Table 1) [2]. Oddly, why in the AJCC publication of 2011 [2] this resection field has been described that could have varied between 1 and 2 cm? [3]. Compared to AJCC, European recommendations have a fixed final field of operational security which, in their view, for melanomas, up to 2 mm thick should be 1 cm [8].

Within the newly introduced OSMS, all cutaneous melanomas (regardless of their thickness) can be operated within one surgical session [3], [4], [5], [6], [7]. When this thickness is between 1.01 - 2.0 mm, the operation can again be performed within one surgical intervention, with a safety margin of 1 cm in all directions combined with SLND (Table 2) [3], [4], [5], [6], [7]. Thus, the AJCC's recommendations on the final security field and SLND are respected and clearly defined but within one intervention [3], [4], [5], [6], [7].

For MM between 2-4 mm tumor thickness, the AJCC recommends that the final operational safety field is 2 cm in all directions, necessarily combined with SLND, which overlaps with the OSMS recommendations (Table 1 and 2). The difference, in this case, comes from the application of the innovative solution of the Bulgarian Society for Dermatologic Surgery (BULSDS) for one step melanoma surgery (OSMS), where preoperative ultrasound measurement of tumor thickness allows to save the need for secondary excision [4]. Thus, the surgical treatment of melanomas is performed with a surgical security field of 2 cm in all directions, combined with SLND during one surgical session [5].

For melanomas with thickness above 4 mm, the recommendations of AJCC and OSMS are similar, and according to the two models, after the removal of the melanocytic lesion, the final safety field should be 2 cm in all directions and in case of pathologically

enlarged lymph nodes to be combined with their removal (Table 1 and 2) [1], [2], [3], [4].

According to both models (for tumors with a thickness above 4 mm), if there is no evidence of pathologically enlarged lymph nodes, the excision of 2 cm is sufficient and SLND is not of paramount importance, as draining lymph nodes may not be affected due to several different reasons [1], [3]. The reason for the lack of involving of the locoregional lymph nodes for thicker melanomas could be due to: 1) the tumor cells have passed the draining lymph node but without stopping in it, 2) primary hematogenous dissemination has already occurred without lymph nodes and pathways being involved, or to have 3) accessory parallel lymphatic pathways surrounding the guard lymph node [3]. The resulting difference between the two types of recommendations-OSMS/AJCC-is that the OSMS model clearly defines 1) the boundaries of surgical security, 2) much stricter with regard to the radical combined approach, also involving the conducting of SLND and 3) leads to a categorically more complex treatment performed within one operational session [3], [4].

In conclusion, preoperative ultrasound measurement of tumor thickness at our patient would allow being performed a single surgical intervention with a direct field of operational safety of 2 cm in all directions without SLND due to a lack of pathological enlargement data.

The presented analysis shows that current AJCC guidelines are not always the most optimal and acceptable solution in therapeutic and cosmetic terms. One step melanoma surgery solves a great deal of the problems and fluctuations that exist in the AJCC's recommendations. The greatest difference and advantage of OSMS versus AJCC is that the treatment of cutaneous melanomas is performed with one surgical intervention.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Dec 20; 6(12):2373-2375.
https://doi.org/10.3889/oamjms.2018.396
eISSN: 1857-9655
Case Report

Secondary Thymoma among Adult Treated For Acute Lymphoblastic Lymphoma/Leukemia: Report of a Case and Review of the Literature

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Citation: Bendari M, Bencharef H, Khoubila N, Cherkaoui S, Lamchahab M, Madani A, Rachid M, Qachouh M, Oukache B, Quessar A. Secondary Thymoma among Adult Treated For Acute Lymphoblastic Lymphoma/Leukemia: Report of a Case and Review of the Literature. Open Access Maced J Med Sci. 2018 Dec 20; 6(12):2373-2375. https://doi.org/10.3889/oamjms.2018.396

Keywords: Lymphoblastic leukaemia; Lymphoma; Thymus; Thymoma

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Received: 22-Jun-2018; **Revised:** 02-Nov-2018; **Accepted:** 03-Nov-2018; **Online first:** 14-Dec-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Concomitant thymoma and T- lymphoblastic/leukaemia lymphoma is possible. Secondary thymoma after treatment for T-lymphoblastic/leukaemia lymphoma was also occasionally reported, although this is quite rare.

CASE REPORT: We report a case of 44-year-old women with secondary thymoma after chemotherapy treatment for T Acute Lymphoblastic leukaemia/lymphoma. Diagnosis of lymphoblastic/leukaemia lymphoma was made in 2015 by morphological and histological study. The patient underwent Moroccan protocol for acute lymphoblastic leukaemia (MARALL) from 2015 to 2017 and achieved complete remission. One year later, the patient developed an anterior mediastinal mass, relapse was suspected, but the surgical biopsy was performed and histological, the mass showed thymoma.

CONCLUSION: At the time of diagnosis of thymoma for a patient treated for T-lymphoblastic/leukaemia lymphoma it is necessary to eliminate a relapse because the distinction between thymoma and T-lymphoblastic/leukaemia lymphoma is sometimes difficult, and the association is possible.

Introduction

Lymphoblastic leukaemia/ lymphoma is a neoplasm of lymphoblasts committed to the T-cell lineage; it frequently shows mediastinal involvement at diagnosis [1] it's required intensive therapy, with chemotherapy, radiation and sometimes stems cell transplantation [2]. On the other hand, thymoma is thymic epithelial tumours composed of varying proportions of neoplastic thymic epithelial cell and non-neoplastic thymocyte, with limited malignant potential, the treatment consists of surgical resection. The distinction between thymoma and lymphoblastic leukaemia/ lymphoma can be problematic because of the immature lymphocytes associated with thymoma may resemble T lymphoblastic leukaemia/lymphoma cells both morphologically and immunohistochemically [1]. We report a rare case of a patient with secondary thymoma which occurred one year after lymphoblastic leukaemia/lymphoma and discuss the cause-effect relationship and pathogenetic mechanism of thymoma and T-cell Lymphoblastic leukaemia/lymphoma and present new method on immunohistochemistry to distinguish T- Lymphoblastic leukemia /lymphoma from thymoma.

Case report

A 44-year-old housewife was diagnosed with T-lymphoblastic leukaemia/lymphoma on 2015; the patient presented four months before her admission fever with the anaemic syndrome and chest pain; the physical exam normal. There were no palpable lymph nodes.

The white blood cells count found normochromic normocytic anaemia with Hb at 10.3 g/dl, WBC count 12 G/L without peripheral blasts, platelet count was at 35 G/L. The chest X-ray and CT scan showed pleural infusion with mediastinopulmonary mass; the lung biopsy showed that the leukemic cells were positive for CD5, CD10, CDBcl2, and negative for CD20, CD79a, CD23 and TDT. The KI-67 was positive at 90%. Bone marrow biopsy showed lymphoblastic cells. Therefore, the lymphoma was classified as precursor T cell lymphoblastic leukaemia/lymphoma. The karyotype was not done. Abdominal ultrasound showed no abnormalities. Renal and liver biochemistry profiles were essentially normal.

The patient underwent chemotherapy for Lymphoblastic leukaemia/lymphoma according to a Moroccan protocol of acute lymphoblastic leukaemia which includes one induction, one consolidation, two intensifications and maintenance therapy for 2 years. Complete remission was achieved; stem cell transplantation was not done. One year later, the patient complained of chest pain with dyspnoea and cough. Computed tomography (CT) scan of the chest demonstrated right gangliotumoral process occupying almost the entire right lung of heterogeneous density containing necrotic zones measuring approximately 113/123 mm, with secondary pleural and probably peritoneal involvement (Figure 1).

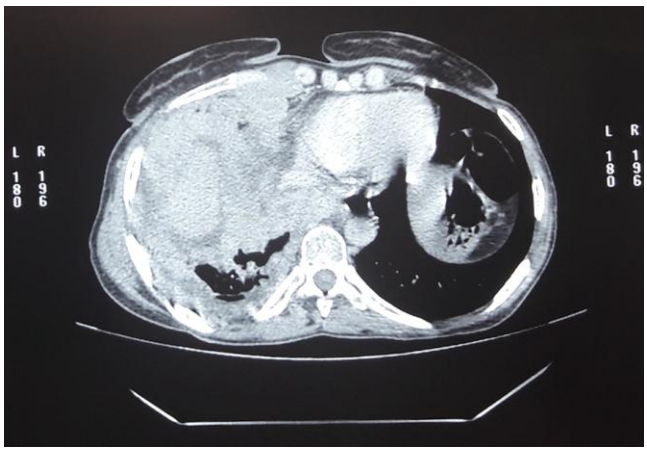

Figure 1: CT scan of the chest showing right tumoral process

The white blood cells count was normal. Because of the possibility of relapse of her Lymphoblastic leukaemia/lymphoma, bone marrow aspiration was performed. Lymphoblastic leukaemia/lymphoma remains in complete remission. Surgical biopsy for the pulmonary mass was performed and the immunohistochemical study objectives malignant tumour proliferation expressing CD3 and Tdt, without expression of CD20, PAX5 and CD79a. Cytokeratin AE1/AE2 revealed a contingent of epithelial cells; The Ki 67 is of the order of 80%, concluding an aspect in favour of a thymoma of type B1, the patient was put under protocol C CAP, she received 2 cures.

Discussion

Thymoma is neoplasm that originates from the thymic epithelial cell and is frequently associated with mature or immature non-neoplastic lymphocytes [1]. This pathology is associated with a variety of disorders notably myasthenia gravis (MG) and pure red cell aplasia (PRCA) [3]. Thymoma may also be associated with Lymphoblastic leukaemia/lymphoma on the same lesion [1] or arise before.

We report the case of thymoma as a secondary neoplasm after chemotherapy treatment of T Lymphoblastic leukaemia/lymphoma. Considering the low survival rate of Lymphoblastic leukaemia/lymphoma the risk of second malignancy remains underestimated. The incidence of second malignancies had been estimated for Lymphoblastic leukaemia/lymphoma at 2.1% at 5 years and 4.9% at 10 years [4]; this incidence is particularly high and strongly linked to cranial irradiation [5].

A study of secondary or concomitant neoplasms among adult with Lymphoblastic leukaemia/lymphoma was done including 1494 patients treated for Lymphoblastic leukaemia/lymphoma, 23 of them (1.5%) developed secondary neoplasms, only one case of secondary thymoma was reported [4]. Our report underlines the rarity of this association. On the other hand, the risk of developing a secondary neoplasm is highest among patients who had undergone transplantation as postremission therapy [4], it was not the case for our patient.

The difficulty in our case is the exclude relapse before retaining the diagnosis of thymoma, because of the resemblance both morphologically and immunohistochemically between lymphocyte-rich thymoma and T-lymphoblastic lymphoma /leukaemia, especially with small biopsy.

Flow cytometric analysis can aid differentiate T-lymphoblastic lymphoma/leukaemia from thymoma [6], [7]. Immunopositivity of cytokeratin is also advisably highlighting a network of epithelial cells in thymoma, in contrast to a few potentially entrapped residual thymic epithelial cells with Lymphoblastic

leukaemia/lymphoma [8].

The study was done and demonstrated that the NOTCH 1 could help to distinguish between thymoma and T lymphoblastic lymphoma/leukaemia. NOTCH1 is the best-characterised member and was discovered from cases of human T-lymphoblastic lymphoma/leukaemia harbouring t(7; 9)(q34; q34.4), which juxtaposes B enhancer/promoter elements on chromosome 7 [9]. NOTCH1 plays a central role in the pathogenesis of the T-lymphoblastic lymphoma/leukaemia. The NOTCH1 signaling pathway is frequently activate in T-lymphoblastic lymphoma/leukemia, it's reported in 50% to 60% of patients [10], [11], [12]. In the same study, all thymoma tested were negative for NOTCH1, but it requires careful interpretation. This IHC marker can be helpful for differential diagnosis; unfortunately, this test is not available in our laboratory.

The other specificity of our case is the short time between the end of chemotherapy and developing thymoma.

In summary, we report a secondary thymoma among adult treated by standard chemotherapy for T-lymphoblastic lymphoma/leukaemia without stem cell transplantation. This case illustrates the rarity of thymoma as a secondary neoplasm and insists to the distinction between thymoma and relapse of T-lymphoblastic lymphoma/leukaemia because of the very different clinical implications and treatment investigations. The use of new marker like NOTCH1 can be helpful for this distinction.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Dec 20; 6(12):2376-2377.
https://doi.org/10.3889/oamjms.2018.516
eISSN: 1857-9655
Case Report

Lymphangiosis Carcinomatosa in a Patient with Giant Cutaneous SCC: Cervicopectoral Advancement Flap in Combination with Tunnel Transposition Flap from the Back as Promising Treatment Approach?

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Citation: Tchernev G, Lozev I, Pidakev I, Temelkova I. Lymphangiosis Carcinomatosa in a Patient with Giant Cutaneous SCC: Cervicopectoral Advancement Flap in Combination with Tunnel Transposition Flap from the Back as Promising Treatment Approach? Open Access Maced J Med Sci. 2018 Dec 20; 6(12):2376-2377. https://doi.org/10.3889/oamjms.2018.516

Keywords: Skin cancer surgery; Single step surgical approach; Survival benefit; Treatment outcome; Tunnel transposition flap; Cervico pectoral flap

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Received: 29-Nov-2018; **Revised:** 03-Dec-2018; **Accepted:** 04-Dec-2018; **Online first:** 18-Dec-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: One of the features characterising cutaneous SCC as high-risk is lymphovascular infiltration. The diffuse lymphangitic spread of carcinogenic cells is defined as the so-called lymphangitis carcinomatosa. In some cases, it is the only and first sign to alert the presence of an underlying malignancy. Therefore, biopsy in patients with clinical data on lymphangiosis carcinomatosa is of paramount importance.

CASE REPORT: We present a 77-year-old man with a progressively growing tumour formation in the area of the right shoulder, clinically suspected for SCC. During the dermatological examination, it was found that the lesion was surrounded by an infiltrated, perilesional relief shaft, which was histologically verified as lymphangitis carcinomatosa. The tumour formation was removed by radical excision and formation of a large skin-subcutaneous defect. To correct the surgical defect, a cervico-pectoral flap was performed, followed by tunnel transposition of the scapular graft through the deltoid muscle. The preoperative, ultrasound-marked artery was the arteria circumflexa scapulae dextra, which was used as the foot of the scapular graft and at the same time ensuring its blood supply. After the performed surgical flaps there remains a small uncovered surgical defect, which was left for subsequent secondary healing or full thickness mesh graft. The subsequent histological examination of the removed tumour formation detected the presence of squamous cell carcinoma.

CONCLUSION: Patients with the simultaneous presence of two different pathological cutaneous changes, located in the immediate proximity often require a multidisciplinary and complex treatment approach. For tumour formations close to the area of the neck, the cervical-pectoral flap provides optimal cosmetic recovery of the surgical defect. The tunnel transposition is an individualised, unconventional and difficult to implement the approach, which however showed a good therapeutic result. On the other hand, the preoperative histological examination of reddish peritumoral localised tentacles leads to 1) diagnosis of lymphangiosis carcinomatosa as well as 2) the subsequent precise determination of the limits of surgical excision, which is a large number of cases saves the need for secondary re-excision in these patients.

Introduction

The therapeutic approach to advanced cancers located near the neck area is a great challenge because of the more complex anatomy and the need for complete and aesthetically acceptable reconstruction [1].

Case Report

We present a 77-year-old patient with a rapidly growing tumour formation in the right shoulder. The lesion appears 10 months ago, progressively increasing its size and beginning to bleed. During the dermatological examination above the level of the right clavicle, an exophytic formation with an erosive surface and 13/7 cm size, clinically suspected of spinocellular carcinoma, was found (Figure 1a and

1b). The lesion was surrounded by an infiltrated, perilesional, reddish embossed shaft (Figure 1a and 1b). The preoperatively performed cutaneous biopsy of the perilesional tissue revealed the presence of lymphangitis carcinomatosa. Radical excision of the tumour formation was performed by forming a large 20/30 cm skin-subcutaneous defect (Figure 1c and 1d).

