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Pharmacokinetics of Nanosomal Form of Levodopa in Intranasal Administration

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Abstract

BACKGROUND: Parkinson's disease is one of the most common neurological diseases. Pathogenesis of the disease is associated with destruction and death of neurons that produce the neurotransmitter dopamine. The precursor to dopamine, which crosses the protective blood-brain barrier, is the amino acid 3, 4-dihydroxy-L-phenylalanine – levodopa, L-DOPA. The investigational drug is a pharmaceutical composition, containing L-DOPA as an active substance, which is distributed in a polymer matrix based on a biodegradable copolymer of lactic/glycolic acids.

AIM: This work aimed to study the main pharmacokinetic parameters for the drug "L-DOPA – PC, nasal drops" and comparator drugs "L-DOPA in oil", "L-DOPA – PC in purified water", reference product – tablets "Madopar 125".

METHODS: To increase the bioavailability of the active substance L-DOPA, a new route of administration was used for the first time – nasal administration. Pharmacokinetics of the innovative drug with the intranasal route of administration was investigated in rabbits. The L-DOPA concentration in blood plasma was determined by high-performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS).

RESULTS: Bioavailability of the drug – nasal drops were 244.4% compared with the drug "Madopar 125".

CONCLUSION: Assay procedure for the determination of L-DOPA in animal blood plasma using liquid chromatography with tandem mass-selective detection (HPLC-MS/MS) was developed and validated.

Introduction

Parkinson's disease is a slowly progressing socially significant chronic neurological disease, characteristic of the older age group [1], refers to the degenerative diseases of the extrapyramidal motor system. The cause of the disease is progressive destruction and death of neurons that produce neurotransmitter dopamine [2], primarily in the black substance, as well as in other parts of the central nervous system. Insufficient production of dopamine leads to the activating influence of basal ganglia on the cerebral cortex. Leading symptoms are muscle rigidity, hypokinesia, tremor, postural instability.

The innovative drug L-DOPA-PC, nasal drops, is an original pharmaceutical composition containing L-DOPA (3, 4-dihydroxy-L-phenylalanine, levodopa) as an active ingredient, distributed in the polymer matrix based on a biodegradable copolymer of lactic/glycolic acids (polylactide glycolide, PLGA 50/50) [3].

L-DOPA's mechanism of action is well studied and described in the literature [4], [5], [6], [7], [8]. L-DOPA eliminates hypokinesia, rigidity, tremor, dysphagia, salivation. L-DOPA is an amino acid, an

immediate metabolic predecessor of dopamine, which unlike dopamine is able to cross the blood-brain barrier and compensate dopamine deficiency in the brain that underlies many clinical manifestations of Parkinson's disease. L-Dopa is captured by the endings of remaining dopaminergic nigrostriatal neurons, undergoes decarboxylation in them, turns into dopamine, which is released into the synaptic cleft, thus maintaining an adequate functional state of the neurons of the striatum and other basal ganglia.

Modern scientists' efforts are concentrated on medical drugs application frequency decreasing, preserving their efficiency [9], [10]. Nasal delivery method is one of the most promising. The most important feature of the medical products intranasal delivery is the opportunity to penetrate them directly into the central nervous system without entering the blood circulatory system. The medical products transportation from the nasal cavity to the central nervous system is implemented without the mucous participation. It is done using an extracellular tract through the epithelial barrier in the course trigeminal and olfactory nerves. It was earlier believed that all L-dopa is entirely utilised in the sympathetic nerves ends and does not get out in the extracellular space. Nevertheless, rather, an important part of L-dopa leaves sympathetic nerves and arrives in the arterial and venous system, which supplies extremities, head, heart, adrenal glands and intestines with the blood [11].

To increase the bioavailability of the active substance L-DOPA, we used the nasal route of administration of the drug [4]. This work aimed to study the main pharmacokinetic parameters for the drug "L-DOPA – PC, nasal drops" and comparator drugs "L-DOPA in oil", "L-DOPA – PC in purified water", reference product – tablets "Madopar 125".

Material and Methods

"L-DOPA-PC nasal drops" was compared with:

1. L-DOPA (3.75% of levodopa substance) in oil.

2. L-DOPA – PC (3.75% of levodopa substance) in purified water.

3. "Madopar 125", dispersible tablets, (levodopa content-100 mg). Composition: levodopa 100 mg, benserazide hydrochloride 28.5 mg. *Excipients:* anhydrous citric acid; corn starch pre-gelatinized; microcrystalline cellulose; magnesium stearate. The tablet was dispersed in 10 ml of purified water and administered orally as a suspension through a feeding tube.

The study was conducted on 24 male rabbits weighing 2900-3200 g. The animals were divided into 4 groups of 6 rabbits each. Groups 1-3 administered the drug intra-nasally; the volume of administration was not more than 200 µl. Group 4 received the drug through oesophagal atraumatic bougie; the volume of administration was not more than 0,5 ml. The dose of administration for L-DOPA was 2.5 mg/kg. Blood samples were taken at the following time points: 0, 0.08, 0,16, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 36, 48 h. Five ml of blood was taken from the ear vein. The design of the study is given in Table 1.

Table 1: Scheme of intranasal and oral administration of drugs based on L-DOPA

Name of the administered sample	Group of animals			
	Group 1	Group 2	Group 3	Group 4
	Nasal oil drops based on L-DOPA-PC	Levodopa suspension in olive oil	L-DOPA-PC suspension in purified water	Tablets "Madopar 125"
Content of Levodopa (L-DOPA)	5.0 %	3.75 %	3.75 %	100 mg
Route of administration		nasally		orally
Rate of application	once	once	once	once
L-DOPA dose	2.5 mg/kg	2.5 mg/kg	2.5 mg/kg	2.5 mg/kg

There are many L-DOPA research methods in various assay samples (HPLC-UV, HPLC-MS/MS and others). Method HPLC-UV is suitable for the determination of L-DOPA in dosage form [12]. HPLC-MS/MS is used for pharmacokinetic studies owing to increased selectivity for the substance [11]. To improve the quality of the study, the method HPLC-MS/MS was chosen.

Determination of L-DOPA concentration in blood plasma was performed by high-performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS). Equipment used for the study – tandem quadrupole mass spectrometric detector, Shimadzu, manufactured in Japan; liquid chromatograph LC-30 Nexera, Shimadzu, Japan.

Statistical data processing was carried out using Statistica 12.0 software.

Results

Four preparations of different compositions were obtained for the study; they contained the amino acid (3,4-dihydroxy-L-phenylalanine, levodopa, L-DOPA) as an active ingredient.

All the blood plasma samples obtained were analysed by HPLC-MS/MS. During the development of the assay procedure of the analyte understudy, a mass spectrum of L-DOPA was obtained. The mass spectrum was obtained in the full scan mode of fragment ions in the range of mass-to-charge ratios from 100 m/z to 500 m/z. Based on the data obtained,

characteristic ion transitions for the analyte under study were determined. The mass spectrum of L-DOPA is shown in Figure 1.

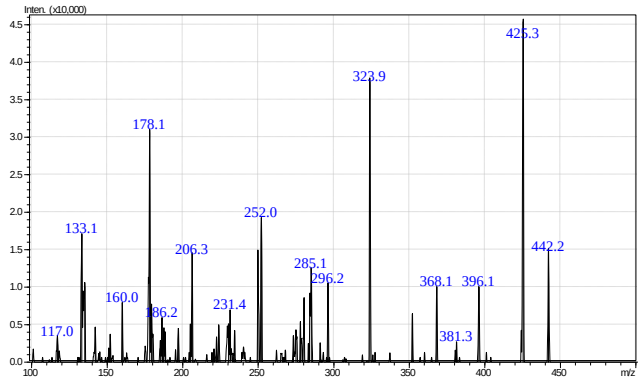
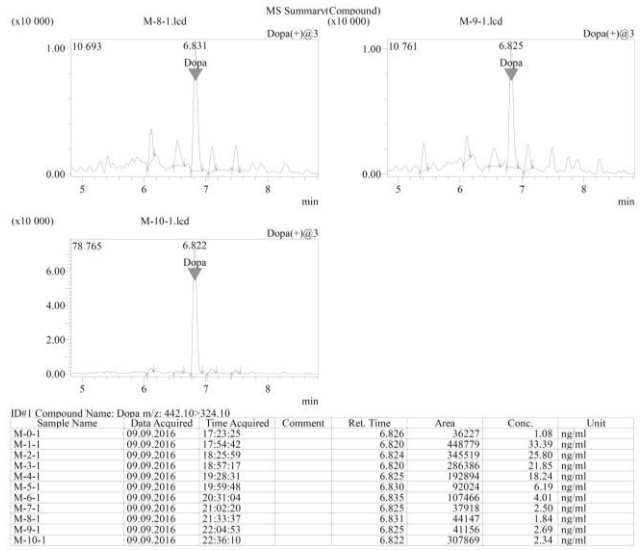


Figure 1: Mass spectrum of L-DOPA

Typical results of L-DOPA concentration determination are shown in Figure 2.



Sample Name	Data Acquired	Time Acquired	Comment	Ret. Time	Area	Conc.	Unit
M-0-1	09.09.2016	17:23:25		6.826	36227	1.08	ng/ml
M-1-1	09.09.2016	17:54:42		6.820	448779	33.39	ng/ml
M-2-1	09.09.2016	18:25:59		6.824	345519	25.80	ng/ml
M-3-1	09.09.2016	18:57:17		6.820	286386	21.85	ng/ml
M-4-1	09.09.2016	19:28:31		6.825	192894	18.24	ng/ml
M-5-1	09.09.2016	19:59:48		6.830	92024	6.19	ng/ml
M-6-1	09.09.2016	20:31:04		6.835	107466	4.01	ng/ml
M-7-1	09.09.2016	21:02:20		6.825	37918	2.50	ng/ml
M-8-1	09.09.2016	21:33:37		6.831	44147	1.84	ng/ml
M-9-1	09.09.2016	22:04:53		6.825	41156	2.69	ng/ml
M-10-1	09.09.2016	22:36:10		6.822	307869	2.34	ng/ml

Figure 2: Mass chromatograms of L-DOPA in the blood plasma of rabbits after a single intranasal administration of levodopa suspension in olive oil (2), sample №1

Figure 3 shows pharmacokinetic profiles of L-Dopa in the blood of rabbits, in linear coordinates, after a single administration of the drugs "L-Dopa-PC nasal drops", "L-DOPA in oil", "L-DOPA-PC in purified water" and "Madopar 125" capsules.

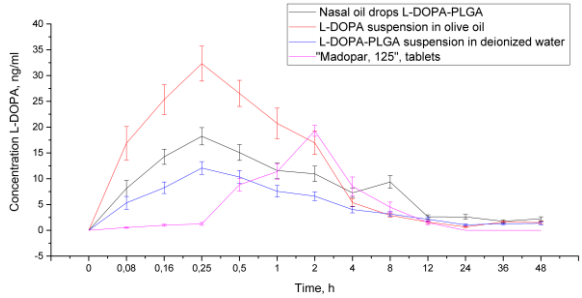


Figure 3: Graph of averaged pharmacokinetic profiles (in linear coordinates) for L-DOPA (observation from 0 to 48 hours)

The main pharmacokinetic parameters are presented in Table 2.

Table 2: Pharmacokinetic parameters calculated from averaged data

Group	Parameter	$T_{½}$	T_{max}, h	C_{max}, ng/ml	$AUC_{0→48}$, ng × h/ml	$AUC_{0→\infty}$, ng × h/ml	MRT, h	$C_{max}/AUC_{0→t}$	$C_{max}/AUC_{0→\infty}$	K_{el} (h^{-1})
(1)	Mean	15.39	0.25	18.2	181.88	231.64	27.3	0.1002	0.0787	0.0452
	Gmean	15.38	0.25	18.1	180.75	230.91	27.3	0.1005	0.0787	0.0451
	Median	15.06	0.25	18.0	175.27	229.30	26.3	0.1026	0.0794	0.0460
	Min	14.53	0.25	15.5	167.81	214.83	25.6	0.0891	0.0721	0.0395
	Max	17.54	0.25	20.3	203.94	254.75	34.5	0.1073	0.0848	0.0477
	SD	1.09	0.00	1.7	13.76	16.52	3.5	0.0068	0.0051	0.0029
(2)	Mean	10.92	0.25	32.31	135.53	159.32	20.19	0.2383	0.2027	0.0636
	Gmean	10.91	0.25	32.16	135.24	159.09	20.12	0.2378	0.2022	0.0635
	Median	11.06	0.25	31.54	134.51	158.05	20.89	0.2426	0.2088	0.0627
	Min	10.12	0.25	28.21	124.73	149.54	17.23	0.2167	0.1788	0.0614
	Max	11.29	0.25	37.02	152.75	171.45	21.70	0.2613	0.2172	0.0685
	SD	0.44	0.00	3.38	9.82	9.46	1.82	0.0169	0.0161	0.0027
(3)	Mean	15.99	0.25	12.08	100.36	131.22	29.00	0.1209	0.0927	0.0437
	Gmean	15.92	0.25	12.03	99.62	129.97	28.96	0.1208	0.0926	0.0435
	Median	15.24	0.25	12.12	98.19	129.16	28.41	0.1215	0.0935	0.0455
	Min	14.73	0.25	10.54	86.43	109.17	26.96	0.1104	0.0825	0.0357
	Max	19.39	0.25	14.01	126.96	169.87	31.76	0.1275	0.0983	0.0471
	SD	1.73	0.00	1.23	14.02	20.73	1.75	0.0058	0.0055	0.0041
(4)	Mean	3.25	2.00	19.28	87.52	94.68	5.00	0.2220	0.2048	0.2265
	Gmean	3.14	2.00	19.26	87.14	94.43	4.95	0.2210	0.2040	0.2204
	Median	2.96	2.00	19.07	89.33	93.46	4.80	0.2210	0.2082	0.2343
	Min	2.50	2.00	17.95	72.91	86.44	4.29	0.1937	0.1769	0.1323
	Max	5.24	2.00	20.73	95.70	104.80	6.47	0.2487	0.2338	0.2777
	SD	1.01	0.00	1.06	8.71	7.56	0.82	0.0225	0.0201	0.0520

Discussion

Comparative analysis of main pharmacokinetic parameters for the investigational drug "L-Dopa-PC nasal drops"(1) and "L-Dopa in oil"(2), "L-Dopa-PC in purified water"(3), "Madopar 125" capsules (4) (table 3) showed that the studied drugs quickly enter systemic circulation, however, at different rates.

Table 3: Relative bioavailability of L-Dopa-PC-based nasal oil drops [1]

Name of the administered sample	Group of animals		
	Group 2	Group 3	Group 4
	L-Dopa suspension in olive oil	L-Dopa-PC suspension in purified water	"Madopar 125", capsules
Dosage	2.5 mg/kg	2.5 mg/kg	2.5 mg/kg
RELATIVE BIOAVAILABILITY	145.2 %	176.3 %	244.4 %
Group 1 L-DOPA – PC based nasal oil drops Dosage - 2.5 mg/kg			

Difference between L-DOPA absorption rate values ($Cmax/AUC_{0→t}$) for investigational drugs (1), (2), (3) and (4) is statistically-valid and for (1) was (0.1002 ± 0.0068) h^{-1}, for (2) – (0.2383 ± 0.0169) h^{-1}, for (3) – (0.1209 ± 0.0058) h^{-1} , for (4) – (0.2220 ± 0.0225) h^{-1}, and the individual variance of values is insignificant - CV was 22-23%. Time to reach maximum L-DOPA concentration (Tmax) for (1), (2), (3) was 0.25 h on average, which indicates that there were no significant differences in the time of reaching maximum concentration (Tmax) between the studied drugs. For the drug (4) time to reach maximum L-DOPA concentration was 2 hours due to the oral route of administration, which shows a significant difference in the time to reach the maximum concentration (Tmax) between the studied drugs (1) and (4).

The average maximum concentration of L-DOPA, determined in the blood plasma of rabbits (Cmax), was for the drug (1) – (18.23 ± 1.68) ng/ml, for (3) – (12.08 ± 1.23) ng/ml, for (4) – (19.28 ± 1.06) ng/ml and (2) – (32.31 ± 3.38) ng/ml. L-Dopa is then slowly excreted from the body and after 48 hours it is still found in the blood plasma of rabbits after administration of drugs (1), (2) and (3), but after oral administration of the drug (4) after 12 hours L-Dopa is not detected. The results of the Student's test for Ln (Cmax) for the values between and within the groups (1-2) ($t = -16.8105$ at $p < 0.05000$), (1-3) ($t = 10.80150$ for $p < 0.05000$), showed a statistically significant difference between groups (1) and (2), (1) and (3). The results of the Student's test for Ln (Cmax) for the values between and within the groups (1-4) ($t = 1.74050$ at $p < 0.05000$) showed that there was no statistically significant difference between the groups.

Analysis of the main parameter characterising the degree and rate of bioavailability of the active substance (L-DOPA) from the dosage form – the area under the pharmacokinetic curve ($AUC_{0\rightarrow t}$) indicates a significant variability of this value. The average value of $AUC_{0\rightarrow t}$ for the drug (1) was (181.88 ± 13.76) ng/ml×h, for (2) – (135.53 ± 9.82) ng/ml×h, for (3) – (100.36 ± 14.02) ng/ml × h, for (4) – (87.52 ± 8.71) ng/ml × h. The average value of $AUC_{0\rightarrow\infty}$ for the drug (1) was (231.39 ± 16.52) ng/ml × h, for (3) – (131.22 ± 20.73) ng/ml × h, for (2) – (159.32 ± 9.46) ng/ml × h, for (4) – (94.68 ± 7.56) ng/ml × h. The results of the Student's test for Ln ($AUC_{0\rightarrow t}$) for the values between and within the groups: (1-2) ($t = 17.83326$ at $p < 0.05000$), (1-3) ($t = 9.041106$ at $p < 0.05000$) (1-4) ($t = 18.05994$ for $p < 0.05000$) showed that there is a statistically significant difference between groups.

As can be seen from Table 3, the relative bioavailability of the drug "L-DOPA–PC nasal drops " (1) relative to the drug "L-DOPA in oil" (2) was 145.2%. The relative bioavailability of the drug "L-DOPA-PC nasal drops"(1) relative to the drug "L-DOPA-PC in purified water" (3) was 176.3%. The relative bioavailability of the drug "L-DOPA-PC nasal drops"(1) relative to the drug "Madopar 125" (4) was 244.4%.

Parkinson's disease is the most frequent neurodegenerative disease after Alzheimer's disease [13], [14]. The disease is common in occurrence. Disease prevalence varies from 120 to 180 cases per 100 thousand of the population; the number of patients increases significantly among the older age group. The prevalence per 100,000 population for the different age groups: 41 for the 40-49 age group; 107 for the 50-59 age group; 173 for the 55-64 age group; 428 for the 60-66 age group; 425 for the 65-74 age group; 1087 for the 70-79 age group; and 1903 for the age group over 80 years [15]. The average age of disease onset is 65.3 ± 2.6 years. The incidence in men is higher than in women [16]. No significant racial differences in the morbidity patterns were found [2].

A new method of administering nanoparticles, containing levodopa, allows rapid delivery of the dopamine neurotransmitter precursor to the brain cells. The most important feature of intranasal administration of drugs is the possibility of their penetration directly into the central nervous system. Transport of drugs from the nasal cavity to the central nervous system is performed without the involvement of the mucosa, extracellularly along the trigeminal and olfactory nerves. Within 10-15 minutes chemical agents administered intranasally are found in the brain. Drugs penetrate the brain only from the olfactory region, where there is a possibility of extra - and intracellular penetration of drugs through the epithelial barrier and getting not into the bloodstream, but directly to meninges.

The study showed that the use of nasal delivery method improves pharmacokinetic parameters of the drug in comparison with oral administration. All the above mentioned allowed to increase the time of drug presence in the blood and, potentially, to use the drug in much smaller doses with a reduction in risk of toxic complications.

In conclusion, a new form of the drug (oil-based drops), containing the precursor of dopamine - L-DOPA, was developed for intranasal administration. Pharmacokinetic parameters of "L-DOPA – PC nasal drops" were studied in comparison with three other potentially active dosage forms, containing L-DOPA in the same concentrations as the study drug: "L-DOPA in oil", "L-DOPA-PC in purified water", "Madopar 125". Assay procedure for the determination of L-DOPA in animal blood plasma using liquid chromatography with tandem mass-selective detection (HPLC-MS/MS) was developed and validated.

The relative bioavailability of the drug "L-DOPA–PC nasal drops" relative to the drug "L-DOPA in oil" was 145.2%; relative to the drug "L-DOPA-PC in purified water" – 176.3%; relative to the drug "Madopar 125" – 244.4%.

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Immunohistochemical Expression of Epidermal Growth Factor Receptor in Astrocytic Tumors in Iraqi Patients

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Abstract

BACKGROUND: Diffuse astrocytomas constitute the largest group of primary malignant human intracranial tumours. They are classified by the World Health Organization (WHO) into three histological malignancy grades: diffuse astrocytomas (grade II), anaplastic astrocytomas (grade III) and glioblastoma (grade IV) based on histopathological features such as cellular atypia, mitotic activity, necrosis and microvascular proliferation. Epidermal growth factor receptor (EGFR) is a 170-kDa transmembrane tyrosine kinase receptor expressed in a variety of normal and malignant cells regulating critical cellular processes. When activated, epidermal growth factor receptor (EGFR) triggers several signalling cascades leading to increased proliferation and angiogenesis and decreased apoptosis and hence associated with aggressive progression of the tumour. Epidermal growth factor receptor (EGFR) level is known to be a strong indicator associated with the aggressive behaviour of the tumour and acts as a prognostic factor for evaluating the survival rate.

AIM: To evaluate the expression of epidermal growth factor receptor (EGFR) in different grades of astrocytoma.

MATERIAL AND METHODS: formalin-fixed paraffin-embedded astrocytic tumours of 44 patients were collected from the archival material of pathology department of Ghazi Al Hariri Teaching Hospital during the period from June to December 2018. Hematoxylin and eosin-stained sections were used to characterise the tumours histologically based on cellularity, nuclear hyperchromasia, polymorphism, mitotic activity, vascular proliferation and necrosis with or without pseudopallisading of tumour cells. Diagnosis and grading of astrocytic tumours in this study were made according to WHO criteria (2016). Using a monoclonal antibody to the epidermal growth factor receptor (EGFR) and immunohistochemical analysis, the expression and distribution of epidermal growth factor receptor in astrocytic tumours were examined.

RESULTS: The study included 1 case pilocytic astrocytoma (grade I), 20 cases diffuse astrocytoma (grade II), 5 cases anaplastic astrocytoma (grade III) and 18 cases of glioblastoma (grade IV). Expression of EGFR was found in 38.88% of the glioblastoma samples (grade IV). However, none of the astrocytomas of WHO grades I, II and III showed immunoreactivity for EGFR protein. Different patterns of immunoreactive cells and significant intratumor heterogeneity of EGFR expression were observed in glioblastomas.

CONCLUSION: The immunohistochemical expression of Epidermal growth factor receptor (EGFR) was restricted only to high-grade astrocytic tumours, namely glioblastoma, thus may use to predict glioblastoma.

Introduction

Primary brain tumours are a heterogeneous group of benign and malignant tumours arising from the brain parenchyma and its surrounding structures. These tumours are an important cause of morbidity and mortality in both adults and children, often generating severe disabilities and producing high burden in both families and health care systems [1], [2].

The world age-standardized incidence rate for all primary brain tumours ranged from 4.3 to 18.6 per 100 000 per year [3].

In the United States, the incidence rate for primary brain and nervous system tumours in adults (aged 20 years or older) is estimated to be 29.9 per 100,000 persons (data from 52 cancer registries, 2011 to 2015) [4]. Approximately one-third of tumours are malignant and the remainder is benign or borderline malignant [4], [5].

Gliomas are tumours of the brain parenchyma that are classified histologically based on their resemblance to different types of glial cells. The major

types of glial tumours are astrocytoma, oligodendrogliomas, and ependymomas [6], [7].

The WHO classification and grading of CNS tumours recognise seven sub-types of astrocytic neoplasms grouped into two major categories. These include diffusely invasive astrocytoma (diffuse astrocytoma, anaplastic astrocytoma, glioblastoma) and the relatively more circumscribed tumours (pilocytic and pilomyxoid astrocytoma, pleomorphic xanthoastrocytoma, subependymal giant cell astrocytoma).

The so-called 'gliomatosis cerebri', a clinicopathologic and radiologic entity, is also a diffuse glioma, usually of the astrocytic type [8], [9].

Diffuse astrocytomas (WHO grades II to IV) account for roughly 40% of primary intracranial tumours, with an annual incidence of 4 per 100,000 person-years. They occur at all ages, although the median age is 30 to 40 for astrocytoma (grade II), 40 to 50 years for anaplastic astrocytoma (grade III), and 50 to 60 years for glioblastoma (grade IV). Glioblastomas are the most frequent, with low-grade examples being comparatively uncommon, particularly in the elderly [10].