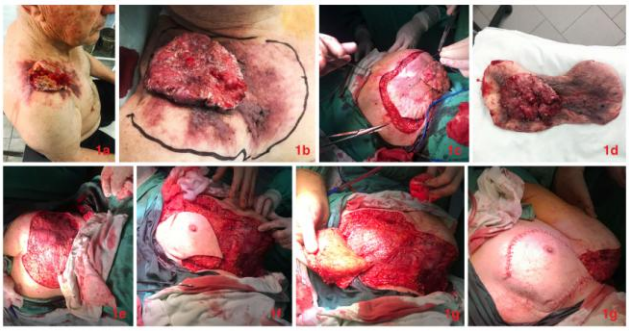

Figure 1: a) and b) Exophytic formation above the level of the right clavicle, with an erosive surface, clinically suspected of spinocellular carcinoma; c) and d) Intraoperative aspect. The tumourous tissue after surgical resection; e), f) and g) Surgical defect recovered by cervico-pectoral flap

The surgical defect was recovered by cervico-pectoral flap (Figure 1e, 1f and 1g), followed by tunnel transposition of the scapular flap through the deltoid muscle (Figure 2a, 2b, 2c, 2d and 2f), the blood supply of which was provided by artery circumflexa scapulae dextra, branch of the subscapular artery. The scapular graft's foot is the separated artery circumflexa scapulae dextra, which was marked ultrasound before surgery (Figure 2a and 2b). There remains a small uncovered stretch of 3/2 cm surgical defect for subsequent secondary healing or full thickness mesh graft (Figure 2e and 2f).

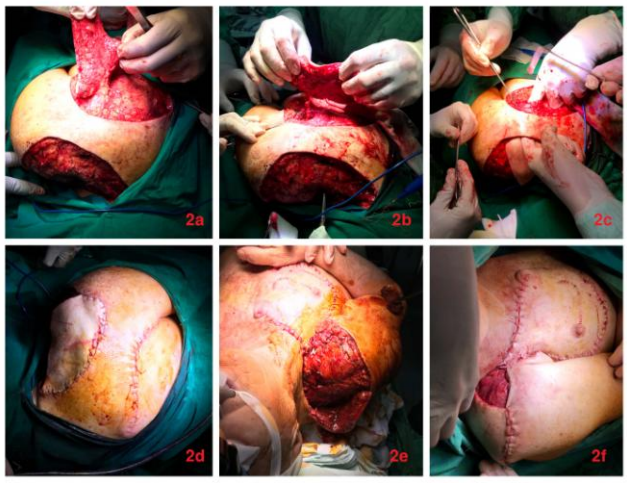

Figure 2: a), b), c) and d) Tunnel transposition of the scapular flap through the deltoid muscle, the blood supply of which was provided by a.circumflexa scapulae dextra (Figure 2b); e) and f) Immediate postoperative result

The subsequent histological examination showed evidence of squamous cell carcinoma, diameter 8 cm, engaging the dermis and hypodermis, resection lines free of tumour infiltration. Staging identified spinocellular carcinoma Stage II (T2N0M0).

Discussion

Advanced cutaneous spinocellular carcinoma hides the risk of metastases and relapses [2]. Apart from tumour thickness more than 2 mm and tumour size of more than 6 mm, other features characterising high-risk cutaneous SCC include poor differentiation, perineural or lymphovascular infiltration [2].

Lymphangiosis carcinomatosa is a disease that shows a diffuse lymphangitic spread of cancer cells [3]. Sometimes lymphangiosis carcinomatosa may be the first dermatological symptom revealing the presence of underlying malignancy [4].

Pre-operative histopathological testing of patients with lymphangious carcinomatosa helps, on the one hand, to precisely determine the limits of surgical excision and, on the other hand, leads to the saving of a possible second operation of the patients.

When it comes to large, advanced tumours near the neck, cervicopectoral flap provides a good therapeutic and cosmetic result [1].

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Dec 20; 6(12):2378-2380.
https://doi.org/10.3889/oamjms.2018.517
eISSN: 1857-9655
Case Report

Valsartan Induced Melanoma?! First Description in Medical Literature!

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Citation: Tchernev G, Temelkova I. Valsartan Induced Melanoma?! First Description in Medical Literature!. Open Access Maced J Med Sci. 2018 Dec 20; 6(12):2378-2380. https://doi.org/10.3889/oamjms.2018.517

Keywords: Valsartan; Cutaneous melanoma; Surgery; Drug-induced melanoma; Survival benefit

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Received: 03-Nov-2018; **Revised:** 03-Dec-2018; **Accepted:** 04-Dec-2018; **Online first:** 18-Dec-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Drug-induced carcinogenesis is a matter of huge popularity and the subject of in-depth research over the last few years. According to the literature, dopamine agonists and acetylsalicylic acid fall into the list of drugs likely to potentiate the development of cutaneous melanoma. However, according to recent data, widely used angiotensin receptor blockers (ARBs) for the treatment of arterial hypertension, also carry a risk of malignancy development. The content of probable carcinogens, such as NDMA or NDEA in the drug valsartan (ARBs), causes the product to be withdrawn from the market. Recent experimental data suggest that another angiotensin receptor blocker-losartan also stimulates cell adhesion and melanoma cell invasion.

CASE REPORT: We present a 70-year-old patient who has been on systemic therapy with a combined drug of amlodipine and valsartan since 2008 and only valsartan from 2015. Three years after the first intake of valsartan (2011), the patient developed a pigment lesion on the right arm. Approximately 2.5 years after doubling the dose of valsartan, the patient observed a progression in the size of the lesion, which was the cause of the dermatological examination and hospitalisation for surgical removal. The melanocytic lesion was removed by radical excision and a surgical field of 0.5 cm in all directions, followed by histological verification, which found the presence of cutaneous melanoma with a tumour thickness of 3 mm. A re-excision was planned with an additional surgical field of 1.5 cm in all directions combined with parallel removal of a draining lymph node.

CONCLUSION: The case is indicative of two things: 1) the possible triggering of melanoma within the systemic treatment with valsartan; and 2) the necessity for optimization of melanoma surgery within the one-step melanoma surgery, which in this case would result in a single surgical excision of the primary lesion, with an operational security field of 2 cm in all directions, along with the removal of a draining lymph node.

Introduction

Numerous data suggest that malignant melanoma may be drug-induced, and various mechanisms are likely to potentiate directly or indirectly carcinogenesis [1], [2], [3], [4], [5]. According to the number of publications in patients with schizophrenia and Parkinson's, the risk of melanoma is probably determined by blood levels of dopamine [1], [2]. Induction of malignant melanoma by acetylsalicylic acid has also been the subject of studies, and it is currently thought that men taking Aspirin on a daily basis are at an increased risk of developing melanoma [3]. According to the latest in vitro data, valsartan and losartan may also potentiate carcinogenesis [4], [5].

We describe a first official case of possible valsartan-induced melanoma following administration of an angiotensin receptor blocker- valsartan, produced by a company still on the market and not on the list of withdrawn products.

Case Report

We present a 70-year-old man, skin phototype II, no history of excessive exposure to UV light and no history of malignancy in the family. The patient suffers from arterial hypertension, which he controls through medication. The systemic cardiologic therapy started with a combined drug of amlodipine and valsartan (10/160 mg), once daily (1-0-0), from 2008 to 2018. Due to unsatisfactory control of

hypertension in 2015 additional valsartan (160mg) has been added to the therapy (0-0-1), which the patient is still receiving at the time of hospitalisation (2018). Three years after the first intake of amlodipine and valsartan (10/160 mg) (2011), the patient developed a pigment lesion on the right arm. Clinically and dermatoscopic (according to anamnestic data and the examinations conducted at that time) there was no evidence of cutaneous melanoma, and surgical treatment was not recommended. However, in 2015, the dose of valsartan was duplicated. Two and a half years later (2018), the patient observed gradual change in the size of the lesion, discomfort, small bleeding, and sensitivity in the area of the lesion.

Reason for hospitalisation of the patient is the progressive increase in the size of the lesion and bleeding observed from its surface over the last few days (Figure 1A). During the dermatological examination, in the area of the right arm, we found the presence of a melanocytic lesion with an uneven surface, a nodular component, uneven borders and partly a bleeding surface, clinically suspect to malignant melanoma (Figure 1A). The lesion was removed by elliptical excision, with an operative safety margin of 0.5 cm in all directions (Figure 1B and 1C). The surgical defect was closed by a single interrupted sutures (Figure 1D). The subsequent histological study showed that it is superficial nodular malignant melanoma, Clark's level III, Breslow's thickness- 3 mm, high mitotic activity and ulceration; abundant lymphocytic stromal reaction; resection lines-no infiltration.

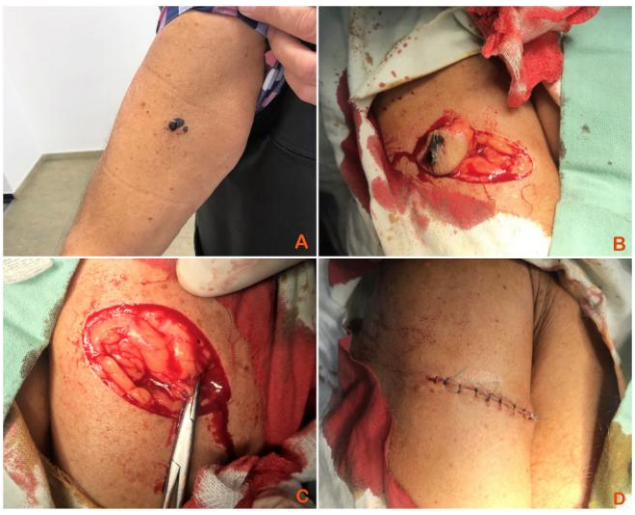

Figure 1: A) Clinical view of the melanocytic lesion with black colour, nodular component and partially bleeding surface; B) Intraoperative finding of the lesion removed by elliptical excision; C) Intraoperative finding of ligation of the blood vessels; D) Postoperative clinical picture of surgical defect closed by single interrupted sutures

Staging showed that it was a malignant melanoma stage IIB (T3bN0M0). The follow-up screening and the PET scan conducted detected the presence of a pathologically enlarged lymph node in the right axilla (suspected for metastatic), with dimensions 6/10 mm. Lymphatic scintigraphy was conducted, which confirmed the findings from the Pet Scan. A re-excision was performed with an operative safety margin of 1.5 cm in all directions, combined with removal of a draining lymph node and axillary lymphadenectomy on the right axilla. A single metastatic lymph node was established histologically; therefore the stage was defined as IIIC (T3bN1M0). A plan was prepared for 1) monthly control review, 2) monthly immunotherapy (vaccines) and 3) abdominal ultrasound at 6 months and once a year (in the winter) X-ray of the lung.

Discussion

Valsartan belongs to a group of medicines called angiotensin receptor blockers (ARBs) that are widely used to treat arterial hypertension [4]. According to randomised controlled trials, this group of blockers is associated with an increased risk of developing cancer, but the individual risk of any drug in this group is not yet known [4]. Lately, research has been increasing on the role of the renin-angiotensin system, and more specifically angiotensin II type 1 and type 2 receptors in the regulation of cell proliferation, angiogenesis and tumour progression [4]. As malignancy, malignant melanoma also falls within the scope of studies about the likelihood of being induced by ARBs [5]. The alleged carcinogenic effect of valsartan is first announced by the US manufacturer, Prinston Pharmaceuticals Inc., which in June 2018 informed the Food and Drug Administration (FDA) that it stopped the production of valsartan-containing products because it detected traces of N-nitrosodimethylamine (NDMA) in the active pharmaceutical ingredient of valsartan (API) provided by a Chinese manufacturer (Zhejiang Huahai Pharmaceutical Co) [6]. NDMA is classified as a chemical that belongs to the family of potent carcinogens and is used for the production of rocket fuel, softeners and other products [7]. According to the US Department of Health and Human Services, exposure to high doses of NDMA may cause liver damage, and NDMA is a likely human carcinogen [8]. Animal studies have shown that NDMA can cause tumours in the liver, kidneys and the respiratory tract, making it potentially harmful for humans as well [8]. Subsequently, the European and American health services have expanded the withdrawal of valsartan after detection of NDMA in medicines manufactured by a second Chinese pharmaceutical manufacturer (Zhejiang Tianyu Pharmaceuticals of Taizhou) and by a manufacturer in India (Hetero Labs Limited, Camber Pharmaceuticals) [9]. Although the FDA declares that not all drugs containing the NDMA ingredient are potentially dangerous, several companies voluntarily withdraw their products with valsartan (Major Pharmaceuticals, Solco Healthcare and Teva

Pharmaceuticals Industries, as well as valsartan/hydrochlorothiazide from Solco and Teva) [10]. In September 2018, information on a second potential carcinogen in the product valsartan-N-nitrosodiethylamine (NDEA) was published [11]. An important publication from 2018 explains the possible mechanism by which angiotensin receptor blockers (ARBs), particularly losartan, are involved in the pathogenesis of development of malignant melanoma [5]. It postulates that losartan inhibits the activity of NHE1 (Na+/H+ exchanger isoform 1) and migration of human melanoma cells (MV3), but at the same time stimulates MV3 cell adhesion and invasion [5].

The case presented by us poses several interesting questions: the newly discovered melanocytic lesion occurred 3 years after the first intake of valsartan, as in the last 2-3 years (2015-2018), the patient observed an increase in its size, which coincides with the introduction of a second product containing valsartan (of the same pharmaceutical company). The inevitable association that occurs is that the progression of melanoma and the likelihood of developing metastases may be dose-dependent [12]. In fact, according to the FDA, on a daily intake of the highest dose of valsartan (320 mg) throughout 4 years, 1/8000 patients are likely to develop cancer, which, according to the FDA, is sufficient reason to withdraw products (containing NDMA) [12]. An open question remains whether the cause lies in NDMA, NDEA or other carcinogens? Or the generic itself?

According to officially shared data from EMA (European Medicines Agency, within our electronic correspondence), there are already 9 reported cases of melanoma in patients taking valsartan, but none of them is officially disclosed. Interestingly, these cases are most likely of valsartan contaminated with NDMA (?) Or drugs that are in the FDA's prohibited lists? While missing detailed EMA data, we present a case with valsartan intake, produced in Germany! A product that is still on the market and does not fall into the list of withdrawn products (produced by a company belonging to the top 10 pharmaceutical giants). This means that the carcinogenic effect may not only be related to the presence or contamination with NDMA but may come directly from the generic substance of valsartan (?) [5)] as well as from the presence of a potential another carcinogen [11]. It should not be excluded that the onset of melanoma and the systemic administration of valsartan may be just a mere coincidence.

It should be noted that older patients, especially men over 65, are at increased risk of developing melanoma [13]. Also, it is believed that 50 years of age and older men are more often diagnosed with melanomas with a thickness ≥ 2.0 mm and that with increasing age, more frequently arise de novo melanomas [13]. Shared information is not an indictment to the respective manufacturer, but contains important clinical observations and analyses based on experimental data in the world literature, as well as from the previously recorded cases of melanomas in patients receiving valsartan -data shared by EMA.