The origin of astrocytic neoplasms may include neural stem cells, progenitor cells, or differentiated glial cells.

Many molecular markers used as prognostic markers in glioma these include IDH mutations, 1p/19q codeletion, MGMT promoter methylation, TERT promoter mutations and EGFR amplification [11].

Isocitrate dehydrogenase (*IDH1, IDH2*) and *TP53* gene mutations are considered to be early events in neoplastic progression. In contrast, allelic loss of chromosome 10 occurs predominantly in glioblastomas. Molecular genetic studies have revealed differences between glioblastomas that evolve over the years from low-grade astrocytoma (secondary) and those that arise *de novo* (primary). In particular, Epidermal growth factor receptor (*EGFR)* overexpression is common in primary glioblastoma, while IDH1 mutations are common in secondary glioblastoma [12].

Epidermal growth factor (EGF) and the epidermal growth factor receptor (EGFR) have long been recognised for their role in tumour growth [13]. There are four transmembrane epidermal growth factor receptors: EGFR (also known as human EGF receptor 1 or HER1), HER2, HER3, and HER4 [14].

The EGFR protein contains an extracellular ligand-binding domain, a transmembrane region and an intracellular domain with intrinsic protein-tyrosine kinase activity. Ligand binding of the EGF receptor activates the EGFR tyrosine kinase which phosphorylates proteins in the signal transduction pathway leading to activation of genes that regulate cell proliferation, angiogenesis, motility, and metastasis [15], [16].

In astrocytoma, overexpression of EGFR or ErbB1 (chromosome 7p11-p12) is a late event promoting malignant progression to a glioblastoma, with amplification and often accompanying activating mutations. EGFR amplification varying in ranges of 0-4%, 0-33% and 34%-64% in grade II, III and IV astrocytomas, respectively. This amplification correlated to the histological malignancy grade and lower overall survival [17], [18], [19], [20], [21].

It has been shown that EGFR amplification promotes invasion, proliferation and resistance to radiotherapy and chemotherapy [22], [23], [24], [25].

We aimed to evaluate the expression of epidermal growth factor receptor (EGFR) in different grades of astrocytoma in a sample of Iraqi patients.

Material and Methods

This cross-sectional study enrolled 44 formalin-fixed paraffin-embedded astrocytic tumours, 17 were females and 27 were males diagnosed with different grades of astrocytic tumours of which 1 case was Pilocytic astrocytoma grade I, 20 cases were diffuse astrocytoma grade II (18 cases were diffuse fibrillary astrocytoma and 2 cases were pleomorphic xanthoastrocytoma), 5 cases were anaplastic astrocytoma grade III and 18 cases were glioblastoma grade IV. Graded according to WHO criteria 2016 [26]. These cases were retrieved from the archival material of pathology department of Ghazi Al Hariri Teaching Hospital during the period from June to December 2018.

All the clinical information, including age, gender and location, had been taken from patients archive files.

All biopsies were obtained through open brain biopsy, from each paraffin block, 2 representative (4 micrometres) sections were obtained, one section stained with hematoxylin and eosin stain and characterized on the basis of cellularity, nuclear hyperchromasia, polymorphism, mitotic activity, vascular proliferation and necrosis with or without pseudo pallisading of tumor cells into different grades and the other section was subjected to immunohistochemical testing for Anti- EGFR antibody, clone (EP38Y) manufactured by Abcam dilution (1:100).

Interpretation of the results of IHC staining

Immunoreactivity was scored based on membranous and / or cytoplasmic staining [27].

A positive stain is indicated by a golden brown coloured precipitate at the site of specific cellular antigen localisation.

The positive control for EGFR was obtained from tonsillar tissue sections, which are known to express EGFR in its basilar squamous epithelial cells was used with each run.

Technical negative control was obtained by omission of the primary antibody (EGFR).

Scoring system

Immunohistochemical stains for EGFR were graded as follows: 0 (no cell stained), 1 + (< 5% tumor cells stained), 2 + (5- 50% cells stained), and 3 + (> 50% cells stained). For statistical analysis, a score of 0 and 1 were considered negative and a score of 2 or 3 was considered positive [28].

All statistical analyses were performed using SPSS ver. 19.0 (SPSS Inc., Chicago, IL, USA). Univariate data were summarised using standard descriptive statistics, tabulation of categorical variables and histograms of numerical variables. Associations between categorical variables were assessed via cross-tabulation and chi-square. T-test and ANOVA were used to compare means of continuous variables.

Spearman correlation was used to measure the association between two continuous variables or when at least one variable was ordered. Exact tests were used to calculate the p value. A p-value of less than 0.05 was accepted as statistically significant.

Results

Frequency distribution of different grades of astrocytoma

Histopathological review of primary brain astrocytic tumours involved in this study revealed the following:

The highest frequency was noticed in low-grade astrocytoma (grade II) which constituted 20 cases (45.45%), (18 cases were diffuse fibrillary astrocytoma and 2 cases were pleomorphic xanthoastrocytoma), followed by glioblastoma (grade IV) which constituted 18 cases (40.9%), anaplastic astrocytoma (grade III) which constituted 5 cases (11.36%) and pilocytic astrocytoma (grade I) with only one case (2.27%) according to WHO criteria 2016 [11].

Among cases with glioblastoma, the majority was primary15 (83.33%), and only 3(16.7%) were secondary (progress from low grade astrocytoma) (Figure 1 and 3).

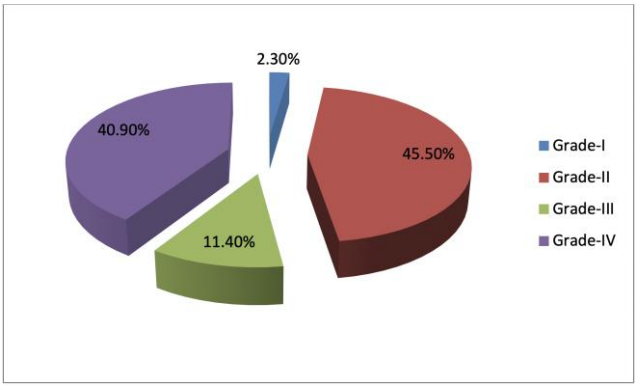

Figure 1: Pie chart showing distribution of cases according to grade of astrocytoma

Age group and sex distribution

Distribution among age groups revealed that the mean age of cases in this study was 37.41 ± 19.02 year.

The mean age of patients with grade IV astrocytoma was 44 ± 17.92 year, grade III 44.4 ± 6.66 year, grade II 30.95 ± 19.56 year and grade I 13 year with no statistically significant difference (p value = 0.073) (Table 1).

Table 1: Mean age of different grades of astrocytoma in the studied cases

Tumor Grade	Mean Age (year)	P value
Grade-I	13	0.073 (N.S)
Grade-II	30.95 ± 19.56	
Grade-III	44.4 ± 6.66	
Grade-IV	44 ± 17.92	
TOTAL	27 (61.4)	

Males constituted 61.4% of total cases. Differences between males and females among different grades of astrocytic tumors showed the following: males constituted 9/18 (50%) of patients with grade IV, 4/5 (80%) of patients with grade III, and 14/20 (70%) of patients with grade II with no statistically significant difference (p value = 0.3) (Table 2).

Table 2: Distribution of different grades of astrocytoma according to the gender of the studied cases

Tumor Grade	Males No. (%)	Females No. (%)	P value
Grade-I	0 (0)	1(100)	0.3 (N.S)
Grade-II	14 (70)	6 (30)	
Grade-III	4 (80)	1 (20)	
Grade-IV	9 (50)	9 (50)	
Total	27 (61.4)	17 (38.6)	

Epidermal growth factor receptor (EGFR) immunohistochemical expression

EGFR expression was positive in 7 (15.9%) of the cases, all of which were of grade IV astrocytoma. All cases of grade I, II, III were negative for EGFR expression 37 (84.1%) of the cases (Figures 2and 3).

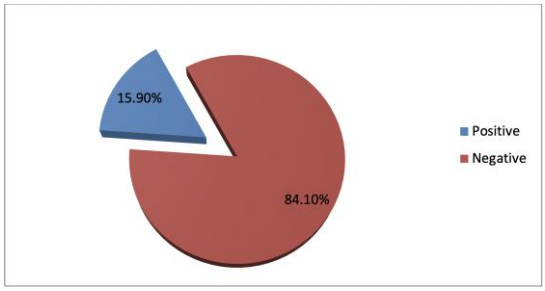

Figure 2: Pie chart showing the distribution of the cases according to the immunohistochemical expression of EGFR

Immunohistochemical expression of EGFR was seen only among those with glioblastoma, 7/18 cases showed EGFR expression (38.88%) as shown in Table 3, four were females (57.7%), and three were males (42.85%) with a mean age of 46.71 years.

Table 3: EGFR immunohistochemical expression according to the grade of astrocytoma

Tumour	No. of positive cases	EGFR expression %
Pilocyte astrocytoma grade I	0 / 1	0%
Diffuse astrocytoma grade II	0 / 20	0%
Anaplastic astrocytoma III	0 / 5	0%
Glioblastoma IV	7 / 18	38.88%
Total	44	

Immunohistochemical expression of EGFR according to the age of the studied cases

The mean age of those with immunohistochemical expression of EGFR was 46.71 years, whereas the mean age of negative cases was 35.64 years. The difference was *statistically significant (P-value = 0.04).* Within grade IV astrocytoma, the mean age of negative cases was 42.27 years compared to 46.71 years for the positive cases. The difference was *statistically insignificant (P-value = 0.06)* (Table 4).

Table 4: Association between immunohistochemical expression of EGFR and Age

Tumour	No.	Mean age (year)	P-value
Astrcytomas with positive expression of EGFR (all grades)	7	46.71	0.04
Astrocytomas with negative expression of EGFR (all grades)	37	35.64	
Glioblastomas with positive expression of EGFR	7	46.71	0.06
Glioblastomas with negative expression of EGFR	11	42.27	

Within grade IV astrocytoma, males constituted 3 cases (42.85%) of EGFR-positive cases and 6 cases (54.54%) of EGFR-negative cases.

The difference in sex regarding EGFR positivity was statistically insignificant (P-value = 0.45) (Table 5).

Table 5: Immunohistochemical expression of EGFR according to the gender of the patient with glioblastoma

EGFR expression in glioblastoma	Gender			P-value
	Male	Female	Total	
Positive	3	4	7	0.45 NS
Negative	6	5	11	
Total	9	9	18	

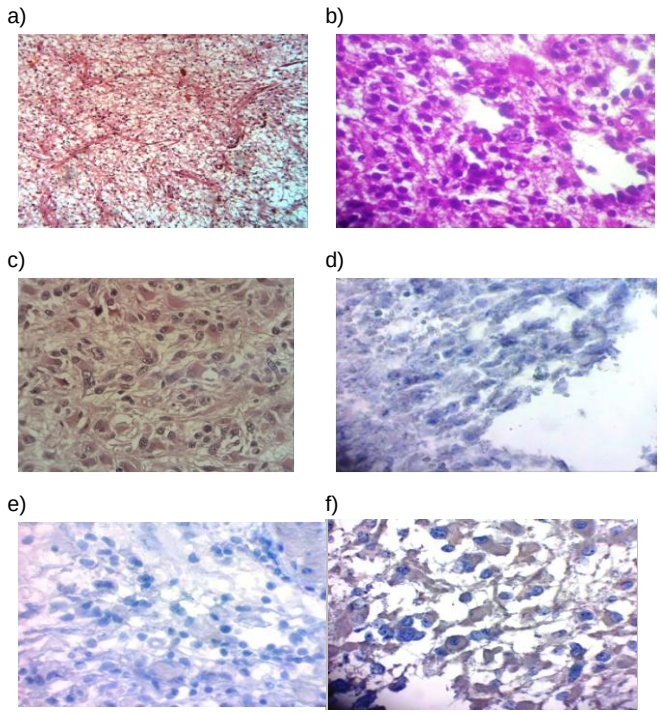

Figure 3: a) Pilocytic astrocytoma shows Rosenthal fibres, note homogenous eosinophilic staining (H & E × 10); b) Anaplastic astrocytoma, grade III. This lesion exhibits increased cellularity, the cytological features of a fully malignant neoplasm (H & E × 400); c) Glioblastoma grade IV, H & E, (x 400); d) Diffuse fibrillary astrocytoma, grade II with negative immunohistochemical expression of EGFR IHC (x 400); e) Anaplastic astrocytoma, grade III with negative IHC expression of EGFR (x 400); f) Glioblastoma (grade IV) stained with anti-EGFR showing positive staining for EGFR (complete membranous and cytoplasmic staining) + 3 (× 400)

Discussion

Epidermal growth factor and its receptor (EGFR) constitute an important and well-characterized mitogenic system in various ectodermal tissues, including glial cells.

Over-expression of the EGFR due to gene amplification has been reported in primary brain tumours of glial origin [29]. This amplification promotes invasion, proliferation and resistance to radiotherapy and chemotherapy thus correlated to the histological malignancy grade and lower overall survival [17], [18], [19], [20], [21], [22], [23], [24], [25]. Based on these features, this marker chooses in this study to predict high-grade astrocytoma.

Astrocytic tumours begin as early as in the first decade of life. Young adults are typically affected by low-grade astrocytoma while glioblastoma shows a peak incidence in the sixth decade. Anaplastic astrocytoma occupies an intermediate position [30]. Glioblastoma is among the most malignant human neoplasms with a mean duration of survival for less than one year.

Extensive research works on the molecular pathogenesis of glioblastoma may facilitate molecular classification of this tumour and predict prognostic markers.

Expression of EGFR is important in molecular classification and is considered as a new prognostic parameter for astrocytic tumors [20], [31], [32], [33].

The current study showed that astrocytic tumours are more common in males than in females, 61.4% compared to 38.6%**.** The mean age of patients was (37.41 ± 19.02) year. The mean age of patients with grade IV and grade III astrocytoma was higher than that of patients with grade I and II astrocytoma; However, these findings were of no statistical significance.

Several previous studies found similar findings regarding the male predominance and mean of age; in Chaloob et al., study the mean age for cases of astrocytoma was 35.98 ± 2.67 years, they also found slight male predominance (53%) compared to females (47%) in astrocytoma [30].

Maiti et al., in their study, found that males constituted 58% of cases with a mean age of 42 ± 13 years [31]. Kordek et al. showed that males constituted 56% of cases with a mean age of 45 ± 11 years [32]. In Agosti et al., study, males constituted 64% of cases and the mean age was 34 ± 14 years [29].

In this study, the majority of cases were of grade II (45.5%) followed by grade IV (40.9%), similarly, Chaloob et al., found in their study that grade II is the most frequent: 7 (13.2%) cases were grade I, 22 (43.1%) cases were grade II, 6 (11.8%) cases were grade III and 16 (31.4%) cases were glioblastomas (grade IV) [30]. On the other hand, other studies showed different findings, Agosti et al. showed that out of 103 cases with astrocytoma, 21 cases were of grade I, 10 cases of grade II, 26 cases of grade III, and the most frequent were of grade IV (46 cases) [29].

Similarly, Maiti et al., in their study that included 40 cases of astrocytoma found that 21 cases were of glioblastoma or Grade IV astrocytoma (52.5%), eight cases of anaplastic astrocytoma or Grade III astrocytoma (20%), six cases of diffuse Grade II astrocytoma (15%) and five cases were grade I (12.5%) [31]. In Kordek et al., study of 56 cases of astrocytoma, 8 cases were of pilocytic (grade I) astrocytoma, 9 cases were of grade (II) fibrillary astrocytoma, 9 cases were of high grade (III) anaplastic astrocytoma and the majority of the cases (30) of glioblastomas (grade IV) [32]. Gaitonde et al. showed in their study that out of 30 cases with astrocytoma, 2 cases were of grade I-II, 11 cases were of the anaplastic type, 13 cases were of glioblastoma type and 4 cases were of other histological types [33].

This difference in grade frequency among different studies may suggest geographical or environmental causes as Iraqi studies show the predominance of grade II while worldwide studies reveal grade IV predominance

EGFR amplification is rare in low-grade gliomas [32], [33], [34], [35], [36] however, many studies have reported EGFR amplification varying in ranges of 0-4%, 0-33% and 34%-64% in grade II, III and IV astrocytomas, respectively [17], [18], [19], [20], [21].

In the recent study, EGFR expression was found in only 7 (38.88%) cases of glioblastomas, while it was negative in all other types, these findings Although other similar studies showed EGFR expression in other grades of astrocytoma, still the expression was higher in higher grades. Kordek et al. showed that 23% of astrocytoma express EGFR and that the immunohistochemical expression of EGFR increased with the grade of malignancy (11.1%, 22.2% and 33.3%) [32]. Gaitonde et al. showed positive EGFR expression in 8 of 30 cases of astrocytoma [33]. Gines et al. found that 53% of primary glioblastoma showed EGFR amplification and 33% of them showed EGFR over-expression [36]. Smith et al., showed that EGFR amplification was present in 17% of anaplastic astrocytoma and 41% of glioblastomas [20].

Stark et al. showed that 64% of glioblastoma showed positive EGFR expression [37].

Maiti et al., and van der Valk et al., in their study, showed that all cases of grade I astrocytoma showed negative immunostaining for EGFR [31], [38]. However, EGFR positivity has been observed in Grades II-IV with increasing expression associated with higher grades of astrocytoma [38]. These results were in agreement with those reported by Smith et al., in their study [20].

Shinojima et al. found that overexpression of EGFR and gene amplification frequently occurs in gliomas and is restricted to high-grade tumours, especially anaplastic astrocytoma and glioblastoma multiforme [19]. In this study, there was a significant association between immunohistochemical expression of EGFR and age of the patients with the mean age of cases with positive expression significantly higher than cases with negative expression; this may be attributable to that the mean age of the patient with glioblastoma is higher where EGFR is over-expressed. Within grade IV astrocytoma, there was no statistically significant association between positive EGFR expression and age of patients, these findings wherein tune with that of Maiti et al., Stark et al., and Bouvier et al., [31], [37], [39].

On the other hand, Kordek et al., showed that higher expression was associated with a younger age group [32]. Smith et al., and Van der Valk et al., showed no significant association of EGFR expression with specific age group [20], [38].

Taking gender of the study sample into consideration, the current study reported no significant association of EGFR expression with the sex of the patients even within those who expressed the EGFR that is in unity with other authors; Maiti et al., and Kordek et al., [31], [32]. On the other hand, Bouvier et al., and Torp et al., showed higher expression of EGFR among female [39], [40], while Smith et al., and Agosti et al., showed more positive EGFR expression in male patients [20], [29]. These differences in relations with age and sex may be due to differences in sample size.

In conclusion, the immunohistochemical expression of Epidermal growth factor receptor (EGFR) was restricted only to glioblastoma, thus may use to predict a high-grade glioblastoma.

EGFR expressed in 38.8% of glioblastoma patients which means 38.8% of these patients tend to arise de novo as primary glioblastoma.

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Effect of Irbesartan-Poloxamer-188 Solid Dispersion on Intercellular Cell Adhesion Molecule-1 and Interleukin-8 on Hypertension Rats

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Keywords: Solid dispersion; ICAM-1; IL-8

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Abstract

BACKGROUND: Based on the Biopharmaceutics Classification System (BCS) system, irbesartan is a drug that belongs to the class II BCS group which has limitations in terms of dissolution rates with low bioavailability of 26% -60%. These limitations to bioavailability can be overcome by solid dispersion with hydrophilic matrices such as Poloxamer. Irbesartan is an angiotensin receptor blocker. At present, it is widely used in dealing with hypertension due to endothelial dysfunction.

AIM: This study aims to determine endothelial function blood markers can be examined, such as adhesion molecules (ICAM-1) and IL-8 pro-inflammatory cytokines.

MATERIAL AND METHODS: Research on the effects of irbesartan-poloxamer-188 solid dispersion on ICAM-1 and IL-8 in hypertensive rats has been carried out. The formation of solid dispersion through dissolution method while induction of hypertension using 2.5% NaCl and prednisone 1.5 mg/Kg BB orally in 3 treatment groups, irbesartan dose was 13.5 mg/kg. The parameters observed were serum ICAM-1 and IL-8 levels.

RESULTS: The result showed that the solid dispersion of irbesartan-poloxamer-188 could reduce ICAM-1 and IL-8 levels in hypertensive rats which differed significantly from the positive control group ($p < 0.05$).

CONCLUSION: This study concluded that the solid dispersion of irbesartan-poloxamer-188 effects and decreases ICAM-1 levels in the serum of hypertensive rats. Solid dispersion of irbesartan-poloxamer-188 can influence and reduce IL-8 in the serum of hypertensive rats.

Introduction

Based on the Biopharmaceutics Classification System (BCS) system, irbesartan is a drug that belongs to the class II BCS group, which has limitations in terms of dissolution rate [1], [2]. Availability of irbesartan is reported to be 26% [3] and 60% [4]. This limitation of bioavailability has been overcome by the solid dispersion of irbesartan made with dextrose water-soluble matrix, the method of making solid dispersion is done by grinding and smelting method [5], formulation and evaluation of irbesartan liquid-solid tablets to improve irbesartan dissolution and bioavailability [2].

Another study of solid dispersion technology using super disintegrant sodium starch glycolate, crospovidone, croscarmellose sodium and microcrystalline cellulose [3]. The latest study was comparing the dissolution rate of 2 methods of making irbesartan tablets namely wet granulation technique and sublimation technique [6].

To improve the effectiveness of treating hypertension using irbesartan, it is necessary to look for new polymers. Poloxamer-188 is often considered a functional excipient because it is an important component in the formulation. Regarding the amphiphilic structure possessed by this surfactant, it is widely used in the industry. In the study of irbesartan, solid dispersion with various comparisons of poloxamer-188 the best dissolution rate was obtained at a ratio of 2:1 [7].

In the inflammatory process, the endothelial surface will express adhesion molecules such as vascular cell adhesion molecule-1 (VCAM), intercellular cell adhesion molecule-1 (ICAM) and interleukin-8 (IL-8) [4]. On the other hand, the effect of dissolution rate and modification of the crystal properties of irbesartan on endothelial cells such as intercellular cell adhesion molecule-1 (ICAM) and interleukin (IL-8) has not been reported.

Material and Methods

Research Materials

Irbesartan (Dr Reddys), poloxamer-188 (Merck), ethanol 96%, prednisone, NaCl, NaCMC and distilled water, ELISA kits for ICAM-1 and IL-8 (USCN).

Instruments

Vacuum ovens, desiccators, digital analytic scales (Denver Instruments), UV-Vis spectrophotometers (UV-1700 PharmaSpec), ELISA reader.

Animal Experiments

White mice were weighing between 200-300 grams many as 24 (*Rattus norvegicus*) Wistar strain (Laboratory of Pharmacology), Faculty of Pharmacy, Andalas University, Padang.

Acclimatised animals

For the next 7 days were grouped into 4 groups. Three groups of experimental animals were given induction with 2.5% NaCl and prednisone 1.5 mg/kg body weight as much as 2 mL orally for 2 (two) weeks; then the experimental animals were given a test preparation orally at a dose of 13.5 mg/kg for 1 (one) week. Each group consisted of 6 rats and treated as follows: Group I as a negative control, was given standard food and drink, group II as a positive control, standard food and drinks were given and given induction, group III as a test group was given standard food and drinks and inducers and irbesartan non-dispersion dose of 13.5 mg/kg, group IV as a test group given standard food and drink and induction and solid dispersion of irbesartan the dose was equivalent to 13.5 mg/kg. Blood is taken from the eye vein by 1.5 ml at certain minutes. Then the separation between serum and blood objects was carried out, the serum was stored in a refrigerator and the storage cabinet for a sample temperature of -40°C for further analysis.

Test for Irbesartan Solid Dispersion Activity against ICAM-1 levels

All reagents are prepared, use well, according to the number of wells plate used and labelled. Well plate for standard solutions determined, blanks and samples, made 7 wells for standards and 1 for blanks, added 100 µL of each standard solution, blanks, and samples into appropriate wells, covered with incubation plate sealers for 2 hours at 37°C. The solution of each well was removed and not washed and added 100 µL of the working solution to detect the reagent in each well and incubated for 1 hour at 37°C after being covered with plate sealer.

The supernatant was removed and washed with 350 µL of wash buffer, with 3 x inverted plates. Next 100 µL of reagent B working solution was added to each well; the plate sealer was closed, incubated for 30 minutes at 37°C. The washing process was repeated up to 5 times, added 90 µL of the substrate solution to each well, covered with a new plate sealer and incubated for 15 minutes at 37°C and protected by the light the solution would turn blue. Fifty µL stop solution is added to each well, so the solution turns yellow. Then the microplate reader is traced with an ELISA at a wavelength of 450 nm to determine its optical density value. The results of ICAM-1 level determination, in each rat serum at a specified time compared to each experimental group.