In conclusion, this case reaches important conclusions, indicating that the systemic intake of valsartan may trigger the development of malignant melanoma and the likelihood of its progression being directly proportional to the dose. One step melanoma surgery, in this case, would be a good therapeutic solution providing removal of the lesion in one surgical session along with the removal of a draining lymph node.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Dec 20; 6(12):2381-2385.
https://doi.org/10.3889/oamjms.2018.488
eISSN: 1857-9655
Public Health

Risk Analysis Characterization of Benzene and Demographic Factors toward Immunoglobulin A

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Citation: Tualeka AR, Jalaludin J, Salesman F, Wahyu A, Tukiran T, Setiawan S, Wibrata DA, Hasyim HN. Risk Analysis Characterization of Benzene and Demographic Factors toward Immunoglobulin A. Open Access Maced J Med Sci. 2018 Dec 20; 6(12):2381-2385. https://doi.org/10.3889/oamjms.2018.488

Keywords: Benzene; Ig-A; Shoe-Maker Worker; Risk Quotient

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Received: 29-Aug-2018; **Revised:** 14-Nov-2018; **Accepted:** 15-Nov-2018; **Online first:** 13-Dec-2018

Funding: This study was supported by Activity Budget Plans 2017, Faculty of Public Health, Airlangga University

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

Abstract

BACKGROUND: Research on risk assessment at industrial sites has experienced growth during the end of this year. But in Indonesia, there is still limited research on risk assessment, especially regarding the importance of measuring non-carcinogenic risk assessment in the workplace. Benzene exposure is believed to reduce levels of immunoglobulin A (IgA) in workers.

AIM: The purpose of this study was to analyse the relationship between risk quotient (RQ) of non-carcinogenic risk assessment of benzene and demographic factors on IgA levels.

MATERIAL AND METHODS: The subjects of the study were shoe craftsmen who were at risk of benzene exposure. The study design was cross-sectional with a total population of 20 workers. Measurement of IgA levels by Immunoturbidimetric Assay with a normal standard of 2-3 mg/ml. Calculation of non-carcinogenic (RQ) risk characteristics with a comparison between risk agent non-carcinogenic intake with RfD or RfC benzene.

RESULTS: The majority of the study subjects aged over 45 years and had a working period of ≥ 25 years. There were 2 location points that had a threshold value exceeding the benzene standard (> 0.05 ppm), and 40% of the subjects had decreased IgA levels. Age and working periods had a significant relationship to IgA levels ($p = 0.027$; $p = 0.047$), while benzene and RQ levels did not have a significant relationship with IgA levels ($p = 0.179$; $p = 0.436$).

CONCLUSION: Increasing age and working period can reduce IgA levels in the body. Further research is needed on risk assessment, especially on the safe limits of benzene concentration in the workplace to find out how long benzene exposure forms a non-carcinogenic or carcinogenic risk in workers' bodies exposed to benzene.

Introduction

Research on risk assessment in industrial environments has begun to develop in recent years. The importance of knowledge about hazard risk is a very important term to avoid accidents. Risk assessment studies such as RQ of noncarcinogenic risk assessment intake, or risk characteristics of carcinogenic effects (ECR) have begun to develop, but in Indonesia, this research is still limited.

Benzene is the most important ingredient in the chemical industry. Benzene is present in solvents for waxes, resins, rubber, plastics, rubber, paint, glue [1], [2]. Benzene is non-polar and insoluble in water, but soluble in organic solvents such as diethyl ether, carbon tetrachloride or hexane [3]. Benzene evaporates into the air very quickly, dissolves little in water, and is highly flammable [1], [4]. Long-term exposure to benzene can reduce levels of Immunoglobin A (IgA).

Immunoglobulin A (IgA) is the main serum

immunoglobulin and a class of antibodies that are predominant in the external secretion that functions to coat the mucosal surface and has a key role in immune protection [5]. IgA levels in the blood serum of adults (aged 16-60 years) are 1.4-4.2 mg/ml for IgA1 and 0.2-0.5 mg/ml for IgA2. Biological half-life for IgA1 is 5-7 days, while the half-life of IgA2 is 4-6 days [6].

Several studies had shown a relationship between benzene exposure and IgA levels. Research on 13 oil tank workers with relatively low benzene exposure (0.01 ppm-0.62 ppm) showed a significant decrease in serum immunoglobulin M (IgM) and immunoglobulin A (IgA) [7]. In studies using a rat as animal subjects, there was a 50% reduction in IgA in female mice with benzene exposure of 30 and 300 ppm [8], [9]. Research on painting workers in Egypt consisted of 81 people exposed to benzene and 83 controls, showed an increase in serum IgM and a decrease in serum IgA and IgG in the exposed group compared with the control group [10]. This is due to the presence of necrosis, and liver dysfunction in the group exposed to benzene which is immune/immune suppressed [10].

However, among all previous studies, there is still no study about the correlation between the RQ of non-carcinogenic benzene and IgA levels. Research in Indonesia regarding risk assessment especially on non-carcinogenic risk characteristics of IgA levels is still limited, especially in the shoe-making industry which has a continuous risk of benzene exposure from organic glue. The previous research explains about calculated RQ at worker exposed to benzene in shoe worker industry, but not gives a correlation finding between RQ and effect of benzene exposure at worker such as decreased level of IgA serum [11], [12], [13].

Therefore, researchers are interested in analysing the relationship between the RQ of non-carcinogenic benzene and IgA levels in Oso Wilangun Tambak Fish Farmers' workers.

Material and Methods

The research subjects were all shoe craftsmen who were in Tambak Oso Wilangun Village, Surabaya with the inclusion criteria were workers in a healthy condition, workers who were not pregnant, and did not have the habit of drinking alcohol.

This study was a cross-sectional study with sampling using a total population (total sampling) of workers located in Tambak Oso Wilangun Surabaya Village, amounting to 20 people with research carried out in November-December 2017. Before data collection, this study was approved by the Faculty of Public Health's ethics committee, Airlangga University, Surabaya with ethics number 516-KEPK.

Independent variables were RQ of non-carcinogenic benzene, while the dependent variable was the IgA level of shoe craftsmen. Measurement of IgA levels was carried out by Immunoturbidimetric Assay by Parahita Laboratory with normal IgA standard values between 2-3 mg/ml. The subjects demographic data were also collected in the form of age, gender, length of work and length of service.

RQ was calculated by dividing the noncarcinogenic risk agent with its RfD or RfC with the equation [14], [15]:

$$RQ = \frac{I}{RfC \text{ or } RfD}$$

Notes:

I: non-cancer intake from the calculation of exposure (mg/kg/day);

RfC: reference concentration (mg/kg/day).

RfC value of benzene is 0.03 mg/m^3 for non-cancer effects and CSF benzene is 5.5 x 10^{-2} for the effect of cancer [16]. However, RfC value must be converted into units of mg/kg/day first by dividing it by the American default weight of 70 kg and multiplying it by the inhalation rate of 20 m^3/hour. So, we get the equation as below:

$$\text{RfC conversion} = 0.03 \frac{mg}{m3} x \frac{1}{70} kg \times 20 \frac{m^3}{day}$$

Calculation of intake was carried out in the exposure analysis by entering the variable values needed in the calculation. The data needed in the intake calculation were benzene concentration, intake rate, body weight, exposure time, the frequency of exposure, and duration of exposure. The concentration of benzene in the air was measured using Flame Ionization Detector gas chromatography (GC/FID) conducted by the Laboratory of the Occupational Health and Safety Technical Implementation Unit (UPT K3) Surabaya with a standard of 0.5 ppm [17]. All of these values were included in the intake formula as below:

$$\text{Intake} = \frac{C \times R \times t_E \times f_E \times D_t}{W_{bx} \, t_{avg}}$$

Notes:

I: Intake of the number of risk agents that individuals received per body weight per day (mg/kg/day); C: Concentration of risk agent mg/m^3; R: The intake rate, US-EPA default: 0.83 m^3/day; tE: Daily, hour/day exposure time; fE: Annual exposure frequency, day/year; Dt: Duration of exposure, real-time or 30 years (default lifespan projection) or 70 years (US-EPA life expectancy default); Wb: Weight, kg; t avg: Average time period, 30 years x 365 days/year (non-carcinogen) or 70 years x 365 days/year (carcinogens).

If the RQ value ≤ 1, then it shows the respondent exposed to benzene is still safe and has no health risk due to benzene exposure. Whereas, if the value of RQ > 1, then this indicates that respondents have health risks due to exposure to benzene.

Data analysis used analysis of Pearson Product Moment, Spearman 's Rank and Contingency Coefficient C with SPSS version 20. Correlation numbers ranged from -1 to +1. The closer to 1 the correlation is getting close to perfect. The interpretation of correlation numbers according to is if the value of r: 0-0.199: very weak; 0.20-0.399: weak; 0.40-0.599: medium; 0.60-0.79: strong and 0.80-0.10: very strong [18].

Results

The gender of men and women is equal to 10 people (50%) as shown in Table 1. Gender had an insignificant relationship with a decrease in IgA concentration (r = 0.386; p = 0.174) as shown in Table 2.

Table 1: Univariate Analysis of Research (n = 20)

Variable	n = 20 (%)	Mean	Min-Max
Demography Characteristics			
Age			
< 45 years	7 (35)	46.50	23-63 years
≥ 45 years	13 (65)		
Gender			
Man	10 (50)	-	-
Woman	10 (50)		
Working Periods			
< 25 years	9 (45)	25.575	2.5-43 years
≥ 25 years	11 (55)		
Working Time			
≤ 8 hours/day	6 (30)	10.25	6-14 hours/day
> 8 hours/day	14 (70)		
Benzene Concentration*			
> 0.5 ppm	2 (20)	-	0.0129 – 2.3330 ppm
< 0.5ppm	6 (80)		
Levels of IgA			
< 2 mg/ml	8 (40)	-	-
2-3 mg/ml	8 (40)		
> 3 mg/ml	4 (20)		
Risk of Non-Carcinogenic			
RQ < 1	7 (35)	-	-
RQ ≥ 1	13 (65)		

*except for the benzene concentration variable, benzene concentration was not measured individually but measured the location of the workplace in 8 locations.

The average age of shoeshine workers in Tambak Oso Wilangun Village was 46.6 years or 47 years. The highest number of workers were in the age range of 46-55 years (40%) as shown in Table 1. The results of the correlation analysis showed that age variables had a significant relationship with a decrease in IgA concentration (r = 0.494; p = 0.027) (Table 2).

The majority of subjects had a work period/duration of exposure ≥ 25 years (55%) (mean ± 25,575 years) as shown in Table 1. Working period variables had a significant relationship with a decrease in IgA concentration (r = 0.449; p = 0.047) as shown in Table 2.

As many as 30% of subjects had a working time of ≤ 8 hours/day, and as many as 14 people had a work duration > 8 hours/day (mean ± 10.25 hours/day; maximum = 6 hours/day; minimum = 14 hours/day) as shown in Table 1. However, the results of the correlation analysis showed that the working time variable had an insignificant relationship with a decrease in IgA concentration (r = - 0.244; p = 0.300) as shown in Table 2.

There were 2 measurement points (25%) with benzene vapour levels exceeding TLV (Threshold Limit Value) and 6 measurement points (75%) with benzene vapour levels below NAB (max-min = .01212 ± 2.3330 ppm) as shown in Table 1. Benzene levels at location 5 (measurement points 5 and 6) exceeded the threshold limit value set by Regulation of the Minister of Manpower and Transmigration No.13/MEN/X/2011. There was no significant relationship between benzene vapour levels and IgA levels (r = 0.313 and p = 0.179) as shown in Table 2.

The value of IgA concentration in blood serum workers in the footwear home industry in Tambow Osowilangun Surabaya, namely 8 workers (40%) experienced a decrease in IgA concentration, 8 workers (40%) had normal IgA concentrations and 4 workers (20%) experienced an increase in IgA concentration as shown in Table 1.

The RQ calculation for the 20 workers was obtained by RQ > 1, which was 65%, meaning that 13 people (65%) had the effect of non-cancer exposure due to benzene exposure as shown in Table 1. There was a significant relationship between the analysis of non-carcinogenic risk characteristics with a decrease in IgA levels (r = 0.567; p = 0.043) as shown in Table 2.

Table 2: Bivariate Analysis of Independent Variables with IgA Levels

Variable	*p-value*	*Pearson correlation* (r)
Age (years)	0.027*	0.494
Gender	0.174	0.386
Working periods (years)	0.047*	0.449
Working time (hours/day)	0.300	-0.244**
Benzene Concentration	0.179	0.313
RQ of Non-Carcinogenic Benzene	0.436	-0.184**

*p-value <0.05; **Correlation is negative with the intention that the independent and dependent variables are antagonistic, for example, the greater the RQ value, the lower the IgA level.

Discussion

The majority of subjects were more than 45 years of age. Age has a significant correlation with decreased IgA levels. This is consistent with other studies which state that one of the determinant factors that affect the immune system is age [19]. Older age is usually also accompanied by a decrease in resistance to toxins and viruses because it is followed by a decrease in the immune system both adaptive and innate immune response [20], [21]. Workers who

are less than 18 years of age should not work in an environment exposed to benzene, because the age of bone marrow resistance to the toxic effects of benzene is still low. The older age of labour, the higher the risk of benzene poisoning [22].

Gender does not have a significant relationship with decreased IgA levels. This is contrary to other studies stating that gender is one of the determinant factors that affect the immune system with women having a stronger immune system than men because of the presence of androgens in men that are immunosuppressive and do not fluctuate to old age [19]. However, this study is in line with other studies that show no effect of IgA values on female workers exposed to benzene in the shoe industry because there are still other variables that have a significant relationship with decreasing IgA concentrations in male workers in this study [23]. Benzene exposure in individuals is different. This is caused by several factors of each itself, including age, gender, weight, endurance, healthy life behaviour, length of exposure, the frequency of exposure, duration of exposure and work that has been done previously do [11].

Working period variables have a significant relationship with a decrease in IgA concentration. This is consistent with other studies stating that one of the factors that influence benzene exposure is the work period/duration of exposure [11]. Benzene exposure for each is influenced by the length of work/exposure time each day [11]. Conversely, the variable duration of work had an insignificant relationship with a decrease in IgA concentration. It can be assumed that there are still other variables that have a significant relationship with a decrease in IgA concentration.

The results of the study stated that the majority of benzene levels were still in the normal range (< 0.5 ppm) with only 2 location points that exceeded the standard limit. RQ calculation shows that the majority of respondents had RQ values greater than normal (RQ > 1). This is consistent with other studies that also in the shoe industry shows 60% of respondents have a normal RQ value above [24].

Toxins or benzene that enters through inhalation will get an initial immune response from lymphocytes and Antigen Presenting Cell (APC) in the lungs before finally being metabolised in the liver and bone marrow. Cells will present antigens (APC), so they can induce CD4-helper T cells which will morphofunctional as Th2-CD4 by inducing IL4. Benzene in the induction of T-CD4 cells is possible to directly cause T cell death and reduce the formation of mature B cells as a site for the formation of immunoglobulin A [19], [25], [26].

Benzene levels and non-carcinogenic risk assessment measurements did not have a significant relationship with the reduction of IgA levels. This has a difference with other studies which stated a decrease in serum IgA and IgG in workers exposed to painting or benzene [10]. The thing that makes the difference is that in this study, the majority of the location points studied is still at the safe benzene threshold. Although there are 2 places that have more benzene levels than TLV (location points 5 and 6), this is not comparable to the number of proportions in 6 locations that are still within normal limits. The limitations of this study also lie in the minimum sample in the study, which is 20 samples, which should be in the study of statistical or correlation analysis of a minimum of 30 research samples [18], [27].

These research also measured at eight locations whereas should be focusing on the riskiest locations. The volatile nature of benzene and the influence of the presence of air ventilation can also affect the absorption of benzene through inhalation [1], [28], [29], [30], [31]. The thing that affects again is the average working period for workers who are still 26 years old. The possibility of having a working period of around 26 years still has not shown the non-carcinogenic effects seen in haematological or decreased IgA levels, although the results showed that there had been a decrease of 8 workers. Therefore, further research is needed to give a new finding based on risk assessment such as calculating safe limits of workers not to be exposed to non-carcinogenic risks, calculation of Excess Cancer Risk (ECR) benzene and its association with IgA serum and other follow-up studies in the form of factors that influence the reduction of IgA in workers exposed to benzene.

In conclusion, the majority of research subjects were over 45 years of age, working ≥ 25 years, working time > 8 hours/day. There were 2 location points that had a threshold value exceeding the benzene standard (> 0.05 ppm), and 40% of subjects had decreased IgA levels. Age and tenure had a significant relationship to IgA levels, while benzene and RQ levels did not have a significant relationship with IgA levels. This research is still limited regarding small samples and benzene levels measurement is not based on per person/research subject but location. Further research is needed on risk assessment, especially the safe limits of benzene concentration in the workplace to find out how long the exposure to benzene poses non-carcinogenic and carcinogenic risks in the body of workers exposed to benzene.

Ethical Clearance

The study was approved by the institutional Ethical Board of the Public Health, Airlangga University with ethics number 516 KEK.

Acknowledgement

Thanks for Wulan Meidikayanti and Fathma Tualeka for helping to edit this article. Some of the results of this article have been previously published in the results of the thesis "Analysis of the Relationship of Exposure to Benzene Vapor and Trans Levels, Trans Muconic Acid Urine with Decreased Immunoglobulin A Workers of Shoe Craftsmen in Tambak Oso Wilangun Village" at http://repository.unair.ac.id/61400/

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Open Access Macedonian Journal of Medical Sciences. 2018 Dec 20; 6(12):2386-2392.
https://doi.org/10.3889/oamjms.2018.370
eISSN: 1857-9655
Public Health

Awareness of Systemic Lupus Erythematosus among Primary Health Care Patients in Riyadh, Saudi Arabia

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Citation: Bin Haikel KA, Al Tulaihi B. Awareness of Systemic Lupus Erythematosus among Primary Health Care Patients in Riyadh, Saudi Arabia. Open Access Maced J Med Sci. 2018 Dec 20; 6(12):2386-2392. https://doi.org/10.3889/oamjms.2018.370

Keywords: Systemic Lupus Erythematosus; Awareness; Health promotion; Rheumatology

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Received: 04-Oct-2018; **Revised:** 06-Nov-2018; **Accepted:** 07-Nov-2018; **Online first:** 19-Dec-2018

Funding: This research did not receive any financial support

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

Abstract

AIM: To measure the level of Systemic Lupus Erythematosus awareness among visitors in PHC at KAMC and to explores the factors which influence the Systemic Lupus Erythematosus awareness.

METHODS: The study was a cross-sectional study conducted between February and September 2018 in four primary health care centers belong to King Abdulaziz Medical City. The study participants were male and female adult visitors to the centers` age from 18 to 60 years of age. The sample size was 400 participants. The participants were enrolled via a random convenience sampling method. Study data was collected using a self-administered questionnaire. Analytic statistics were done using the Chi-square (χ^2) test for associations and/or the difference between two categorical variables. A P-value ≤ 0.05 was considered statistically significant.

RESULTS: The awareness about Systemic Lupus Erythematosus among male and female was not statistically significant as (P = 0.304), but there was a statistically significant difference according to education level. Visitors with high school education are aware of Systemic Lupus Erythematosus than those with a lower level of education (Primary & Middle school) who are not aware of the Systemic Lupus Erythematosus by (P = 0.023).

CONCLUSION: The study shows that this survey is valuable and beneficial to the community as it helps people to assess their knowledge about Systemic Lupus Erythematosus and become aware of this disease, as well as awareness of Systemic Lupus Erythematosus should be promoted among the community.

Introduction

Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease that occurs when the immune system assaults the tissues of the body and its organs. Systemic means that SLE can affect the body's various organs, including the skin, joints, brain, blood cells, kidneys, lungs, and heart. Lupus refers to the butterfly rash on the patient's face, which is similar to the whitish outline marking on the middle of wolves' faces. Erythematosus means the rash is reddish to purplish in color. Accordingly, four types of lupus can be identified: systemic lupus erythematosus, neonatal lupus, drug-induced lupus, and cutaneous lupus erythematosus. However, lupus erythematosus is the most common classification [1], [2].

The epidemiological studies was estimated that the prevalence of SLE was 241 per 100,000 people in North America which is the highest and the lowest incidence was 0.3 per 100,000 person yearly noticed in South Africa which is the lowest. SLE affected more women than men for all age and ethnic groups. Women of reproductive age (15-45) are often affected, and the rate of females to males is 9 to 1 [1], [2], [3].

Likewise, scientists trust that human genetic studies will structure our understanding of the premise of individual hereditary vulnerability to SLE. These studies will help to detect the person who will have a high probability of having SLE in the future [1].

Treatment of SLE will help to control disease progression and inhibit further complications. However, at the moment, there is no definitive medication or drug for treating SLE. SLE is usually very active when diagnosed for the first time. At this stage, high doses of drugs like glucocorticoids may be required to restrain the disease's progression and stop organ harm, and this is the cornerstone of the treatment. However, despite advanced treatment SLE-associated infection and renal failure still increase mortality and this remains a major burden in management plans for patients [5], [6].

Addressing patient worries and fears about drug therapy, including treatment costs, side effects, and complications can increase the treatment adherence, fulfillment, and enhanced wellbeing results alongside psychological and behavioral interventions, which can be effective for decreasing weariness as well as enhancing strength in patients with SLE. Social help can assist patients with SLE accomplish positive thinking and a feeling of becoming stronger.

Despite numerous advances in the diagnosis and treatment of SLE and associated comorbid conditions, such as loss of physical, social, and emotional functioning, this disease remains a source of significant morbidity and mortality. Indeed, even with the expected changes in general mortality over the last five decades, a patient diagnosed at 20 years of age still has a one in six chance of dying by the age of 35 and a higher probability of a shortened lifespan; in addition, SLE has a severe and pervasive influence on patients' self-esteem, resulting in emotional challenges, fear of rejection, stigmatization, social isolation, and loss of independence [8], [9].

On the other hand, there are some studies that stress the importance of raising awareness and educating the public about SLE by clarifying the nature of the disease, its direct causes, treatment methods, and control mechanisms; for example, awareness activities directed to both the overall society and health care workers in particular can advise them to reduce delays in diagnosis and ensure early transfer to specialist care.

In Riyadh, Saudi Arabia a study conducted in 2015 among students at King Saud University showed that the level of awareness about SLE among students is low. Workshops or campaigns are required to enhance awareness and correct misunderstandings [10].

In Taif, Saudi Arabia a study undertaken in 2017 that involved many of the Kingdom's regions reported a high prevalence of less than optimal awareness and knowledge of SLE among the Saudi adult population [11].

In India, a study (2017) among the general population to assess their SLE awareness and knowledge found that the majority of participants had insufficient knowledge of its status as a rare disease that occurs in the population [12].

A 2018 study conducted among students at King Faisal University in Al-Hassa, Saudi Arabia found that most students have low awareness and some misunderstandings regarding SLE. Awareness programs are presumed able to increase awareness about SLE, as these campaigns have confirmed that greater public awareness may be useful in managing and controlling the disease and its associated complications [13].

Along with awareness activity, qualitative research is thought to be able to elicit patient knowledge and viewpoints, such as patients' philosophies, misconceptions about the disease, and perceptions about new therapy or interventions, including *monoclonal antibodies* (mAb) therapy or immuno-suppression medications [7].

The main aim of the study is to evaluate the awareness of SLE among patients to primary health care clinics at King Abdulaziz Medical City (KAMC) in Riyadh and highlight the factors that influence SLE awareness, health promotion, information needed, and educational support.

Methods

The study was a cross-sectional study conducted between February and September 2018 in four primary health care centers belonging to KAMC: the Health Care Specialty Center (HCSC); King Abdulaziz Housing Clinics (Iskan); the National Guard Comprehensive Specialized Clinic (NGCSC), and King Abdulaziz Housing Clinics (Dirab).

Each of the four centers delivers primary curative and preventive health services, and consists of walk-in services, a minor emergency room setting, and an appointment booking system for patients to obtain treatment and counseling for acute and chronic medical conditions. The study participants were male and female adult visitors to the centers aged between 18 and 60.

The sample size was calculated by the Openepi website. The population of the KAMC-PHC as per the latest report was found to be around 420,000 people in 2017. They were distributed as follows: 200,000 patients in the HCSC, 100,000 patients in the NGCSC, 70,000 patients in King Abdulaziz Housing Clinics (Dirab), and 50,000 patients in King Abdulaziz Housing Clinics, (Iskan). Using a 95% confidence interval and 5% margin of error, the calculated minimum sample size was expected to be 384, which was modified to 400 to compensate for incomplete questionnaires.

The proportion of participants from each of the four centers was based on the clinic catchment

area as follows: HCSC serves a population of around 200,000 people, thus accounts for approximately 47.6% of the sample size, equal to 190 participants; NGCSC serves around 100,000 people, accounting for approximately 23.8% of the sample size, equal to 95 participants; Dirab center serves around 70,000 people, accounting for approximately 16.6% of the sample size, equal to 67 participants; and Iskan serves around 50,000 people, accounting for approximately 11.9% of the sample size, equal to 48 participants.

Study data were collected using a self-administered questionnaire. The questionnaire used was developed by Sullivan [2], and was adopted by the researchers to meet the study main objectives after a literature review of similar studies.

The questionnaire was written in simple Arabic, and included the following sections: demographic data, and questions about knowledge, attitudes, and beliefs regarding SLE. Two research experts revised the questionnaire, and a pilot study was conducted with ten participants to confirm and verify the instrument's validity.

The participants were enrolled via a random convenience sampling method. Patients to the four primary care centers were approached while sitting in the waiting area. The investigator explained the purpose of study to each participant and obtained verbal and documented consent to participate in the study before administering the questionnaire. All data were coded, entered, and analyzed using the Statistical Package for the Social Sciences, version 23 (SPSS 23). Continuous variables were reported in terms of means and standard deviation, while categorical variables were described using frequencies and percentages. Analytic statistics were performed using the chi-squared (χ^2) test for associations and/or the difference between two categorical variables. A *P* value of ≤ 0.05 was considered statistically significant.

Approval for the study was obtained from King Abdullah International Medical Research Center, the Ministry of the National Guard, Kingdom of Saudi Arabia. The participants were fully informed regarding voluntary participation, privacy, anonymity, and confidentiality of the data were ensured and the Declaration of Helsinki ethical rules were followed.

Results

Table 1 show that the majority of PHC patients included in the study were female (58.7%), while males made up 41.3% (Figure 1A).

Table 1: Frequencies, distribution, and percentages of socio-demographic variables

VARIABLES	NO	%
Sex		
Male	165	41.3
Female	235	58.7
Age		
Mean (SD)	35.89 ± 12.04	
Min age	25	
Max age	66	
Occupation		
Student	43	10.8
Housewife	91	22.8
Government employee	124	31.0
Non-government employee	50	12.4
Unemployed	72	18.0
Retired	20	5.0
Education		
Primary school	18	4.5
Middle school	10	2.5
High school	190	47.5
College	23	5.8
Graduated	93	23.3
Post-graduation studies	21	5.3
Can read and write name only	16	4.0
Non-literate	29	7.3
Marital status		
Single	131	32.8
Married	203	50.7
Widowed	22	5.5
Divorced	44	11.0
No. of dependent family members		
0-1	169	42.3
2-3	62	15.4
4	35	8.8
5	31	7.8
6	56	14.0
≥ 7	47	11.7

The mean age of patients was 35.98 yrs. old (± 12.04) with most being government employees (31.0%), followed by housewives, the unemployed, non-government employees, students, and the retired at 22.8%, 18%, 12.4%, 10.8%, 5%, respectively (Figure 1B).

The majority of PHC patients (47.5%) included in the study had some high school; they were followed by graduates, the non-literate, college students, post-graduates, primary school, those able to read and write only their names, and those with middle school at 23.3%, 7.3%, 5.8%, 5.3%, 4.5%, 4%, and 2.5%, respectively (Figure 1C).

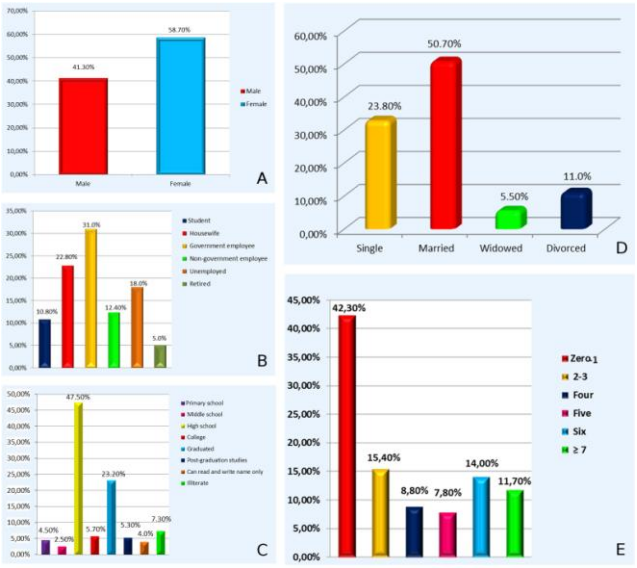

Figure 1: Distribution of visitors according to A) gender; B) occupation; C) education; D) marital status; E) number of dependents

Most study participants were married (50.7%), while single, divorced, and widowed participants

constituted 32.8%, 11%, and 5.5%, respectively (Figure 1D).

The majority of participants (42.3%) had no or only one dependent family member while those with 2–3, 4, 5, 6, or equal to or more than seven made up 15.4%, 8.8%, 7.8%, 14%, and 11.7% of the participants, respectively (Figure 1E).

As shown in Table 2**,** 56.8% of patients had heard the term "SLE." The most common way participants recognized the disease was from the internet (online resources) (35.2%) as the majority of them had not been diagnosed with SLE (85.5%).