Examination of Levels of IL-8

All reagents are prepared and use wells according to the number of wells used and labelled. Well for standard solutions, blanks and samples are determined, 7 wells for the standard (1000, 500, 250, 125, 62.5 and 31.25 pg/mL) are made and 1 for blanks, 100 µL of each standard solution is added, blank and the sample into the right well, covered with a sealer plate and incubated for 1.5 hours at 37°C. The solution of each well was removed and not washed and added 100 µL of the working solution to detect reagent (biotin-labelled antibody) into each well and incubated for 1 hour at 37°C after being covered with plate sealer. The supernatant was removed and washed with 350 µL wash buffer, doing 3x inverted plates. Then added 100 µL of the working solution (SABC) into each well, covered with a plate sealer and incubated for 30 minutes at 37°C. The washing process was repeated up to 5 times, added 90 µL TMB substrate solution to each well, covered with plate sealer new and incubated for 15 minutes at 37°C and protected by the light the solution would turn blue. 50 µL stop solution is added to each well, so the solution turns yellow. The microplate reader is then run by using ELISA at a wavelength of 450 nm to determine its optical density value. The results of IL-8 level determination, in each rat serum at a specified time compared to each experimental group.

Statistical analysis

The results of the non-dispersion evaluation of irbesartan and solid dispersion of irbesartan-poloxamer-188 on serum ICAM-1 and IL-8 levels were analysed using statistics IBM SPSS version 19. Normal distribution tests are carried out using the Saphiro Wilk test. If the value of sig. > 0.05, then the data is normally distributed. For further analysis, one-way parametric (ANOVA) is conducted. To determine the significance of the treatment, the Bonferroni Post Hoc test was carried out.

Research Ethics Requirements

The approval of the Ethics Commission of the Faculty of Medicine, Unand Padang with ethical clearance No. 395/KEP/FK/2017.

Results

Effect of giving irbesartan solid dispersion on ICAM-1 levels

The measurement results of the average ICAM-1 levels of each negative control group rat were: 5.97 ± 2.32 ng/mL, the positive control group were: 16.68 ± 1.30 ng/mL, the group of mice given the preparation irbesartan non-dispersion is 13.11 ± 2.48 ng/mL and the group of rats given irbesartan-poloxamer -188 solid dispersion preparation is 9.54 ± 1.04 ng/mL.

Table 1: Results of ICAM-1 examination in rat serum

NO	I (ng/mL)	II (ng/mL)	III (ng/mL)	IV (ng/mL)
1	7.36	17.01	12.99	8.63
2	7.62	18.42	9.82	10.79
3	3.56	17.36	13.43	10.13
4	7.62	16.61	16.83	9.65
5	2.46	17.19	14.50	7.97
6	7.22	14.45	11.06	10.046
X ± SD	5.97 ± 2.32	16.68 ± 1.3	13.11 ± 2.48	9.54 ± 1.04

These results showed that after induction, there was an increase in ICAM-1 levels in the positive control group whereas after irbesartan administration both non-dispersion and solid dispersion ICAM-1 levels decreased, as shown in Table 1.

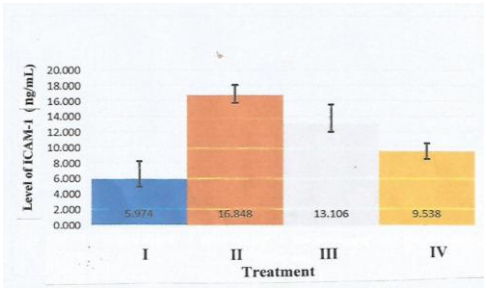

Figure 1: Levels of ICAM-1 in treatment animal group; I) Negative control; II) Positive control; III) Non-dispersion of irbesartan; IV) Solid dispersion of irbesartan

Effect of treatment on IL-8 levels

The administration of inducible substances in the positive control group caused an increase in IL-8 levels in the blood.

Table 2: Results of IL-8 examination in rat serum

NO	I (pg/mL)	II (pg/mL)	III (pg/mL)	IV (pg/mL)
1	43.88	52.29	49.47	41.56
2	38.19	50.04	46.65	41.01
3	39.89	50.04	50.60	44.96
4	39.32	50.60	42.71	45.53
5	40.45	53.98	46.09	45.53
6	43.27	52.86	42.14	47.22
X±SD	40.83 ± 2.26	51.63 ± 1.65	46.27 ± 3.43	44.30 ± 2.46

Decreased level of IL-8 was found in the treatment group after induction and administration of irbesartan non-dispersion and solid dispersion of irbesartan (Table 2 and Figure 2).

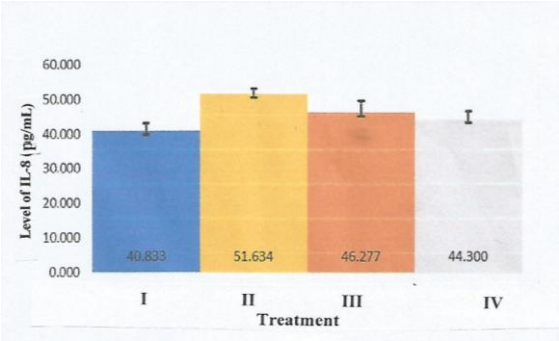

Figure 2: Levels of IL-8 in treatment animal group; I) Negative control; II) Positive control; III) Non-dispersion of irbesartan; IV) Solid dispersion of irbesartan

Discussion

ICAM-1 level

The results showed that after induction, there was an increase in ICAM-1 levels compared to negative controls of 16.85 and 5.47 ng/ml. Hypertension is an inflammatory process that involves the migration and accumulation of cells from innate and adaptive immune responses into the interstitium of blood vessels by releasing cytokines and increasing oxidative stress. Oxidative stress which plays a role in the pathogenesis of endothelial dysfunction in hypertension is from nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, integrin kinase 1 as a mechanism for the reduction in vascular superoxide production due to hypertension [8], [9].

The inflammatory process that occurs with changes in blood vessels that arise quickly and in the short term. This response occurs because the mediators of bradykinin and histamine produced by local mast cells cause vasodilation and increased permeability. This permeability phase occurs within a few minutes. After 30-60 minutes, there is a marginalisation of neutrophils which group along the

endothelium in the injury area, followed by the migration of blood fertilisers. The migration process occurs through the process of margination, adhesion, rolling (grinding), stop rolling and diapedesis [10].

At the stage of the process of marginalisation, the activated macrophages will produce mediators of proinflammatory cytokines, namely IL-1 and TNF-α. Furthermore, TNF-α will induce vascular endothelial cells to express adhesion molecules, namely selectin, integrin, immunoglobulin superfamily (ICAM-VCAM, mucin-like molecule, and CD44. There are three types of ICAM namely ICAM-1, ICAM-2, and ICAM-3. ICAM -1 and ICAM-2 are expressed by endothelial cells and also APC, where these bonds allow lymphocytes to migrate through the walls of blood vessels and there is a weak involvement between the molecules so that the leukocytes attach to endothelium [10].

In the rolling stage, the weak attachment between leukocytes and endothelium will be stronger, so that the strength of the bloodstream cannot release this bond. The attachment between leukocytes and endothelium becomes increasingly strong because of activation by chemotactic factors such as leukotriene B4, platelet-activating factor and IL-8 by increasing the affinity of leukocyte adhesion molecules for endothelial adhesion molecules The higher the blood pressure, the inflammatory response that occurs will also increase to protect the body from cell injury and a sustained inflammatory response will increase various proinflammatory cytokines, ICAM molecules and chemotactic factors IL-8 [10], [11].

The mean ICAM-1 levels from the group given irbesartan non-dispersion and solid dispersion of irbesartan-poloxamer-188, decreased by 13.106 ng/mL and 10.2014 ng/mL when compared to positive controls (16.847 ng/ml), but the results of the decline ICAM-1 levels did not reach the same value as the negative control (5.474 ng/ml). This is because animals that have experienced endothelial dysfunction so that recovery takes time.

Irbesartan, as one of the angiotensin blocker receptor drugs in the form of non-dispersion and solid dispersion, can inhibit the angiotensin II response binding to specific angiotensin receptors. Finally, the administration of this drug can improve endothelial function, increase vasodilator mediators and increase NO bioavailability and reduce the concentration of CRP (C-reactive Protein) and inflammatory markers such as IL-6, IL-1, ICAM and other factors like chemokine IL-8 [12].

The results of one-way analysis of variance analysis (ANOVA) on examination of ICAM-1 levels in experimental animals showed a significant difference between the groups given irbesartan to the positive control group. Based on the results of the Post Hoc Bonferroni statistic, there was a significant effect between negative controls with positive controls and non-dispersion groups and no significant effect on the solid dispersion group.

The results of the research that have been conducted show that the solid dispersion with poloxamer-188 carriers has an effect of decreasing the average ICAM-1 serum level with ICAM-1 levels lower than irbesartan non-dispersion. This is related to changes in the physicochemical properties of irbesartan can increase the rate of dissolution of the drug, with an increase in the dissolution rate of a drug and absorption of the drug so that the expected effect is also achieved [13].

Level of IL-8

The mean value of IL-8 levels from the group given irbesartan non-dispersion preparation and solid dispersion of irbesartan-poloxamer-188 decreased by 46.28 pg/mL and 42.80 pg/mL when compared to positive controls (51.63 pg/ml), but the result of a decrease in IL-8 levels has not reached the same value as the negative control (40.83 pg/ml).

The results of the research that have been carried out show that the solid dispersion with poloxamer-188 carriers has an effect of decreasing the serum levels of IL-8 with IL-8 levels which are lower than non-dispersible irbesartan. This is because there has been a change in the physicochemical properties of irbesartan so that solubility, dissolution, and bioavailability have increased [13]. The powder X-ray analysis showed the decreasing in peak intensity at $2\theta = 12.34°$ from 6345.9 to 2915.3, which indicates the formation of a crystal lattice that has a degree of symmetry. Decreasing the intensity of the interference peak shows changes in the degree of crystallinity so that it will increase its solubility [7].

The results of one-way analysis of variance analysis (ANOVA) on IL-8 levels in experimental mice showed a significant difference between the groups given the preparation to the positive control group. Based on the results of the Post Hoc Bonferroni statistic with positive and non-dispersion controls, the significant influence between negative controls with positive control and non-dispersion irbesartan was significant ($p < 0.05$) and did not significantly influence the solid dispersion group ($p > 0.05$).

In this study, the result showed that the solid dispersion of irbesartan could affect the physicochemical properties of irbesartan, decreasing the level of ICAM-1 and IL-8 levels in the serum of hypertensive mice. Decreased levels of ICAM-1 and IL-8 showed a decrease in the level of inflammation in endothelial cells so that the permeability of endothelial cells be better. This is proven by previous studies of a decrease in mice blood pressure and an increase in serum NO levels [7].

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Larvicidal Activity of Ketapang Leaf Fraction (*Terminalia catappa* L) on *Aedes aegypti* Instar III

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Keywords: Ketapang leaf fraction; Terminalia catappa L; Larvicidal; Aedes aegypti larvae

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Abstract

BACKGROUND: Mosquito control is essential in preventing mosquito-borne diseases. Natural substances originated from plants possessed the great potential of insecticidal properties, the resistance occurs at a slower rate compared to the synthetics, with less toxicity to other living creatures.

AIM: This study aimed to identify the fraction of ketapang leaf (Terminalia catappa L) with larvicidal activity on Aedes aegypti instar III larvae, the concentration of ketapang active leaf fraction comparable to temephos, and Lethal Concentration 50 (LC50).

METHODS: This study was experimental in vitro. The experiment was performed with five different concentrations of ketapang leaf water-ethanol fraction, i.e. 1200 ppm, 1400 ppm, 1600 ppm, 1800 ppm, 2000 ppm. Observation of morphological damage of mosquito larvae was conducted with the microscope and ImageJ application. Data analysis was performed using One Way ANOVA. LC50 was determined with probit analysis.

RESULTS: Phytochemical screening revealed a water-ethanol fraction of ketapang leaf contained tannin, saponin, and flavonoid compounds. The water-ethanol fraction with 1800 ppm concentration possessed significant larvicidal effect comparable to temephos. Probit test revealed an LC50 value of 1563.082 µg/ml.

CONCLUSION: Water-ethanol fraction of ketapang leaf possessed high biolarvicidal activity against Ae. aegypti larvae instar III comparable to temephos.

Introduction

Aedes aegypti mosquitoes are responsible for the epidemics of dengue in populations, with several affecting factors comprising climatic conditions (tropics and subtropics), population growth and travels. In recent decades, the incidence has rapidly arisen around the world. It is estimated there are 390 million dengue infections per year (95% credible interval 284 – 528 million), with clinical manifestations of 96 million (67 – 136 million). In another study, 3.9 billion people, in 128 countries, are at risk of dengue infection with an estimated 2.5% annual case fatality [1].

Mosquito control is essential in preventing mosquito-borne diseases. Mosquitocidal factors that interrupt vector ecology are ovicidal, larvicidal, pupicidal, and adulticidal and include organochlorides, organophosphates, and synthetic pyrethroids [2]. One dominant practice in mosquito control is the use of synthetic insecticides such as organochlorine and organophosphate compounds. However, continuous and repeated use of synthetic insecticides can lead to environmental pollution, the death of various species of living creatures, and it is possible to raise resistances of various species of mosquitoes as diseases vector. Interest in alternative methods of mosquito control with less environmental damage has commenced. Natural substances originated from

plants possessed the great potential of insecticidal properties, the resistance occurs at a slower rate compared to the synthetics, with less toxicity to other living creatures [3], [4].

Abundant prior studies had proven the potential of natural sources as an alternative in insecticides. Hirota *et al.* performed a study to investigate the larvicide activities of *Smilax larvata Griseb*. (Smilacaceae) extracts against *Ae. aegypti* larvae. The crude ethanolic extract presented larvicidal effect on instar III *Ae. aegypti* larvae [5]. A study by Ashwini *et al.* discovered *Acalypha indica* leaf extract possessed larvicidal activities against dengue vector *Aedes albopictus*. Instar III larvae of *Ae. albopictus* were exposed to 1000, 2000, 3000, 4000 and 5000 ppm concentrations of petroleum ether, chloroform, ethyl acetate, n-butanol, ethanol and aqueous extracts of *A. indica* [6].

El-Akhal *et al.* revealed the properties of larvicidal activity of essential oils of *Thymus vulgaris* and *Origanum majorana* family of Lamiaceae against the larvae of the malaria vector *Anopheles labranchiae* [7]. Ramar *et al.* found the efficacy of essential oils (EOs) as anti-mosquito agents and its adulticidal prospective of the essential oils against *Culex quinquefasciatus* [8].

These studies further develop natural sources as an alternative to chemicals in insecticide. Ketapang possesses the properties of antimicrobial, antioxidative, anti-inflammatory, hepatoprotective, antidiabetic, anticarcinogenic, antimalaria, and antinociceptive [9], [10], [11], [12], [13], [14], [15], [16], [17]. Natural products isolated from ketapang comprising of triterpenoids (ursolic acid, Asiatic acid), squalene but no caffeine, flavonoids (isovitexin, vitexin, and rutin), gallic acid, hydrolysed tannins such as punicalagin anomers as a major component, punicalin, terflavins A and B, tergallagin, tercatain, chebulagic acid, geranin, granato B, and corilagin [12], [18].

This study aimed to identify the fraction of ketapang leaf (*Terminalia catappa L*) with larvicidal activity on *Aedes aegypti* instar III larvae, the concentration of ketapang active leaf fraction comparable to temephos, and Lethal Concentration 50 (LC50).

Material and Methods

This study was experimental in vitro performed from February to March 2018. Ketapang leaf extraction and fractionation were conducted at Biomolecular Laboratory, Faculty of Medicine, Sriwijaya University, Palembang. Ketapang leaf was obtained from Cibanteng Village, Ciampea District, Bogor, Indonesia. The dried leaves were mashed with a blender and sieved to obtain the fine powder. The fine powder was macerated with 96% ethanol for 2 x 24 h. The macerate was evaporated using a water bath for 2 h with a temperature of ± 80°C until thickened and pasty mass was formed. The fractionation process was carried out by liquid-liquid fractionation method in which the active fraction was partitioned in the separation funnel.

The efficacy test of ketapang leaf fraction against *Ae. aegypti* larvae were performed at Entomology Laboratory of Lokalitbang P2B2, Baturaja, Ogan Komering Ulu Regency, South Sumatra Province. The efficacy test was performed with a preliminary test on *Ae. aegypti* instar III larvae with three ketapang leaf fractions using n-hexane, ethyl acetate and water-ethanol at 2000 ppm concentration and observed for 24 h. The preliminary test exhibited that water-ethanol fraction possessed the highest larvicidal activity on *Ae. aegypti* instar III larvae with larvae death of 96.67% at 2000 ppm concentration. The water-ethanol fraction was selected to undergo further investigation with five concentrations. After obtaining the fraction concentration in the preliminary test, the experimental group were treated with five different concentrations of water-ethanol ketapang leaf fraction, i.e. 1200 ppm, 1400 ppm, 1600 ppm, 1800 ppm, 2000 ppm. Temephos (Abate®, BASF, Ludwigshafen, Germany) 1000 ppm dose was used as a positive control and aqua destilata as the negative control. In each experimental glass, 30 *Ae. aegypti* larvae instar III and 100 ml of water was inserted. To obtain the optimal concentration to kill the larvae, the process was repeated three times.

Observation of morphological damage of mosquito larvae before and after treatment was performed under an Axioplan (Zeiss) microscope and images were captured with the digital camera AxioCam HRC (Zeiss) and ImageJ application. Data analysis was conducted using One Way ANOVA with SPSS 21.0 software (SPSS Inc., Chicago, USA), followed by Games Howell post hoc test. LC50 was determined using probit analysis.

Results

Phytochemical screening of ketapang leaf on n-hexane fraction revealed the contents of steroid, terpenoid, saponin, and flavonoid compounds, while ethyl acetate and water-ethanol fraction contained tannin, saponin, and flavonoid compounds. The preliminary test exhibited that water-ethanol fraction possessed the highest larvicidal activity on *Ae. aegypti* instar III larvae with larvae death of 96.67% at 2000 ppm concentration. The water-ethanol fraction was selected to undergo further investigation with five concentrations. Figure 1 exhibited the death of *Ae.*

aegypti larvae were at its highest (100%) at 2000 ppm ketapang leaf concentration.

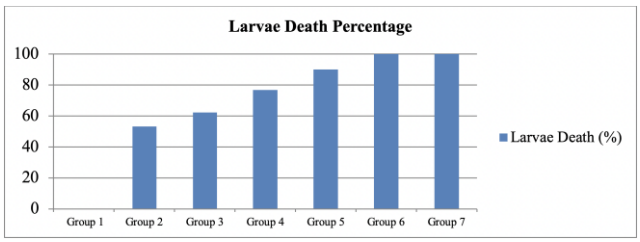

Figure 1: Percentage of Ae. aegypti instar III larvae deaths 24 h after administration of water-ethanol fraction of ketapang leaf (T. catappa L); Group 1: aqua destilata; Group 2: ketapang leaf 1200 ppm; Group 3: ketapang leaf 1400 ppm; Group 4: ketapang leaf 1600 ppm; Group 5: ketapang leaf 1800 ppm; Group 6: ketapang leaf 2000 ppm; Group 7: temephos

The average percentage of morphological damage of instar III larvae such as head, piston, abdomen, siphon, and anal papillae from various ketapang leaf concentrations and controls were presented in Table 2.

Table 2: Percentage of morphological damage to Ae. aegypti instar III larvae after administration of water-ethanol fraction of ketapang leaf (T. catappa L)

Group	Mean percentage (%) of *Ae. aegypti* larvae damage				
	Head	Pectoral	Abdomen	Siphon	Anal papillae
1	1.64	1.39	1.69	1.67	1.69
2	69.56	60.36	50.37	54.57	30.54
3	67.19	56.73	48.56	53.67	30.53
4	63.20	55.98	45.83	51.94	28.18
5	59.58	44.98	31.51	49.29	21.35
6	59.76	43.16	29.87	47.53	21.88
7	70.64	65.39	55.69	55.67	36.69

Group 1: aqua destilata; Group 2: ketapang leaf 1200 ppm; Group 3: ketapang leaf 1400 ppm; Group 4: ketapang leaf 1600 ppm; Group 5: ketapang leaf 1800 ppm; Group 6: ketapang leaf 2000 ppm; Group 7: temephos.

Table 2 showed the highest occurrence of morphological damage of the larvae was at 1200 ppm concentration aside from the positive control and was the lowest at 2000 ppm concentration.

Table 3: Lethal Concentration (LC50) from a water-ethanol fraction of ketapang leaf

LC50 (µg/ml)	1563.082

There were significant differences in regards to the number of *Ae. aegypti* instar III larvae death between different concentrations of a water-ethanol fraction of ketapang leaf ($p < 0.05$), followed by Games-Howell post-hoc test with a p-value at 1800 ppm concentration 0.134 ($p > 0.05$). Ketapang leaf fraction larvicidal activity at 1800 ppm concentration was comparable to temephos. Probit test exhibited Lethal Concentration (LC50) from a water-ethanol fraction of ketapang leaf at 1563.082 µg/ml.

Discussion

To protect themselves from herbivores, plants possess the evolving secondary metabolites that transform into active toxic ingredients, which insects feed on them. It is potential for insects to be exposed to these toxic secondary metabolites and affected physiologically with various impacts on targets ranging from proteins (enzymes, receptors, signalling molecules, ion-channels and structural proteins), nucleic acids, biomembranes, and other molecular components. It is potential for the receptor sites to be affected, including the alteration of neurotransmitter synthesis, storage, release, binding, and re-uptake, also other metabolic processes such as receptor functionality, enzymes in signal transduction pathway, etc. [19], [20], [21].

Secondary metabolites affected the insect physiology through several mechanisms include essential oils through inhibition of acetylcholinesterase (AChE), disruption of morphogenesis and alteration in the behaviour and memory of cholinergic system, thymol through gamma-aminobutyric acid (GABA) gated chloride channel and octopamine receptors, pyrethrin through sodium and potassium ion exchange disruption, and rotenone through inhibition of cellular respiration, ryanodine through the blockage of calcium channels, sabadilla through nerve cell membrane action, azadirachtin through hormonal balance disruption and mitotic poisoning. Out of several mechanisms, AChE inhibition holds a pivotal role in attenuating neurotransmitter through the synaptic pathway. Altered AChE is the mechanism of insect pests resistance and AChE has been reported to be organophosphorus and carbamate resistant [19], [20], [21].

Phytochemical screening of ketapang leaf revealed water-ethanol fraction contained tannin, saponin, and flavonoid compounds. Tannins can interfere with an insect's ability to digest food and absorb protein, through binding to proteins essential for growth [22]. High cytoplasmic vacuolation, absence of cytoplasmic limits, apical vesicle formation with the release of cytoplasmic contents of the cells, increased intercellular space and detached cells from the basement membrane, are major toxicities caused by tannins in mesenteron cells of *Ae. aegypti* instar III. The mechanisms resemble the processes of insects encountering toxic substances. When tannic acid is utilised against Diptera larvae, histopathological processes initially occurs in the anterior region of the midgut, progressing to the median and posterior regions [23].

On the other hand, saponins exerted membrane-permeabilising and haemolytic properties. Saponins are freely soluble and can be extracted in both aqueous and organic solvents. Saponins attack the cuticle membrane of the larvae, disturbing the membrane, which leads to larval death [24]. Saponins

increase mortality levels, lower food intake, causing weight reduction, retardation in development, disturbances in development and decreased reproduction in pest insects. The suggested mechanisms underlying these are that saponins act as a repellent on food, or to cause digestive problems due to moulting defects or toxic effects on cells [25]. Flavonoid compounds also possess promising larvicidal potential. Mechanism of action of the compound on mosquito larvae are not yet established, but previous studies demonstrated that chemicals interfered with the mitochondria at the proton sites [26].

The LC50 value of ketapang leaf water-ethanol fraction to the death of *Ae. aegypti* instar III larvae were at 1563.082 µg/ml. LC50 value of the water-ethanol fraction of ketapang leaf was categorised as having an effective larvicidal power, due to its value was still below the WHO standard of concentration value [27].

In conclusion, phytochemical screening revealed a water-ethanol fraction of ketapang leaf contained tannin, saponin, and flavonoid compounds. The water-ethanol fraction of ketapang leaf possessed high biolarvicidal activity against *Ae. aegypti* larvae instar III. The water-ethanol fraction with 1800 ppm concentration possessed significant larvicidal effect comparable to temephos.

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Basic Science

The Probiotic Bacterium Isolated from Bekasam (Traditional Fermented Food), *Lactobacillus Sp*. Induces Activation of Gut Mucosal Immune System in Rat

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Keywords: Bekasam fermented food; Lactobacillus sp.; Immunoglobulin A; CD4; CD8

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Abstract

BACKGROUND: Bekasam is one of the traditional foods in South Sumatra, Indonesia, a mixture of fermented fish containing Lactic Acid Bacteria (LAB), *Lactobacillus sp.* Non-commensal bacteria and probiotics can induce intestinal mucosal immune responses.