Table 2: Awareness of systemic lupus erythematosus frequencies and percentages

NO	Questions	N	%
	Have you ever heard of the term "systemic lupus erythematosus"?		
7	Yes	227	56.8
	No	148	37.0
	Don't know	25	6.50
	Through which of the following sources did you hear about systemic lupus erythematosus? (You can choose more than one option)		
	Heard from a doctor	31	13.7
	Internet/online resources	80	35.2
8	TV	11	4.9
	Radio	14	6.2
	Newspaper or magazines	8	3.5
	Friend or colleague	65	28.6
	Other (specify) _____	18	7.9
	Have you been diagnosed with systemic lupus erythematosus?		
9	Yes	58	14.5
	No	343	85.5
	Do you know anyone who has systemic lupus erythematosus?		
10	Family member	44	11.0
	Relatives	62	15.5
	Colleague	46	11.5
	No one	246	61.5
	Others (please specify) _____	2	0.5
	Is systemic lupus erythematosus contagious?		
11	Yes	99	24.8
	No	108	27.0
	Don't know	193	48.2
	Is systemic lupus erythematosus fatal?		
12	Yes	141	35.3
	No	85	21.2
	Don't know	174	43.5
	Systemic lupus erythematosus is an autoimmune disease, which means:		
13	There is no cause (the body fights itself)	103	25.8
	There are tumors	64	16.0
	It is hereditary	119	29.8
	Don't know	110	27.4
	Other (please specify) _____	4	1.0
	Does systemic lupus erythematosus mostly affect females?		
	Yes	156	39.0
14	No	46	11.5
	Don't know	198	49.5
	Does systemic lupus erythematosus affect any organ/part in the body?		
15	Yes	174	43.5
	No	48	12.0
	Don't know	178	44.5
	What organs can be affected by systemic lupus erythematosus? (You can choose more than one option)		
	Kidney	106	26.8
	Blood	29	7.3
	Heart	23	5.8
	Eyes	5	1.3
16	Liver	47	11.8
	Skin	30	7.5
	Joints	3	0.8
	Lungs	64	16.0
	All of above	92	23.0
	Don't know		
	Others (Please specify) _____		
	The following are symptoms of systemic lupus erythematosus.		
	Rash	57	14.3
	Alopecia	42	10.5
	Joint pain	59	14.8
	Hematuria	9	2.3
17	Photosensitivity	12	3.0
	Don't know	114	28.5
	All of the above	107	26.8
	Systemic lupus erythematosus can be diagnosed with a single blood test.		
	Yes	139	34. 8
	No	95	23.7
18	Don't know	166	41.5
	Systemic lupus erythematosus can be prevented.		
19	Yes	162	40.5
	No	79	19.7
	Don't know	159	39.8
	Systemic lupus erythematosus is a treatable disease.		
20	Yes	165	41.2
	No	83	20.8
	Don't know	152	38.0
	Systemic lupus erythematosus is an illness with few complications.		
21	Yes	157	39.2
	No	82	20.6
	Don't know	161	40.2
	The treatment for of systemic lupus erythematosus is:		
	Chemotherapy	104	26.0
	Steroids	73	18.2
22	Malaria medications	77	19.3
	Combination of above medications	47	11.7
	Others	1	0.3
	Don't know	98	24.5
	Can lupus erythematosus disease affect the fertility of men and women?		
	Yes	165	41.3
23	No	59	14.8
	Don't know	176	44.0
24	**Systemic lupus erythematosus causes fetal abnormalities or recurrent abortions in the affected mother.**		
	Yes	201	50.3
	No	52	13.0
	Don't know	147	36.7
	Should awareness of systemic lupus erythematosus be promoted?		
25	Yes	345	86.3
	No	9	2.3
	Don't know	46	11.4

Most of the participants did not know anyone with SLE (61.5%) and the majority did not know that SLE is not contagious (48.2%) but could be fatal (43.5%). Most believed that SLE was a hereditary disease and classified SLE in this way (29.8%).

The majority did not know SLE mostly affects females (49.5%). They also did not know that SLE can affect any organs in the body (44.5%). Indeed 26.8% believed that only the kidney could be affected by systemic lupus erythematosus.

Most of the patients did not know the major symptoms of SLE (28.5%) and 41.5% did not know the main laboratory tests for SLE; 40.5% did not know whether SLE could be prevented although most believed that SLE is a treatable disease (41.2%).

The majority of participants did not know the complications of SLE (40.2%) and 26% believed that a treatment of SLE was chemotherapy. Most did not know that SLE can affect the fertility of men and women (44.0%), although they knew that SLE causes fetal abnormalities or recurrent abortions in the affected mother (50.3%).

Finally, the majority of participants believed that awareness of SLE should be promoted (86.3%); the score for awareness of SLE (n = 400) indicated that 64.7% of participant were unaware of SLE, while 35.3% were aware of SLE.

Table 3: Correlations between socio-demographic variables and awareness

Socio-demographic variables	Awareness of systemic lupus erythematosus				$\chi 2$	P value
	Aware		Unaware			
	No	%	No	%		
Gender						
Male	63	44.7	102	39.4	1.058	0.304
Female	58	54.9	157	60.6		
Education						
Primary school	12	8.5	31	12.0		
Middle school	28	19.9	63	24.3		
High school	42	29.8	82	31.7		
College	21	14.9	29	11.2		
Graduated	28	19.9	44	17.0	9.457	0.023*
Post-graduation studies	10	7.1	10	309		
Can read and write name only	0	0	0	0		
Illiterate	0	0	0	0		
Do you know anyone who has systemic lupus erythematosus?						
Family member	16	11.3	28	10.8		
Relatives	18	12.8	44	17.0		
Colleague	15	10.6	31	12.0	10.082	0.020*
No one	92	65.2	154	59.5		
Others (please specify)	0	0	2	0.8		

Table 3 shows the correlations between socio-demographic variables (gender, education, do you know anyone who has systemic lupus erythematosus?) and awareness of SLE. Awareness about SLE by gender was not statistically significant (P = 0.304), but there was a statistically significant difference according to education level. Participants with high school education were more aware than those with a lower level of education (primary and middle school) (P = 0.023).

Furthermore, there was a statistically

significant correlation between knowing a person with SLE and awareness (P = 0.020); this means that if the participants did not know a person with SLE before they will be unaware of SLE and its nature or manifestations.

Thus, the major factors that affect SLE knowledge and attitude are education level and knowing someone who have been diagnosed previously with SLE and is coping with it.

Discussion

The main aim of this study was to assess the awareness of the patients in the primary heath care of KAMC about SLE, its clinical manifestations, diagnosis, treatment, complications, and comorbidities as well as explore the possible factors that influence SLE awareness, such as adequate health promotion and clinical awareness-raising.

The study was carried out in four primary health care centers belonged to KAMC in Riyadh. The centers are the HCSC; King Abdulaziz Housing Clinics (Iskan); the NGCSC, and King Abdulaziz Housing Clinics (Dirab). As found in a similar local study [10], 40% have previously heard the term SLE. As well as the 2nd local study [13], 55.9% have previously heard the term SLE.

The 400 participants in this study were chosen from the waiting area in the clinics using random convenience sampling. The fact that females are more frequently reported having the disease than males. This is attributable to the fact that women are more expected to get SLE than males. Most of the participants were government employees (31.0%) and the majority had studied at high school; 50.7% of participants were married with/without dependent family members.

The study clarifies that 56.8% of participants had heard the term “systemic lupus erythematosus” and the most common way that they recognized the disease was through the internet (online resources).

The vast majority had not been diagnosed with SLE and did not know that SLE is not contagious and fatal. However, they believed that the kidney is the only organ that can be affected by SLE. As found in a similar local study11, more than 50% of participant believed that SLE don’t have renal manifestation while some study conclude that one of major reason of death among SLE patients is renal failure.

The onset of the disease is slow with new symptoms occurring over weeks, months, and sometimes years. The most common initial symptoms of lupus in patients are unspecified complaints of fatigue and health malaise. Many patients experience sporadic or persistent high temperatures with gradual loss of weight and poor appetite. Ultimately, patients progress to developing certain clinical symptoms because one or more parts of the body are affected. Skin and mucous membranes are usually the most affected by the disease. Symptoms may include various forms of skin rash, photosensitivity, and mouth ulcers. In one-third of the affected patients, the characteristic butterfly rash occurs in the center of a patient`s face [4], [5].

SLE has been found to have serious complications in previous studies, which range from renal failure to death. Early death is common in males with skin manifestations who are under 16 years old at the time of diagnosis while the possibility of late death is frequently seen in patients over 30 years at the time of diagnosis. Overall death was significantly high in older patients and in those with established kidney disease [6], [7].

In terms of SLE diagnostic patterns, the participants did not know the possible laboratory tests for diagnosing SLE; they believed that SLE is a treatable sickness where the main treatment is chemotherapy. Moreover, a great number did not know the minor or major complications of SLE. Diagnosis depends on the availability of a range of signs and symptoms with some laboratory tests involving blood and urine, and sometimes biopsy to exclude further disease. Symptoms and signs sometimes do not appear observable at any time, which makes it difficult to diagnose the disease in a timely manner. The American College of Rheumatology has listed 11 criteria to differentiate SLE from other diseases; if many of them are reported in clinical settings they may indicate the presence of SLE. This criterion roughly signifies the common clinical symptoms of people with this disease. To diagnose this disease, at least four of these 11 criteria must be met at any given time. However, experienced physicians are able to diagnose it even if patients present with fewer than four criteria.

Most of the participants in our study were unaware of SLE. This indicates poor knowledge about the disease. There were no statistically significant differences in awareness about SLE between males and females (P = 0.304), but there was statistically significant differences according to education levels (P = 0.023), and between knowing a person who had been diagnosed with SLE and caring for him or her (P = 0.020).

Finally, most participants believed that awareness of SLE should be promoted in the community, whether in hospitals or schools, to enhance knowledge about the disease and its nature in a different way; it was recommended that clinical workshops be conducted, short videotapes or medical flyers be used, and face-to-face discussions be held along with support groups and online resources.

Limitations: This study was geographically limited and only included the patients to primary healthcare clinics at KAMC in Riyadh. Therefore, it may not be possible to generalize the results. Recall bias is also possible.

*Recommendations:*There is a need for public awareness activities to educate people about SLE, its clinical manifestations, diagnosis, treatment, and complications; although it is not a contagious disease that requires isolation, it may be fatal if neglected.

In conclusion, the study shows that this survey is valuable and beneficial to the community as it helps people to assess their knowledge about SLE and become more aware of the disease.

Acknowledgments

A sincere appreciation to King Abdulaziz Medical City and to the participants who supported the research. Furthermore, we would like to thank eScienta (www.eScienta.com) for English language editing.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Dec 20; 6(12):2393-2394.
https://doi.org/10.3889/oamjms.2018.453
eISSN: 1857-9655
Letter to the Editor

Neutrophilic Dermatosis in Pregnancy: An Uncommon Course

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Citation: Villani A, Fabbrocini G, Napolitano M, Costa C, Megna M, Ferrillo M. Neutrophilic Dermatosis in Pregnancy: An Uncommon Course. Open Access Maced J Med Sci. 2018 Dec 20; 6(12):2393-2394. https://doi.org/10.3889/oamjms.2018.453

Keywords: Pyoderma gangrenosum; Pregnancy; Subcorneal pustular dermatosis; Neutrophilic dermatoses

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Received: 05-Sep-2018; **Revised:** 26-Oct-2018; **Accepted:** 27-Nov-2018; **Online first:** 18-Dec-2018

Funding: This research did not receive any financial support

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

Abstract

Pyoderma gangrenosum (PG) is a neutrophilic-mediated inflammatory skin disease characterised by the rapid onset of painful, hemorrhagic pustules developing into necrotic ulcers occurring predominantly in women aged 20-50 years. According to the literature, all patients reported no change or worsening of the disease during pregnancy. We herein present the case of a 34-year-old woman that developed a neutrophilic dermatosis of the hand reporting complete resolution of the skin disease during pregnancy.

Dear Editor,

Pyoderma gangrenosum (PG) is a neutrophilic-mediated inflammatory skin disease characterised by the rapid onset of painful, hemorrhagic pustules developing into necrotic ulcers [1]. PG occurs predominantly in women aged 20-50 years. Its onset can be spontaneous or can be triggered by surgery and traumas (pathergy phenomenon) [2]. To date, only a few cases describing pyoderma gangrenosum during pregnancy have been published, and no therapeutic approaches for the treatment of PG in pregnant women have been validated. According to the literature, all patients reported no change or worsening of the disease during pregnancy, and the majority of them reported immediate postpartum disease flares. We report the case of a 34-year-old woman with a 4-year history of subcorneal pustular dermatosis that came to our attention in 2017 for the onset of an ulcerated lesion with violaceous borders and surrounding erythema on the right hand. Similar lesions were also present on the lower extremities (Figure 1). According to clinical and histopathological findings were consistent with neutrophilic dermatosis of the hand [3]. The patient performed several traditional topical and systemic therapies: topical and oral corticosteroids (prednisone 50 mg/daily for a month); cyclosporine (150 mg/daily) in association with tacrolimus 0,1% ointment for five months with partial improvement of skin manifestations. On December 2017, the patient informed us that she was in the 7th week of her third pregnancy. So, we decided to interrupt cyclosporine treatment. Complete laboratory and instrumental tests were performed, and no abnormalities were documented. Despite the interruption of therapy, the patient showed a total resolution of the disease during the second trimester of pregnancy. She had a healthful delivery in June 2018 (Figure 2). To date, the patient is still without therapy, and no recurrence of the disease has been noticed [4]. Pyoderma gangrenosum is rarely associated with pregnancy. The underlying mechanism of the association between PG and pregnancy is still unknown, but an

alteration in the immune system during pregnancy might be a common factor. Pregnant women show a progressive neutrophilia during pregnancy, due to the increasing levels of proinflammatory factors, such as granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage-colony-stimulating factor (GM-CSF) and T helper (Th) - 17, which may explain neutrophil hyper-reactivity [5].

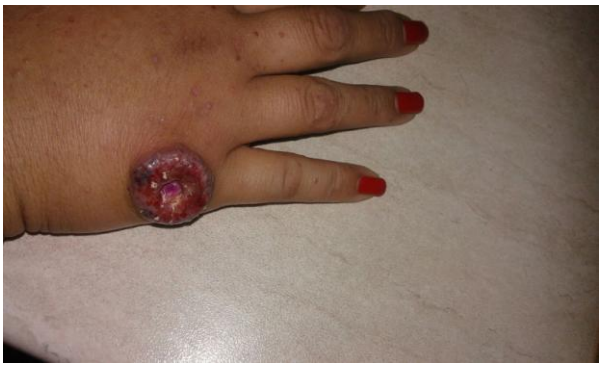

Figure 1: Pyoderma gangrenosum. An area of ulceration with violaceous borders and surrounding erythema

According to the literature, a wide range of treatment options for neutrophilic dermatoses is available with usually a positive response and almost complete remission of the disease. As reported in the literature, most cases occurred during the second and third trimester of pregnancy or after caesarean section.

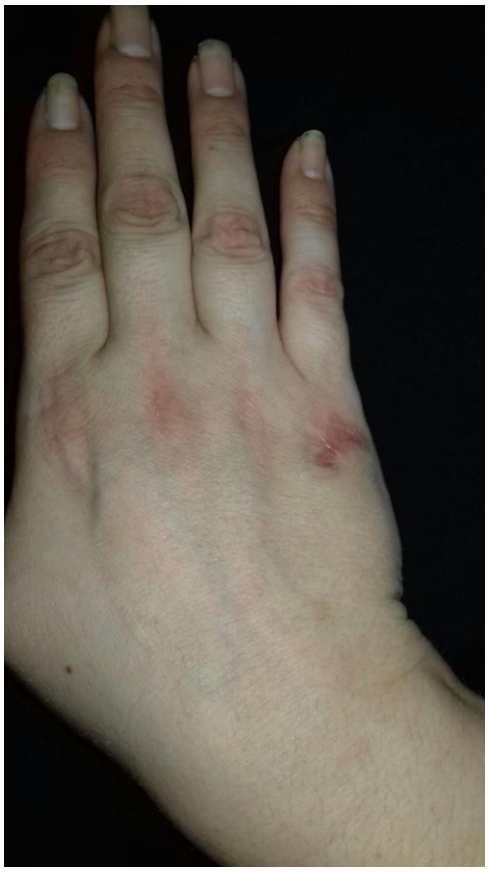

Figure 2: Outcome of pyoderma gangrenosum after pregnancy

Our patient reported a complete remission of the disease also after the caesarean section and is, to the best of our knowledge the first reported case with remission of the neutrophilic dermatosis of the hand during and after gravidity. Although several cases regarding the onset of neutrophilic dermatosis during gestation period has already been described, there are no validated guidelines, and consensus in management is lacking.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Dec 20; 6(12):2395-2401.
https://doi.org/10.3889/oamjms.2018.371
eISSN: 1857-9655
Dental Science

Computer-Aided Design/Computer-Aided Manufacturing Cutting Guides for Odontectomy of Deeply Impacted Mandibular Third Molars

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Citation: Ahmed M, Salah MK, Khairy N. Computer-Aided Design/Computer-Aided Manufacturing Cutting Guides for Odontectomy of Deeply Impacted Mandibular Third Molars. Open Access Maced J Med Sci. 2018 Dec 20; 6(12):2395-2401. https://doi.org/10.3889/oamjms.2018.371

Keywords: Deeply impacted mandibular third molar (DIMTM); Inferior alveolar nerve (IAN) injury; Computer guided cutting guide; External oblique ridge

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Received: 06-Oct-2018; **Revised:** 06-Nov-2018; **Accepted:** 09-Nov-2018; **Online first:** 19-Dec-2018

Funding: This research did not receive any financial support

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

Abstract

AIM: To evaluate a new technique for surgical removal of deeply impacted mandibular third molars (DIMTM), using computer-guided cutting guide to maintain inferior alveolar nerve (IAN) integrity and the covering buccal bone.