AIM: This pilot study aimed to see the efficacy of *Lactobacillus sp.* to the immune response of the intestinal mucosa by assessing the levels of IgA in the intestinal fluid and markers of T cell populations, such as CD4 and CD8 in the intestinal mucosa.

METHODS: This study was an in vivo experimental study. As many as 30 rats were grouped into 3 treatment groups (doses 107, 108, and 109 CFU/rat/day, for 7 days) and 2 groups of controls (negative control, 10% non-fat milk, and positive control, *Lactobacillus casei* 108 CFU/rat/day for 7 days). At the end of the treatment, the intestinal mucosa was taken to examine the levels of IgA, CD4 and CD8 using the Enzyme-Linked Immunosorbent Assay (ELISA) method, according to the manuals of each ELISA kit. All displays of research data were presented with means ± SD. T-test was used to assess the significance of differences.

RESULTS: Secretion of Ig A increased with the addition of *Lactobacillus sp.* from bekasam. Administration of *Lactobacillus sp.* yielded no effect on helper T cell level (CD4 markers), as well as on cytotoxic T cell levels (CD8 markers).

CONCLUSION: *Lactobacillus sp.* probiotic from bekasam improved the intestinal mucosal immune system by increasing the production of Ig A, but exhibited no effect on T lymphocyte cells.

Introduction

The immune system plays a role in the body's defence against the threat of invasion or infection from various pathogenic microorganisms. The immune system consists of innate and adaptive, both of which will induce a systemic and mucosal immune response. In the intestinal mucosa, the innate immune response acts not only as an initial defence of microorganisms but also induces activation of the adaptive immune response. Non-commensal bacteria and probiotics can induce intestinal mucosal immune responses [1].

Macrophages and dendritic cells play a major role in initiating innate immune responses, which will specialise into phagocytic cells. Phagocytic cells possess receptor patterns able to recognise pathogenic molecules found on the surface of pathogenic cells. These receptors will be activated by pathogens [2].

One family receptor that operates in the introduction of pathogen molecules is the Toll-like receptor (TLR). TLR plays an important role in the body's defence warning system against the presence of pathogenic material [3]. TLR activates innate immune responses, especially inflammation, before the activation of the adaptive response system [4], [5]. TLR-2 can recognise microbial components such as lipoprotein/lipopeptides from various pathogens and able to detect the presence of peptidoglycan and lipoteichoic acid from gram-positive bacteria. TLR-2 can recognise lipopolysaccharide (LPS) from enterobacteria such as *Leptospira interrogans,*

Porphyromonas gingivalis and *Helicobacter pylori* [6], [7].

Another family that operates in the introduction of molecular pathogens is the mannequin receptor family. CD-206 is a mannose family receptor that plays a role in the clearance of self-antigens such as endogenous proteins, myeloperoxidase, lysosomal hydrolase and several hormones that contain groups of sulfated carbohydrate [8]. This receptor binds to a group of carbohydrates containing mannosyl/fucosyl residues so that it can recognise proteoglycans from microbial origins [9].

Bekasam is one of the traditional foods in South Sumatra, Indonesia. This food is a mixture of fermented fish. Bekasam contains Lactic Acid Bacteria (LAB), *Lactobacillus sp.* [10]. Several studies show the role of probiotic, especially LAB, as a major component in maintaining health and preventing various diseases. Consumption of probiotics is useful in managing diarrhoea, including antibiotic-induced diarrhoea and diarrhoea in children commonly caused by rotavirus [11], [12], [13]. Innate immune components interplay with intestinal epithelial cells and bacteria [14]. Probiotics had also been proven to benefit in urogenital health [15], improvement of the periodontal condition [16], chronic colitis [17], inflammatory bowel disease [18], [19], and even to prevent or reduce the incidence of oral and respiratory tract infections in paediatrics [20].

Probiotic microorganisms are supplements of living bacteria to maintain the balance of bacteria in the intestine. *Lactobacillus sp*. is a microorganism commonly used as probiotics. Probiotics can stimulate the secretion of immunoglobulin (Ig) A in the intestine. Also, probiotics can increase the secretion of proinflammatory cytokines such as tumour necrosis factor (TNF) alpha and increase regulatory inflammatory cytokines such as interleukin (IL)-4 and IL-10 [21].

This pilot study aimed to see the efficacy of *Lactobacillus sp.* to the immune response of the intestinal mucosa by assessing the levels of IgA in the intestinal fluid and markers of T cell populations, such as CD4 and CD8 in the intestinal mucosa.

Material and Methods

This study was an in vivo experimental study. As many as 30 rats (age 10 weeks, body weight 180-200 gr) *Rattus norvegicus* were used in this study. Each treatment and control group consisted of 6 rats. Each rat was supplied with food and drink in ad libitum. This study was approved by the research ethics committee of the Faculty of Medicine, Universitas Sriwijaya-RSMH (kpt fkunsri-rsmh no. 113/2018). Furthermore, rats were grouped into 3 treatment groups (doses 10^7, 10^8, and 10^9 CFU / rat/day, for 7 days) and 2 groups of controls (negative control, 10% non-fat milk, and positive control, *Lactobacillus casei* 10^8 CFU / rat/day for 7 days). Probiotics were administered by 1 mL of sterile 10% non-fat milk.

Bekasam was composed by mixing 1 kg of fish with 3% salt and 250 grams of rice. Furthermore, the mixture was wrapped with plastic tightly and left for 8 days at room temperature (28-34°C). Bacteria derived from samples were grown into MRSA media. Of these bacterial cultures, colonies that grew dominant with different morphology, both in terms of color, shape as observed from the top, and shape of the protrusion were taken into the media of Man Rogosa Sharpe Agar (MRSA) and morphology the cell was observed. At the end of the treatment, the rat was killed by anesthesia, and the intestine was evacuated. Furthermore, the intestinal mucosa was taken to examine the levels of IgA, CD4 and CD8 using the Enzyme-Linked Immunosorbent Assay (ELISA) method, according to the manuals of each ELISA kit (Cloud-Clone Corp®, Texas, USA).

Statistical analysis was conducted with SPSS for Windows (SPSS Inc., Chicago, Illinois, USA). All displays of research data were presented with means ± SD. T-test was used to assess the significance of differences between treatment and control groups.

Results

Secretion of Ig A increased with the addition of *Lactobacillus sp.* from bekasam. Efficacy of *Lactobacillus sp.* in increasing levels of Ig A at 10^9 CFU dose was greater than the positive control group with *Lactobacillus casei* at a dose of 10^8 CFU. In the group with *Lactobacillus sp.* at a dose of 10^8 CFU possessed the effect of increased Ig A production almost comparable to the control group with *Lactobacillus casei* at a dose of 10^8 CFU, although statistically, the increase in the positive control group was slightly greater than the treatment group receiving *Lactobacillus sp.* at dose of 10^8 CFU. Table 1 showed that *Lactobacillus* probiotic administration elevated Ig A secretion in the intestinal mucosa.

Table 1: Level of IgA in Intestinal Mucosa

Group	Level (ng/mL)	p-Value
Treatment 10^7 CFU	94.18±6.21	0.021*. 0.027#
Treatment 10^8 CFU	124.83±8.55	0.001*. 0.043#
Treatment 10^9 CFU	189.11±10.17	0.001*. 0.001#
Negative control	91.23±3.45	0.001#
Positive control	125.23±9.23	0.001*

*Independent T test VS negative control; # Independent T test VS positive control.

Lactobacillus sp. probiotic possessed no effect on T cells in the intestinal mucosa (Table 2). Administration of *Lactobacillus sp.* yielded no effect on helper T cell level, which was characterised by CD4

markers, as well as no effect on cytotoxic T cell levels, which was marked by CD8 markers.

Table 2: Level of CD4 and CD8 in Intestinal Mucosa

Group	Level CD4 (ng/mL)	Level CD8 (ng/mL)	p-Value CD4	p-Value CD8
Treatment 10^7 CFU	23.18 ± 8.28	22.18 ± 6.28	0.17*. 0.11#	0.37*. 0.31#
Treatment 10^8 CFU	23.63 ± 7.15	24.83 ± 7.15	0.11*. 0.32#	0.21*. 0.31#
Treatment 10^9 CFU	23.91 ± 7.76	23.11 ± 8.16	0.15*. 0.18#	0.11*. 0.21#
Negative control	21.43 ± 8.01	21.23 ± 6.01	0.14#	0.21#
Positive control	22.73 ± 6.29	22.23 ± 5.29	0.21*	0.16*

*Independent T test VS negative control; # Independent T test VS positive control.

Discussion

Epithelial surface of the intestine is the area contacting with intestinal commensal microorganisms, which play a role in optimising the immune system. The surface of the gastrointestinal epithelium also always confronts various external microorganisms. This condition causes a significant role of the intestinal mucosa as a defence against invasion from various microorganisms. An effective defence mechanism is in dire need to maintain the homeostasis of the intestinal mucosa. One defence mechanism in the intestinal mucosa is through the production of IgA antibodies. Whereas, protective microflora will protect the intestinal mucosa from the invasion of pathogenic microorganisms. Protective microflora in the intestinal mucosa will stimulate the proliferation of epithelial cells and the development of the mucosal immune system [6], [14].

Intestinal epithelial cells are the first defence against various bacterial invasions and various products of pathogenic bacteria, especially in apical epithelial cells. Whereas at the basolateral side of epithelial cells, immune cells are present. Epithelial cells are capable of introducing components of bacterial structures that are in contact with the intestine so that they will be able to recognise these bacteria as pathogens or non-pathogens [3]. Non-pathogenic probiotic bacteria originating from food can affect the mucosal immune system in the intestine. Contact between these microorganisms and epithelial cells as well as immune cells in Peyer's patches of the intestine will trigger immune cells such as monocytes/macrophages and dendritic cells to initiate an innate immune response and antigenic stimulation. The basic functions of the mucosal immune system include protection from various pathogens, preventing penetration of various foreign antigens, inducing oral tolerance to various antigens and maintaining mucosal homeostasis. The main difference between the mucosal immune system and the adaptive immune system is in the presence of innate immunity and the activation of B cells rather than T cells [22].

Probiotic species alone do not result in a clinical effect; rather, they facilitate modulation of the gut microbiota composition and metabolic activity, thereby influencing the immune response [23]. Probiotic bacteria interact with the intestinal epithelial cells (IECs) or immune cells associated with the lamina propria, through Toll-like receptors, and induce the production of different cytokines or chemokines. Macrophage chemoattractant protein 1, produced by the IECs, sends signals to other immune cells leading to the activation of the MIS, characterised by an increase in immunoglobulin A+ cells of the intestine, bronchus and mammary glands, and the activation of T cells. Specifically, probiotics activate regulatory T cells that release IL-10. Interestingly, probiotics reinforce the intestinal barrier by an increase of the mucins, the tight junction proteins and the Goblet and Paneth cells. Another proposed mechanism of probiotics is the modulation of intestinal microbiota by maintaining the balance and suppressing the growth of potentially pathogenic bacteria in the gut [24].

This study was the first study to observe the efficacy of *Lactobacillus sp.* from typical fermented foods of South Sumatra, Indonesia (Bekasam) to the optimisation of the mucosal immune system in the intestine. This study showed *Lactobacillus sp.* probiotic elevated Ig A production in the intestinal mucosa but possessed no effect on T lymphocyte cells, which was characterised by no significant effect of *Lactobacillus sp.* probiotic to the expression of CD4 and CD8 in the intestinal mucosa. The administration of *Lactobacillus casei* in this study also exhibited a similar result to the administration of *Lactobacillus sp.* from bekasam. They improved the mucosal immune system by increasing the secretion of IgA but exhibited no effect on T lymphocyte cells.

In conclusion, *Lactobacillus sp.* probiotic from bekasam improved the intestinal mucosal immune system by increasing the production of Ig A. Efficacy of *Lactobacillus sp.* in increasing levels of Ig A was comparable to *Lactobacillus casei*. *Lactobacillus sp.* probiotic possessed no effect on T cells in the intestinal mucosa. Administration of *Lactobacillus sp.* yielded no effect on helper T cell level, which was characterised by CD4 markers, as well as no effect on cytotoxic T cell levels, which was marked by CD8 markers.

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Basic Science

Comparison of Clinico-Pathological Presentations of Triple-Negative versus Triple-Positive and HER2 Iraqi Breast Cancer Patients

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Abstract

BACKGROUND: Breast cancer remains the most common malignancy among the Iraqi population. Affected patients exhibit different clinical behaviours according to the molecular subtypes of the tumour.

AIM: To identify the clinical and pathological presentations of the Iraqi breast cancer subtypes identified by Estrogen receptors (ER), Progesterone receptors (PR) and HER2 expressions.