PATIENTS AND METHODS: Eighteen cases indicated for removal of DIMTM. Cone-beam Computed Tomography (CBCTs) used to determine the tooth's relation to the IAN. Computer-guided software used for fabrication of surgical cutting guide stent to expose the impacted tooth and repositioning of bone after odontectomy without fixation. Clinical assessment included a neurosensory deficit of IAN, pain using a visual analogue scale (VAS), facial swelling, and maximal mouth opening (MMO). CBCTs were taken immediately and six months postoperatively to evaluate position and healing of bone.

RESULTS: None of the patients showed a permanent neurological deficit of IAN while all patients showed normal parameters of pain, facial swelling and MMO.

CONCLUSION: this technique has shown the accurate determination of the bony window cuts with subsequent preservation of IAN and external oblique ridge.

Introduction

Impaction of mandibular third molars is a commonly encountered condition that often proves to be problematic and with a higher incidence of iatrogenic complications during its removal. Newly published data revealed that 72, 2% of the entire world's population has at least one impacted tooth (usually lower third molar) [1], [2], [3], [4]. Santosh P in 2015 [5] stated that IAN injury post third molar extraction is a well-recognised complication with the reported risk of 0.26–8.4%. Tingling, numbness, burning or throbbing sensations in the ipsilateral lower lip, chin and gingiva are customary manifestations. The contributing factors reported include; age, infection history, rotatory tool utilisation, operator skills and location of the impaction. However, the most important factor is the anatomic approximation of the molar roots to the nerve [5], [6], [7].

Nowadays 3d cone beam computed tomography (3D CBCT) are becoming preferable for proper treatment planning and assessment of difficulty indices to digital panoramic radiographs [8], [9].

Numerous methods were proposed for surgical removal of deeply impacted mandibular wisdom including the lingual split-bone technique, coronoidectomy technique, orthodontic extraction technique and even an extra-oral approach.

Nonetheless, the two most encountered complications are still excessive bone removal and high possibility of inferior alveolar nerve (IAN) affection [10], [11], [12], [13], [14], [15], [16].

To simultaneously preserve the buccal bone at the external oblique ridge and afford accessibility for deeply impacted third molars; many case reports have been published that suggested sagittal split ramus osteotomy (SSRO) for odontectomy, followed by fixations of bony segments [18], [19], [20], [21].

The technique published by Ahmed M. in 2016 [22] was innovative in combining two different surgical procedures through harvesting the overlying buccal plate of bone at the external oblique ridge and its relocation after odontectomy without fixation. Although CBCTs were suitable in determining the position and relation of impacted molars to the IAN, linear measurements were made and transferred to the rectangular design of the osteotomized bony segment targeting to expose the impacted tooth till its furcation adequately. However, determining the position of the osteotomy and the angulation was based on arbitrary measurements [22].

Computer-aided design/computer-aided manufacturing (CAD/CAM) techniques are virtual planning software tools which provide anatomically accurate samples of a patient's hard tissue structures. The CAD/CAM cutting guides fabrication can provide adequate information regarding proper cutting planes and maintain the IAN integrity; thus, they may help in treatment planning and outcome prediction of a wide variety of oral and maxillofacial surgeries [22], [23], [24].

In the present study, we aimed to evaluate CAD/CAM cutting guide fabrication for simple osteotomy positioning and segment realignment during odontectomy of deeply impacted third molar tooth for adequate tooth exposure and maximum nerve protection.

Patients and Methods

We confirm that the present study runs in concordance with international ethical guidelines and applicable local regulatory laws. The ethical approval was obtained from the institutional review board (IRB) of Oral and Maxillofacial Surgery Department, Faculty of Dentistry, Cairo University (ethical approval number: 17-11-10). Informed written consent was obtained from every eligible patient before study enrollment.

The present prospective cohort study was conducted on eighteen cases selected from outpatient clinics of the Oral and Maxillofacial Surgery Department, Faculty of Dentistry, Cairo University. The sample size was determined by the medical biostatistics unit (MBU) in the Faculty of Dentistry, Cairo University. Patient's age ranged from 20 to 29 years with a mean of 24.5 years. We included adult patients who met the following criteria: 1) patients with deeply impacted mandibular third molar tooth who were complicated for extraction according to Juodzbalys and Daugela [3]; 2) patients who were classified as American Society of Anesthesiologist's (ASA) [1]; and

3) patients who were fit for surgical removal under general anesthesia. Patients with medically compromised conditions or pregnant women were excluded from the study.

Preoperatively, patients were subjected to detailed medical history and full clinical examination. Then, preoperative CBCTs (Scanora 3D Soredex Finland 85kv-15Ma) were obtained for diagnosis, determining IAN proximity, and developing the cutting guide fabrication respectively (Figure 1A and B).

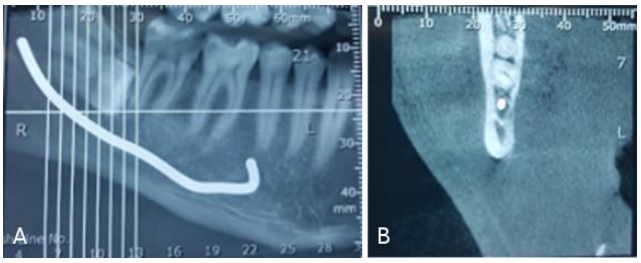

Figure 1: A) and B) Radiographic pictures of pre-operative CBCTs to determine the position of the impacted molar about the IAN

The CBCT-based DICOM images were imported into virtual surgical planning software (Mimics Edition15, Materialise Innovations, Leuven, Belgium). The area of interest at the mandibular external oblique ridge was delineated, and three virtual cutting (two vertical and one horizontal) planes were designed simulating the positions of the two vertical and inferior osteotomies of the bony window. These virtual cuts were planned guided by the position of the impacted molar about the IAN, and the adjacent second molar aiming to expose the crown till the furcation area (Figure 2A).

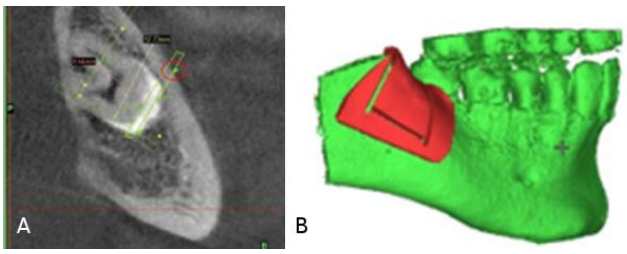

Figure 2: A) CBCT as DICOM files with virtual surgical planning determining the vertical cutting plans; B) Matic software with bone – borne cutting guide with the virtual cutting slots

The two vertical cuts were planned with lingual convergence. This step was followed by exporting the surgical plan to the next specialised software, 3-Matic (Materialise Innovations, Leuven, Belgium), this software was used to design a bone – borne cutting guide with the three virtual osteotomies slots (Figure 2B). 3D printing by Fused Deposition Modeling (FDM) technique (Ultimaker 2 3D printer, model number: UM2) was used to fabricate a 2 mm thick guide of polylactide (PLA); this thickness offered enough rigidity while not interfering with the radius of the cutting disc. The overall average working time of virtual planning and 3D printing was 70-85 minutes.

The cutting guide was designed by the Corresponding author using Mimics Edition15 and 3-Matic software. It took 20-30 minutes to finish the design, and the printing of the cutting guide cost about 15 dollars for each case.

All surgical procedures were performed by the same operator, to minimise the technical variables. Preoperatively, patients received a loading dose (1.5 gm) of Ampicillin/Sulbactam intravenously and local infiltration of 4% articaine HCL with 1:100,000 vasoconstrictor (Septanest SP, Septodent pharmaceutical Industries, France). A three-line mucoperiosteal flap was performed to expose the field. The surgical guide was fixed in place using a single 2.0 mini screw (Figure 3A). Two vertical cuts with lingual convergence were made through the buccal bony plate, using a reciprocating saw (Figure 3B). The inferior horizontal cut was performed perpendicularly or with slight downward divergence, connecting the two vertical cuts, using 0.3mm thickness FRIOS Micro Saw disc. The radius of the Micro Saw disc is 3.2 mm; this permitted monocortical bone osteotomy (Figure 3C). To finish off the rectangular design, the surgical guide was removed, and the superior horizontal cut was done using the reciprocating saw. Lastly, the disc and saw were used to revise all the cuts. Adopting the same technique of Ahmed M [22], chisels were used for splitting the bony window (Figure 3D and E).

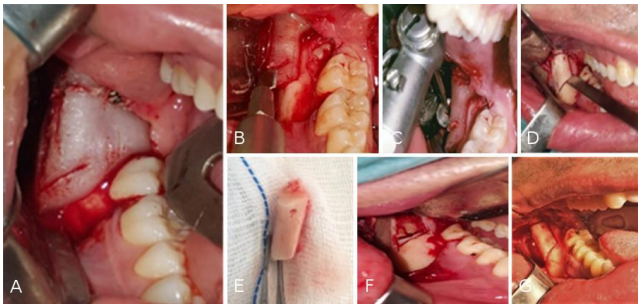

Figure 3: A) The surgical cutting guide in place; B) the reciprocating saw; C) The FRIOS micro saw disc; D) The bony window cuts and the chisel for splitting the segment; E) The osteotomized bony segment; F) The impacted molar with the furcation after separation; G) The repositioning of the osteotomized bony segment after odontectomy

Following odontectomy, the surgical site was debrided, irrigated; and accurate repositioning of the osteotomized bone segment was carried out without fixation (Figure 3F and G). Closure of the flap was performed with 3-0 Vicryl sutures. Postoperative instructions were given, and patients were prescribed antibiotics, analgesics, and mouthwash.

Patients were followed-up postoperatively at 3, 7 and 14 days. Neurosensory testing, including an objective assessment of patients using the pinprick and light touch tests, were used, at 7th day postoperatively and the results were categorised as normal or abnormal [25]. The study's outcomes were:

1) The radiographic parameters for the osteotomy position and bone healing which were evaluated using CBCTs immediately and six months postoperatively.

2) The severity of postoperative pain using visual analogue scale (VAS), the VAS is a psychometric 0-10 scale with '0' being 'No Pain' and '10' being 'Most Severe Pain [26].

3) Facial swelling was recorded using 2-0 nylon threads and a millimetre ruler. Measurements were done bilaterally from the tip of the tragus to the gonium, three times for each patient and the average value recorded in centimetres [27].

4) Maximal mouth opening (MMO) which was measured using a digital millimetre scale pre and postoperatively on the 3rd, 7th and 14th day, values were recorded in centimetres [27].

5) Incidence of alveolar osteitis and wound dehiscence.

Data were analysed using SPSS software (Statistical Package for the Social Sciences, version 24, SSPS Inc, Chicago, IL, USA). Frequency tables with percentages were used for categorical variables, and descriptive statistics (either means with standard deviation or median with range) were used for numerical variables, according to the normality of the data. Paired t-test was used to compare the change in VAS scores, while repeated measures analysis of variance (ANOVA) test was used to compare facial swelling and the change in MMO. This was followed by Tukey's post hoc test when ANOVA revealed a significant difference for numerical variables. A p-value less than 0.05 was considered statistically significant.

ANOVA was used for statistical analysis as the data were numerical data about facial swelling and maximal mouth opening while paired t-test was used for non-numerical data about visual analogue scale.

Results

The present study included 18 cases with a mean age of 24.5 ± 3.4 years. The majority of patients were females (61%). Intraoperatively, the cutting guide fit accurately in all cases without additional adjustment or further flap extension. The bony segment at the external oblique ridge was successfully harvested as one piece in all cases without fracture, offering adequate exposure of the crown to the root furcation. After odontectomy, the harvested bone was repositioned and was stable and self-retentive with no need for fixation. Uneventful primary wound healing was observed in all patients; no signs of infection, flap dehiscence or bone exposure were seen. Postoperatively, lower lip paresthesia was only encountered in three patients, probably owing to pressure during root removal. The patients were followed up until recovery which was achieved within three months.

Table 1: VAS score pre-and post-operatively and significance of the difference using Paired t-test for Equality of Means

VAS Time	Mean	SD	T	P value
Pre-operative	5.6667	2.30089	5.279	< 0.0001*
14th day post-operative	2.3333	0. 7199		

Significance level p < 0.05, * significant

Regarding the pain score, the Visual Analogue Scale mean (VAS) demonstrated improvement by decreasing from 5.67 ± 2.33 pre-operatively to 2.33 ± 0.72 two weeks postoperatively. Paired t-test revealed a statistically significant difference at (p < 0.0001), (Table1, Figure 4).

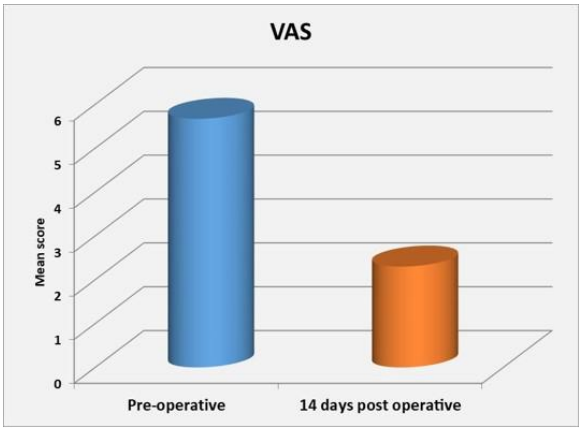

Figure 4: Column chart showing mean VAS score pre-and post-operatively

The maximum mouth opening's mean score decreased from 3.98 ± 0.36 pre-operatively to 2.27 ± 0.11 after three days postoperatively. However, a gradual increase to 3.08 ± 0.049 till the 7th postoperative day was seen, and then finally reached 3.93 ± 0.034 at 14 days postoperatively. Repeated measures analysis of variance (ANOVA) test revealed that the difference was statistically significant at (p < 0.0001). While, Tukey's post hoc test revealed no significant difference between values recorded at 7 and 14 days postoperatively (Table 2, Figure 5).

Table 2: MMO (cm) pre-and post-operatively and significance of the difference using repeated measures analysis of variance (ANOVA) test

MMO	Mean	SD	P value
pre-operative	3.9833[a]	.35687	
3rd-day post-operative	2.2722[b]	.46628	< 0.0001*
7th day post-operative	3.083[c]	.04938	
14th day post-operative	3.928[c]	.03461	

Significance level p < 0.05, * significant; Tukey's post hoc test: means with different superscript letters are significantly different.

Facial Swelling; the mean value increased from 13.76 ± 1.15 pre-operatively to 15.26 ± 1.21 after three days, but decreased to 13.68 ± 1.18 after 7 days postoperatively, repeated measures analysis of variance (ANOVA) test revealed that the difference was statistically significant at (p < 0.0001).

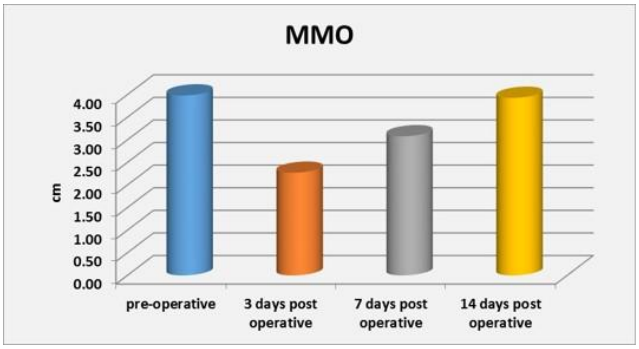

Figure 5: Column chart showing mean MMO (cm) pre-and post-operatively

However, Tukey's post hoc test revealed no significant difference between values recorded pre-operatively and at 7 days postoperatively (Table 3, Figure 6).

Table (3) Facial swelling (cm) pre-and post-operatively and significance of the difference using repeated measures analysis of variance (ANOVA) test

Facial swelling	Mean	SD	P value
pre-operative	13.7611[b]	1.14950	
3rd day post-operative	15.2556[a]	1.20987	
7th day post-operative	13.6778[b]	1.17851	< 0.0001*

Significance level p<0.05, * significant; Tukey's post hoc test: means with different superscript letters are significantly different.

Reviewing the radiographic results; immediate postoperative CBCTs showed proper repositioning of the harvested bony segment (Figure 7). Six months postoperative CBCTs showed adequate cortication of the buccal plate of bone at the external oblique ridge and normal bone healing of the sockets in all cases (Figure 8A and B).

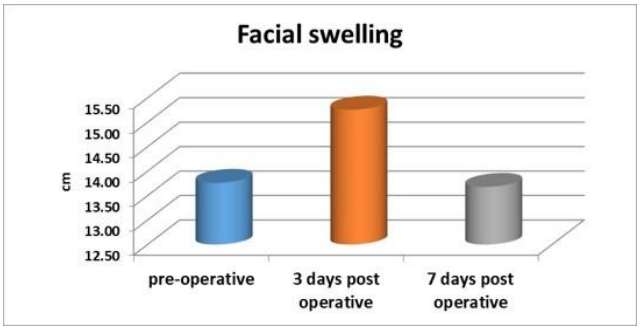

Figure 6: Column chart showing mean facial swelling (cm) pre-and post-operatively

Discussion

Deeply impacted mandibular third molar (DIMTM) removal is surgery with difficulty ranging from excessive bone removal to the high liability of injury to IAN either directly or indirectly with subsequent hypoesthesia of the lower lip. This study was conducted to assess the novel idea of harvesting bone covering the DIMTM and the use of a CAD/CAM cutting guide in its removal, aiming at preserving the integrity of the IAN and repositioning the harvested external oblique ridge after odontectomy [28], [29], [30], [31], [32].

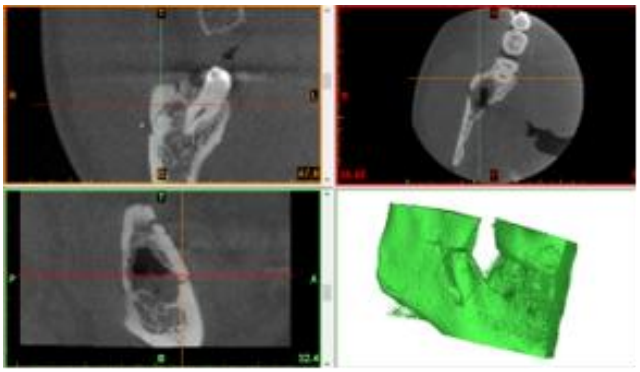

Figure 7: Immediate postoperative CBCTs radiograph

Although CBCTs were very useful in determining the spatial relation of DIMTMs to the IAN, [22] however, the introduction of the CAD/CAM cutting guide guaranteed accurate osteotomy positioning, maximum protection of the IAN and adequate exposure of the impacted tooth making this procedure a more applicable alternative for oral and maxillofacial surgeons [33].

In the present study, a CAD/CAM cutting guide was used to plan out the rectangular bony window with proper dimensions and directional orientation of the osteotomies to ensure adequate exposure of the impacted molar. This was achieved using the FRIOS Micro Saw disc, with 0.3 mm thickness, keeping the thickness of the inferior osteotomy to a minimum. Also, the maximum cutting depth of the Micro Saw disc is 3.2 mm; this permitted monocortical bone osteotomy with preservation of the IAN integrity.

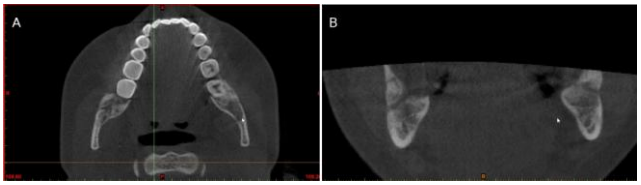

Figure 8: A), B) CBCTs; A) axial and B) coronal cuts 6 months postoperative showing normal bone healing

The thickness of the cutting guide was 2 mm. This particular thickness was chosen to ensure enough rigidity of the stent, while at the same time allowing the inferior osteotomy to be made with no interference. The orientation of the osteotomies' bevel converges lingually to allow bony segment removal as one piece and making it self-seated and retentive without any means of fixation upon its repositioning [28]. Normal bone healing was confirmed radiographically by the presence of bony trabeculae six months postoperatively which indicated graft revascularization and consolidation. This was also supported clinically by the absence of any signs and symptoms of infection or wound dehiscence [34].

Previous reports have shown that the surgical extraction of mandibular third molars is associated with considerable frequency of minor and severe complications, especially with extended operative time and deep impaction. Extend operative time, and excessive flap reflection causes a significant rise in the level of inflammatory mediators, which in return aggravate postoperative pain, facial oedema and limit mouth opening. The average operative time was reported to be around 25 minutes (range from 10 to 40 minutes). In the present study, we performed a classical flap extension to allow seating of the cutting guide which was valuable in reducing the surgical time in relation to position of the impacted molar; the new approach reduced the operative time by 20% lower than the average operative time for extraction of deeply impacted third molar tooth [2], [7], [35], [40].

The current published literature shows that the surgical extraction of impacted third molars usually leads to postoperative pain, facial swelling, and reduced mouth opening; with subsequent deterioration in health-related quality of life [41], [42]. Yuasa and Sugiura [43] reported that the occurrence of postoperative pain and swelling depend on patients age and difficulty index. In the present study, there was a significant improvement in postoperative pain; while the maximal mouth opening and facial swelling were within the normal range 14 days postoperatively. Such favourable postoperative

outcomes may be attributed to the short operative time assisted by the using of CAD/CAM cutting guides [1], [2], [7], [31], [32], [36].

In conclusion, this study proposes the joining of two new, different modalities in lower wisdom odontectomy, the first of which is the temporary harvesting of bone at the external oblique ridge, to provide access and a path of removal of the impacted tooth. The second proposition is the use of computer-guided technology in the fabrication of the surgical cutting guide, with measurements that eliminate chances of IAN injury and allows repositioning of this harvested bone without fixation. In addition to decreasing operative time, postoperative pain, facial swelling and trismus, this technique preserved the external oblique ridge's bone for future use in oral cavity augmentation. Although there was no nerve injury and good graft consolidation, which reflects the success of the technique, it is necessary to mention, however, that computer-guided surgery does not eliminate the operator dependent part for tooth exposure and odontectomy regarding the pressure exerted by the apical fragments on the nerve.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Dec 20; 6(12):2402-2408.
https://doi.org/10.3889/oamjms.2018.353
eISSN: 1857-9655
Dental Science - Review

The Success of Dental Veneers According To Preparation Design and Material Type

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Citation: Alothman Y, Bamasoud MS. The Success of Dental Veneers According To Preparation Design and Material Type. Open Access Maced J Med Sci. 2018 Dec 20; 6(12):2402-2408. https://doi.org/10.3889/oamjms.2018.353

Keywords: laminate veneers; anterior teeth; restorative procedure; survival rate of dental veneers

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Received: 01-Aug-2018; **Revised:** 03-Nov-2018; **Accepted:** 04-Nov-2018; **Online first:** 14-Dec-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Due to their high aesthetic outcome and long-term predictability, laminate veneers have become a common restorative procedure for anterior teeth. However, because of the variety in the preparation designs and the material types, the clinician faces a dilemma of which approach to use.

AIM: To compare the survival rate of dental veneers according to different preparation designs and different material types. The sub-aim is to reach a favourable preparation design and material based on scientific evidence.

METHODS: Comprehensive electronic search of the dental literature via PUBMED, MEDLINE and Scopus databases was performed using the following keywords: “porcelain veneers”, “composite veneers”, “all-ceramic veneers”, “success of porcelain veneers”, “preparation design”, “preparation geometry”, “patient’s satisfaction”. Additionally, references from the selected studies and reviews were searched for more information.

RESULTS: Under the limitations of the available literature, the clinician preference is the decisive factor for choosing the preparation design. Nonetheless, incisal overlap preparation seems to have the most predictable outcome from all the preparation designs.

CONCLUSION: Porcelain veneers show excellent aesthetic results and predictable longevity of the treatment, while composite veneers can be considered as a good conservative option, but with less durability.

Introduction

Since 1930s dental veneers have been used to improve the aesthetic and protection of teeth (Calamia, 1988) [1], the indications of dental veneers include: 1) discoloured teeth due to many factors such as tetracycline staining, fluorosis, amelogenesis imperfect, age and others 2) restoring fractured and worn teeth 3) abnormal tooth morphology 4) correction of minor malposition 5) Intra-oral repair of fractured crown and bridge facings [2], [3], [4]. Unfavourable conditions of dental veneers include 1) patients with parafunctional habits such as bruxism 2) edge to edge relation 3) poor oral hygiene 4) insufficient enamel [5], [6]. Many studies reported positive clinical outcomes veneers, with a survival rate of 91% in 20 years [7] dental veneers are considered a predictable aesthetic correction of anterior teeth.

The materials of dental veneers have evolved remarkably, early materials that had been used had many disadvantages such as the materials needed to be too thick to cover any discolouration, difficulty to polish which can cause abrasion of the opposing dentition and easy to stain [8], [9]. Researchers and dental material manufacturers have aimed to develop new materials with better aesthetic characteristics through the years. In 1975 laminate veneers were introduced as a better material of choice to mask the dentition, the restorations were 1 mm in thickness and were made from a cross-linked polymeric veneer [10]. The use of laminate veneers resulted in a better aesthetic outcome and less chair time [11]. The progress of developing new materials reached porcelain in the 1980s when enamel was etched, and the porcelain surface was treated to improve the bonding [12], [13].

The desire for more durable aesthetic outcomes did not confine to improve the material type only; new preparation designs were introduced to the

field of dental veneers. There are four different main designs of teeth preparation commonly mentioned in the literature (Figure 1): 1) window preparation: in which the incisal edge of the tooth is preserved 2) feather preparation: in which the incisal edge of the tooth is prepared Bucco-palatable, but the incisal length is not reduced 3) bevel preparation: in which the incisal edge of the tooth is prepared Bucco-palatable, and the length of the incisal edge is reduced slightly (0.5-1 mm) 4) incisal overlap preparation: in which the incisal edge of the tooth is prepared Bucco-palatable, and the length is reduced (about 2 mm), so the veneer is extended to the palatal aspect of the tooth [14], [15], [16], [17].

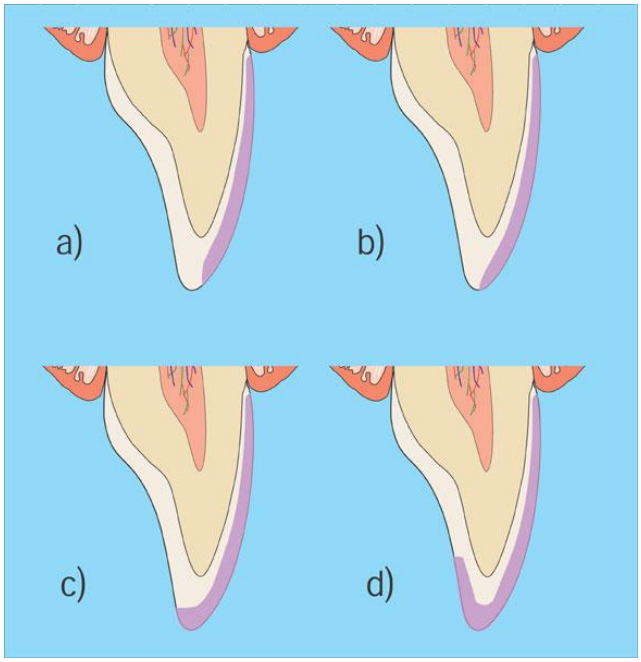

Figure 1: Showing common veneer preparations a) window b) feather c) bevel d) incisal overlap [17]

Influence of preparation design on the survival of dental veneers

Different opinions have been reported about superior preparation design over the others. In fact, due to the great variety in the materials, preparations designs and luting cement, favourable approaches to restore teeth with veneers have been controversial.

This review aims to compare the survival rate of dental veneers according to different preparation designs and different material types. The sub-aim is to reach a favourable preparation design and material based on scientific evidence.

One important aspect to investigate is the tooth preparation of dental veneers and how it might affect the fracture resistant of the material and reinforcement of the abutment tooth. Unfortunately, clinical trials that investigate the survival rate of dental veneers according to preparation designs are few, the criteria of investigation would include more than one factor which can affect the outcome of the treatment [16], [18]. In contrast, many *in vitro* studies have been conducted to evaluate the influence of different preparations design. Although such studies do not mimic the actual clinical environments and factors, they can provide criteria and guidelines for the clinician and further clinical investigations [5]. Table 1 illustrates the results of multiple *in vitro* studies regarding the influence of preparation design.

Table 1: *In vitro* studies that investigated the influence of preparation design on dental veneers

Study	Preparation design	Method of loading	Number of samples	Survival probability	Conclusion	Remarks
(Highton & Caputo 1987) [26]	Incisal overlap-chamfer FL Window preparation Slight labial preparation only Unprepared	Four directions: Central vertical Distal vertical Central inclined Distal inclined	4 (one of each)	High Moderate Low Lowest	Labial, proximal, incisal and gingival reduction is recommended.	Samples were photoelastic teeth
(Castelnuovo *et al.* 2000) [14]	Incisal overlap (1mm)-chamfer finish line Butt joint incisal reduction (1mm) Feather edge preparation Deep incisal overlap(4mm) Unprepared	Static loading at a 90-degree angle to the palatal surface of the sample	50 (10 each)	Moderate High High Low Control	Butt joint incisal reduction and feather edge prep. Provide the best retention to the restoration. Deep incisal overlap is not recommended	-
(Stappert *et al.* 2005) [16]	Incisal overlap (2mm) butt joint Deep incisal overlap (3mm)-butt joint Window preparation Unprepared	Dynamic loading and thermal cycling 135-degree angle in the masticatory stimulator	64 (16 each)	High Low Low Control	Incisal overlap provides the best support. Deep preparation is not necessary.	-
(Zarone *et al.* 2005) [28]	Incisal overlap-chamfer FL Window preparation	Static loading at the long axis of the tooth	4	High Low	Incisal overlap is a better design than window prep.	Samples were 3D computerised models
(Schmidt *et al.* 2011) [31]	Incisal reduction – chamfer FL Incisal reduction – butt joint	Static loading at a 90-degree angle to the palatal surface of the sample	32 (8 each)	Low High	Having a chamfer FL increase the failure rate of the veneer	Amount of existing tooth structure was considered in the study
(Lin *et al.* 2012) [23]	Incisal reduction – butt joint Three quarter preparation	Static loading at a 125-degree angle of the palatal surface of the sample	48 (12 each)	High Moderate	Three-quarter prep. Requires stronger material for support	Influence of restorative materials was included in the study
(Alghazzawi *et al.* 2012) [32]	Incisal reduction – butt joint Three quarter preparation	Dynamic loading at a 135-degree angle of the palatal surface of the sample	60 (30 each)	High High	No significant difference between the two preparations	-

General concepts

Some features of the preparation design are highly recommended in the majority of the literature and lab studies. For example, restricting the preparation to enamel is considered to be a critical factor for a favourable bonding strength, thus more durable outcome [6], [18], [19], [20]. Additionally, preserving the interproximal contact is recommended in most of the literature and studies, this is due to preserving more enamel and tooth structure, allowing a positive seat for cementation in a conservative approach [16], [21], [22], [23]. However, the clinician might face certain situations where removing the interproximal contact can provide better aesthetic results such as malaligned teeth or diastema [24], [25]. Moreover, the amount of labial reduction concurrent at 0.4-0.7 mm for ceramic veneers [1], [3], [15]. This is due to the enamel thickness in the anterior teeth, according to Ferrari *et al.,* (1991) [3],

the enamel thickness of 114 extracted anterior teeth was 1.0 to 2.1 mm at the incisal third, 0.6 to 1.0 mm at the middle third and 0.3 to 0.5 at the gingival third, therefore, minimal preparation is advisable.

Preparation designs

Although there are different opinions and different results in studies that investigate the influence of preparation design on the survival of the restoration. It seems that incisal overlap preparation provides the best support for the restoration and distributes occlusal forces over a larger surface area. In the window preparation, the occlusal stress is highly concentrated on the incisal third which may lead to fracture of the restoration. Also, incisal translucency can be better achieved when the incisal edge is reduced [14], [16], [23], [26]. However, it is controversial whether it is favourable to add a chamfer finish line palatable or have a shoulder finish line (butt joint). Troedson and Dérand (1999) [27] and Zarone *et al.,* (2005) [28] reported that it is required to have a chamfer finish line palatable for the restoration to tolerate the occlusal stress.

In contrast, Castelnuovo *et al.,* (2000) [14] suggested that having a chamfer finish line doesn't add to the longevity of the restoration.

Additionally, they reported that veneers with butt-joint finish line could provide more than one path of insertion (Figure 2). However, having a single path of insertion can be considered as an advantage because it prevents any displacement of the veneer during cementation. Eventually, the study stated that an overlap preparation with chamfer finish line does not decrease the longevity and predictability of the treatment.

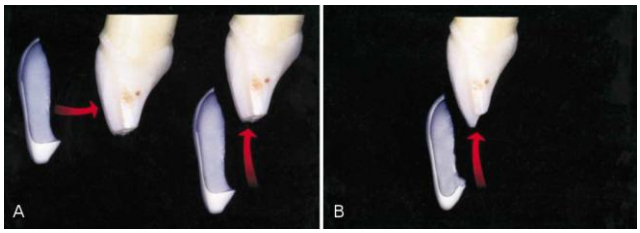

Figure 2: Incisal overlap with shoulder finish line (A) provide more than one path of insertion while incisal overlap with chamfer finish line (B) provide only one path of insertion (Castelnuovo et al., 2000) [14]

Ultimately, the biting force of the anterior teeth is considered to be low (100 – 200 N) (Carlsson 1973) [29] and with the absence of a strong well-conducted clinical study, the decision of preparation design is the clinician preference mainly, while incisal overlap can always be chosen to re-establish anterior guidance (Hahn *et al.,* 2000) [30].

Influence of material type on the survival of dental veneers

A range of materials are available in the market to restore aesthetic/functional complications by the mean of veneering teeth; the most common material is porcelain, resin composite. Each material type has its unique composition, optical characteristics and fabrication process. Thus, it can be expected that the treatment outcome and longevity will differ according to the material used (Font *et al.* 2006) [33]. Table 2 shows multiple clinical studies that investigated the survival rate of dental veneers with a variety of material types.

Table 2: Clinical studies are illustrating the survival rate of dental veneers. Adapted from Peumans et al., (2000) [18]

Study	Type of study	Number of veneers (number of patients)	Observation period	Survival rate	Remarks
Porcelain laminate veneers (PLVs)					
(Peumans *et al.,* 1998) [43]	Prospective	87 (25 patients)	5 years	93%	-
(Meijering *et al.,* 1998) [61]	Prospective	263 (112 patients)	2.5 years	100%	-
(Dumfahrt & Schäffer 2000) [62]	Retrospective	191 (72 patients)	1 – 10 years	91% in 10 years	Failure increase when PLVs are bonded to dentin
(Magne *et al,.* 2000) [63]	Prospective	48 (16 patients)	4.5 years	100%	-
(Smales & Etemadi 2003) [48]	Retrospective	110 (50 patients)	Up to 7 years	95%	Compared two different preparation designs as well
(Chen *et al.,* 2005) [64]	Retrospective	546 (not mentioned)	2.5 years	99%	All patients had tetracycline staining
(Granell *et al.,* 2010) [65]	Prospective	323 (70 patients)	3 – 11 years	87% over 11 years	Failure increased with the presence of composites and bruxism
(Beier *et al.,* 2011) [47]	Retrospective	318 (84 patients)	Up to 20 years	94% in 5 y. – 93% in 10 y. - 82% in 20 y.	50% of the patient were diagnosed with bruxism
(Layton & Walton 2012) [7]	Prospective	499 (155 patients)	Up to 21 years	96% in 10 y. 91% in 20 y.	Bonding to enamel is a critical factor for survival
Resin composites- direct and indirect (DC –IC)					
(Peumans *et al.,* 1997) [59]	Prospective	87 (23 patients)	5 years	89%	DC-Main failure due to wear
(Meijering *et al.,* 1998) [61]	Prospective	263 (112 patients)	2.5 years	90% for IC - 74% for DC	Results for DC and IC
(Wolff *et al.,* 2010) [54]	Retrospective	327 (101 patients)	5 years	79%	Result for DC
(Gresnigt *et al.,* 2012) [60]	Prospective	96 (23 patients)	3.4 years	87%	Split mouth design- no difference between composite type- all DC

Porcelain veneers

One of the most common materials that are used to fabricate laminate veneers is feldspathic porcelain (Figure 3).

The main component of feldspathic porcelain is feldspar; a naturally occurring glass which contains silicon oxide, aluminium oxide, potassium oxide and sodium oxide (Layton & Walton 2012) [7]. Feldspathic porcelain has many advantages; the material is very thin so it can be almost translucent which result in an appearing natural restoration. Also, it requires minimal tooth preparation. Therefore enamel can be preserved. Moreover, it is possible to etch feldspathic porcelain with hydrofluoric acid which gives a great bonding strength to the remaining enamel (Calamia 1982, Nicholls 1988, Stacey 1993, Layton & Walton 2012) [7], [12], [34], [35]. Nevertheless, feldspathic porcelain has some disadvantages. The fabrication of feldspathic porcelain can be done by two methods: the refractory die technique and the platinum foil technique (Horn 1983, Plant & Thomas 1987, Clyde & Gilmour 1988) [13], [15], [36], these methods are technique sensitive and the fabricated veneer requires good care prior to bonding (Layton & Walton 2012) [7]. Additionally, masking heavy discoloured teeth can be difficult because the porcelain is very thin. Moreover, it was reported that etching the inner surface of the porcelain can cause micro-cracks which can lead to decrease the flexural strength of the porcelain and eventually fracture the veneer (Yen *et al.,* 1993) [37].

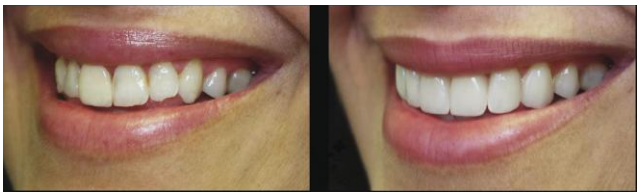

Figure 3: A case showing before and after the treatment with porcelain veneers (Nalbandian & Millar 2009) [38]

New ceramic systems have been developed recently such as IPS e.max press from Ivoclar Vivadent©, leucite is added to the glass matrix in order to increase the strength of the ceramic (Rasetto *et al.,* 2001) [39], however, such new systems lack well-conducted clinical studies that investigate the success of using them as laminate veneers. Thus, future studies in this field are required.

Adhesion complex

The adhesion complex between porcelain, luting composite and enamel is considered to be a great advantage of porcelain veneers. It has been reported that the bonding strength of that complex is around 63 MPa while the bond between composite and enamel is about 31 MPa and between composite and porcelain alone is 33 MPa (Stacey 1993) [35]. Also, some *in vitro* studies suggest that extracted teeth that are restored with porcelain veneers have regained their original strength (Andreasen *et al.,* 1992, Stokes & Hood 1993) [40], [41]. This can explain the low failure rate (0 – 5%) in clinical studies due to debonding of the porcelain veneer especially when parafunctional habits are missing, (Rucker *et al.* 1990, Kihn & Barnes 1998, Peumans *et al.,* 1998) [42], [43], [44]. Respectively, some authors reported that when porcelain veneers are bonded to composite rather than enamel, porcelain veneers tend to have a higher failure rate (Dunne & Millar 1993, Shaini *et al.,* 1997) [45], [46].

Longevity of porcelain veneers

Many studies investigated the longevity of porcelain veneers. Beier *et al.,* (2011) [47] reported in a retrospective clinical study a survival rate of 94.4% after five years and 93.5% after ten years; they found the main reason for failure is a ceramic fracture. A randomised clinical trial done by Layton and Walton (2012) [7] showed similar results, with a survival rate of 96% after ten years and 91% after 20 years. Also, Smales and Etemadi (2003) [48] reported a survival rate of 95% for porcelain veneers throughout 7 years. It is essential to stress that these studies and others that reported high survival rate of porcelain veneers had a strict assessment of remaining enamel and bonding systems. As a result, careful, conservative preparation and optimum isolation during cementation are required to ensure predictable outcomes.

There are other studies which reported a lower survival rate for porcelain veneers. A retrospective study of 2,563 veneers in 1,177 patients done by Burke and Lucarotti (2009) [49] reported a survival rate of 53% over 10 years. The material type of the veneers was not reported. Moreover, the study evaluated veneers that were done by the general dental service, and thus, it is possible that preparations of teeth did not meet the criteria of specialists' level. Another retrospective study was done by Shaini *et al.,* (1997) [46] reported a survival rate of 47% in 7 years. The veneers were done by undergraduate students and staff member at Birmingham University in the United Kingdom. The study reported that over 90% of veneers were placed on unprepared teeth, this can be a reason for high failure rate as it is suggested that the bond to aprismatic enamel is much weaker than prepared enamel (Perdigão & Geraldeli 2003, Layton & Walton 2012) [7], [50].

The high survival rates that are reported by well-designed clinical studies suggest that feldspathic porcelain can act as a reliable and effective material to restore anterior teeth.

Resin composite

Resin-based composites are restorative materials that have mainly the following three compositions: 1) resin matrix 2) inorganic filler 3) coupling agent. The most commonly used monomer in the resin is Bis-GMA which has a higher molecular

weight than methyl methacrylate resins. Therefore, the polymerisation shrinkage of Bis-GMA (7.5%) is significantly less than that of methyl methacrylate resins (22%). Wide range of fillers such as quartz have been added to composites through the years, the addition of fillers offers many advantages like: 1) reduction of the polymerisation shrinkage 2) reduction of coefficient thermal expansion of the monomer 3) improve mechanical characteristics 4) some metallic fillers such as barium provide better radiopacity. The bonding between the resin and the filler is achieved by the use of coupling agents i.e. salines, the most commonly one that is used in resin composite is γ-MPTS. Dental composites can be categorised according to the particle size of the filler traditional composites have a mean particle size of 10-20 μm, on the other hand, micro filled composites have a mean particle size of 0.02 μm. new generations of composites are introduced by the dental company through the years, aiming for better aesthetic and physical properties (Bonsor & Pearson 2012, Van Noort 2013) [51], [52].

It was thought once that composites in the anterior area would be replaced with porcelain veneers due to their success (Garber 1989) [53]. However, the aesthetic and physical properties of resin composite have improved remarkably lately. Thus, it has been used extensively in clinical practice (Wolff *et al.* 2010) [54]. The main advantage of composite veneer is that it can be used directly, resulting in less chair time with good initial aesthetic. Nonetheless, composite veneers are more prone to discolouration and wear (Wakiaga *et al.* 2004) [55]. Additionally, the clinician skill in placing, finishing and polishing the composite plays a major factor in the aesthetic outcome.

Composite veneers do not require heavy preparations. Therefore enamel can be preserved for good adhesion. It is documented that the bonding strength between etching porcelain and enamel is greater than resin composite and enamel (Lacy *et al.,* 1988, Nicholls 1988, Lu *et al.,* 1992) [34], [56], [57]. Correspondingly, it has been reported that composite veneers do not significantly restore the stiffness of the prepared tooth (Reeh & Ross 1994) [58]. Although composite veneers can be made indirectly in dental laboratories, the used composite is essentially the same one that is applied directly. Thus, it shares the same physical properties and limitations of direct composite restorations such as polymerisation shrinkage (Van Noort 2013) [52].

Longevity of composite veneers

The survival rate of composite veneers in many clinical studies is constant. Peumans *et al.* (1997) [59] placed 87 direct composite veneers for 23 patients; they reported a survival rate of 89% after 5 years. Wolff *et al.,* (2010) [54] did a retrospective study on 327 direct composite veneers for 101 patients; the estimated survival rate was 80% after 5 years. A recent randomised control trial to compare two different types of composites reported a survival rate of 87% in over 3 years (Gresnigt *et al.,* 2012) [60]. The use of resin composite to veneer the anterior teeth is justifiable; it is a fast procedure with the good aesthetic outcome and reasonable longevity (Figure 4).

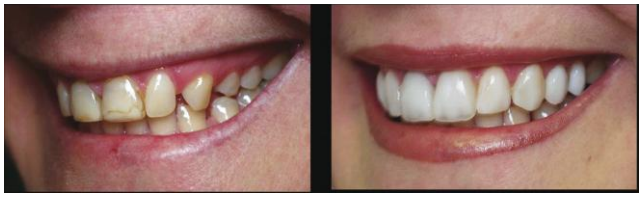

Figure 4: A case showing before and after treatment with direct composite veneers (Nalbandian & Millar 2009) [38]

Patients' satisfaction

Generally, aesthetic satisfaction is a complex process as it is considered subjective [38], [61]. However, some factors may play an important role in patients' satisfaction such as the durability of the final aesthetic outcome, the required amount of teeth preparation for the material type and the cost of the treatment.

Many clinical studies that evaluated the longevity of porcelain veneers have also considered patients' satisfaction of the treatment, the range of satisfaction in these studies is 80-100 % [43], [44], [46]. Other studies have been conducted to evaluate patients' satisfaction with different material types for veneers. Meijering *et al.,* (1997) [67] compared patients' response to three different types of veneers restorations after two years: feldspathic porcelain, direct composite and indirect composite. Porcelain veneers had the best response from patients (93%) followed by indirect composite veneers (82%) and lastly direct composite veneers (67%). In contrast, Nalbandian and Millar (2009) [38] found no statistical difference between patients' response to composite veneers and porcelain veneers. These two studies might be subjected to bias, the degree of preoperative discolouration or malposition can affect the grade of transformation postoperatively, and thus, affect the response of the patient.

From the result of the previous studies, it can be concluded that porcelain veneers can provide a predictable aesthetic acceptance, while composite veneers can be the treatment of choice for patients who appreciate minimally invasive approaches.

Conclusion

The influence of preparation design and material type on the success of dental veneers is controversial. Usually, the clinician preference decides the preparation geometry. Nevertheless, veneers with incisal coverage seem to have better aesthetic and more predictable outcomes, while having a chamfer finish line palatable seems to be unnecessary and limiting the preparation to a butt-join finish line is more sensible. According to multiple clinical studies, porcelain veneers have excellent aesthetic results, the longevity of the treatment and patient's satisfaction; the most critical factors to ensure a successful treatment are to obtain bonding to enamel and absence of parafunctional habits. Respectively, composite veneers provide good aesthetic outcome and patient's satisfaction; however, due to its physical properties and to the bonding strength when compared to porcelain veneers, composite veneers tend to fail significantly faster than porcelain veneers. Further clinical trials are needed to evaluate different types of composites and new ceramic systems for longer observation time.

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