PATIENTS AND METHODS: The present study comprised 486 Iraqi female patients diagnosed with breast cancer. ER, PR and HER2 contents of the primary tumours were assessed through immunohistochemical staining; classifying the patients into five different groups: Triple Negative (ER/PR negative/HER2 negative), Triple Positive (ER/PR positive/HER2 positive), Luminal A (ER/PR positive/HER2 negative), HER2 enriched ((ER/PR negative/HER2 positive) and all other subtypes.

RESULTS: The major registered subtype was the Luminal A which was encountered in 230 patients (47.3%), followed by the Triple Negative (14.6%), Triple Positive (13.6%) and HER2 Enriched (11.5%). Patients exhibiting the Triple Negative subtype were significantly younger than the rest of the groups and presented with larger size tumours. A significant difference in the distribution of the breast cancer stages was displayed ($p < 0.05$); the most advanced were noted among those with HER2 enriched tumours who exhibited the highest frequency of poorly differentiated carcinomas and lymph node involvement.

CONCLUSION: The most significant variations in the clinicopathological presentations were observed in the age and clinical stage of the patients at diagnosis. Adoption of breast cancer molecular subtype classification in countries with limited resources could serve as a valuable prognostic marker in the management of aggressive forms of the disease.

Introduction

The burden of breast cancer, as the most prevalent malignant neoplasm among females globally [1] and in Iraq specifically [2], has been illustrated comprehensively in numerous surveys that emphasised the importance of its early detection and control [3], [4], [5], [6], [7], [8]. Evidence suggests that patients diagnosed with the disease exhibit different clinical behaviour according to the various pathological and biological characteristics of the tumour; thus, recommending different therapeutic strategies.

Earlier analytic studies on gene expressions revealed that the response of the mammary carcinoma cell to treatment is determined by intrinsic molecular characteristics that could be probed. The pioneer molecular portrait presented by Sorlie et al., classified breast cancer into five intrinsic subtypes with distinct clinical outcomes, i.e., luminal A, luminal B, HER2 over-expression, basal and normal-like tumours [9], [10]. Later on, immunohistochemical (IHC) assays categorised breast cancer into three major adopted phenotypes; the Luminal, HER2 enriched and Triple Negative (TN).

TN breast cancer is considered a heterogeneous group that comprises the basal subtypes which are reported to be more frequent among younger age patients and exhibit more aggressive nature with limited therapeutic options and high risk of recurrence [11], [12], [13]. On the other

hand, the luminal-like tumours, that express hormone receptor, are the most common subtypes that carry a good prognosis. They usually respond well to hormonal treatment but poorly to conventional chemotherapy. While Luminal A could be adequately controlled with endocrine treatment, luminal B (triple positive TP) phenotypes often tend to be more proliferative, yielding relatively higher grade and recurrent tumours; thus, recommending combined chemotherapy and hormonal treatment [10], [14].

Whereas few previous studies registered the various breast cancer IHC subtypes among Iraqi patients [5], [6], [15], [16], [17], [18], reported data on their different behaviours and outcomes to remain scanty.

This study aims at providing further information on the clinical and pathological presentations of TN Iraqi breast cancer patients as compared to the other variants focusing on the triple positive (TP) and HER2 enriched phenotypes.

Material and Methods

The present study comprised 486 Iraqi female patients with a histopathologically confirmed diagnosis of mammary carcinoma registered between 2015 and 2017 at the Oncology Teaching Hospital and the National Cancer Research Center, Baghdad, Iraq. The studied clinical and pathological parameters were obtained from the case sheet records and pathology reports of the corresponding patients and included the age at diagnosis, tumour type, grade and size, lymph node status and the stage of the disease at presentation. Written consents were obtained from all the patients enrolled in the present work to use the relevant recorded information and approval of the study design was given by the Ethical Committee of the National Cancer Research Center of Baghdad University following the ethical standards laid down by the Declaration of Helsinki.

The reported data were reassessed to document the requested variables. Pathologically, breast cancers were typed according to the WHO classification [19] and graded following the modified Nottingham Bloom-Richardson categorisation [20]. The UICC TNM System was adopted to classify the clinical stage of the disease at presentation [21]. Evaluation of the Estrogen receptor (ER), Progesterone receptor (PR) and HER2 contents of the primary tumours was performed through IHC staining of the formalin-fixed paraffin-embedded tissue blocks using Dako kits (Denmark) including the specific monoclonal antibodies [14], [15].

The major registered receptor-defined subtypes were: - Luminal A (ER/PR positive/HER2 negative); - Luminal B/Triple Positive (ER/PR positive/HER2 positive); - HER2 Enriched (ER/PR negative/HER2 positive); and - Triple Negative (ER/PR negative/HER2 negative).

Other recorded subtypes included: - ER (positive)/PR (negative)/HER2 (positive); - ER (negative)/PR (positive)/HER2 (positive); - ER (positive)/PR (negative)/HER2 (negative); and - ER (negative)/PR (positive)/HER2 (negative).

Accordingly, patients were classified into five groups: TN (Group I); TP (Group II); Luminal A (Group III); HER2 enriched (Group IV); and all other subtypes (Group V).

Statistical correlation to compare the demonstrated clinical and pathological features of the different breast cancer subtypes was carried out using SPSS version 16.0 statistical program. Categorical data were presented by frequencies and percentages. Chi-square test was used to assess the association between the different variables. *P* values, less or equivalent to 0.05, were considered significant.

Results

IHC examination of the diagnosed breast cancer tissue specimens belonging to 486 patients revealed that the total rates of ER, PR and HER2 positive tumour contents were equivalent to 68.5%, 66.2% and 29.6% respectively. The major registered subtype was the Luminal A (E+/P+/H-) which was encountered in 230 patients (47.3%), followed by Triple Negative (E-/P-/H-) in 71 patients (14.6%), Luminal B/Triple Positive (E+/P+/H+) in 66 patients (13.6%) and HER2 Enriched (E-/P-/H+) in 56 patients (11.5%). Other encountered phenotypes were rare and included in order of frequency: (E+/P-/H-) in 22 cases (4.5%); (E-/P+/H-) in 19 cases (3.9%); (E+/P-/H+) in 15 cases (3.1%) and (E-/P+/H+) in seven cases (1.4%) (Figure 1, Table 1).

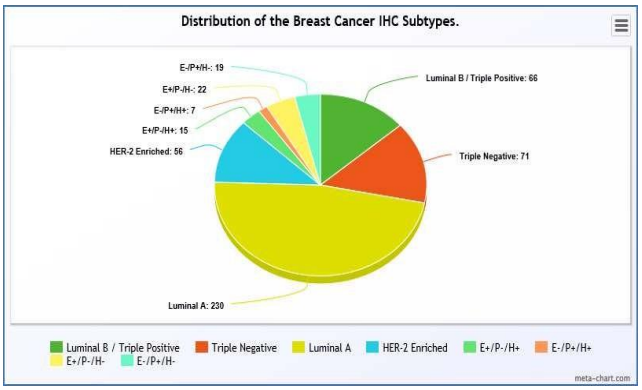

Figure 1: Frequency distribution of Iraqi breast cancer IHC subtypes

Table 2 illustrates the clinical and pathological presentations of the TN breast cancer subtype compared to the TP/Luminal B, HER2 enriched and

Luminal A phenotypes. Significant differences were noted among the different studied groups concerning the age of the patients at presentation ($p < 0.05$). Patients exhibiting the TN subtype were significantly younger than the rest of the groups (69% under the age of 50 years versus 52% overall).

Table 1: Overall rates of the reported breast cancer subtypes among Iraqi patients

	IHC Subtype	ER* Status	PR** Status	HER2*** Status	IHC Phenotypes	Total No.	Total %
1	Luminal B/Triple Positive	E+	P+	H+	E+/P+/H+	66	13.6
2	Triple Negative	E-	P-	H-	E-/P-/H-	71	14.6
3	Luminal A	E+	P+	H-	E+/P+/H-	230	47.3
4	HER-2 Enriched	E-	P-	H+	E-/P-/H+	56	11.5
5	E+/P-/H+	E+	P-	H+	E+/P-/H+	15	3.1
6	E-/P+/H+	E-	P+	H+	E-/P+/H+	7	1.4
7	E+/P-/H-	E+	P-	H-	E+/P-/H-	22	4.5
8	E-/P+/H-	E-	P+	H-	E-/P+/H-	19	3.9
Total						486	100

*Total Estrogen Receptor rate: 68.5%; **Total Progesterone Receptor rate: 66.2%; ***Total HER2 rate: 29.6%.

Although the rate of Lobular carcinoma was higher among group III (Luminal A), yet the differences in frequencies of the various histologic types of breast cancer were not statistically significant.

Table 2: Clinical and tumour characteristics categorized according to the examined breast cancer subtypes

	Group I E-/P-/H-TN*		Group II E+/P+/H+TP** Luminal B		Group III E+/P+/H- Luminal A		Group VI E-/P-/H+Her2- Enriched		Group V All other Subtypes		Overall Total Subtypes		p-value
	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	
Age (years)													11.863.
< 50	49	69.0	33	50	107	46.5	28	50	36	57.1	253	(52.1)	P=0.0184
=>50	22	31.0	33	50	123	53.5	28	50	27	42.9	233	(47.9)	Significant
Tumour Type													
Ductal	56	78.9	53	80.3	171	74.4	48	85.7	49	77.8	377	(77.6)	
Lobular	5	7	4	6.1	21	9.1	1	1.8	3	4.8	33	(6.8)	7.259
In situ	0	0	2	3.0	11	4.7	1	1.8	1	1.6	11	(2.3)	p=0.848
Others/mixed	4	5.6	2	3.0	13	5.7	2	3.6	4	6.3	18	(3.7)	NS
Unknown	6	8.4	5	7.6	14	6.1	4	7.1	6	9.5	47	(9.7)	
Tumour Grade													
I	2	2.8	0	0	14	6	0	0	0	0	16	(3.3)	13.0145
II	50	70.4	41	62.1	153	66.5	35	62.5	39	61.9	318	(65.4)	p=0.016
III	13	18.3	12	18.1	32	13.9	15	26.8	14	22.2	86	(17.7)	NS
Unknown	6	8.4	13	19.7	31	13.5	6	10.7	10	15.9	66	(13.6)	
Tumour Size													
T1	11	15.5	10	15.1	48	20.9.	6	10.7	13	20.6	88	(18.1)	
T2	34	47.9	43	65.2	128	7	35	62.5	39	61.9	279	(57.4)	16.321
T3	18	25.3	9	13.6	31	13.5	8	14.2	5	7.9	71	(14.6)	P=0.4308
T4	4	5.6	2	3	10	4.3	2	3.6	2	3.2	20	(4.1)	NS
Tx	4	5.6	2	3	13	5.6	5	8.9	4	6.3	28	(5.8)	
LN Status													
N0	20	28.1	20	30.3	79	34.3	14	25	20	31.7	153	(31.5)	2.045
N+	42	59.1	40	60.6	135	58.7	38	67.9	39	61.9	294	(60.5)	p=0.563
Nx	9	12.7	6	9	16	7	4	7.1	4	6.3	39	(8.0)	NS
Stage													
I & II	37	57.8	37	60.7	133	61.9	20	38.5	35	59.3	262	(53.90	9.6886.
III & IV Unknown	27	42.2	24	39.3	82	38.1	32	61.5	24	40.7	189	(38.9)	P=0.0460
	7		5		15		4		4		35	(7.2)	Significant
Total	71	(14.6)	66	(13.6)	230	(47.3)	56	(11.5)	63	(13.0)	486	(100)	

*Triple Negative; **Triple Positive

Likewise, whereas the highest frequency of poorly differentiated mammary carcinomas was observed among group IV (HER2) subtype (26.8% versus 17.7% overall), such difference was not significant. Larger size tumours (T3 and T4) were more common in patients with the group I (TN) phenotype (30.9% versus 16.6%, 17.8%, 17.8% and 11.1% in groups II, III, and IV and V respectively). More than two-thirds of patients in group IV (HER2) presented with metastatic lymph node involvement (67.9%); that rate was higher than the other subtypes. Nevertheless, Chi-square statistics failed to reveal any significant variations between the studied groups regarding tumour size or nodal status. On the other hand, a statistical difference in the distribution of breast cancer stages was displayed ($p < 0.05$); with significantly more advanced stages (III and IV) noted in patients with HER2 enriched subtypes (61.5%) compared to 42.2%, 39.3% and 38.1% in groups I, II and III respectively.

Discussion

Cancer is currently forming a major public health concern in Iraq; being responsible for the second cause of death among the general population [2], [22], [23]. Iraqi studies, in particular, have displayed the emerging dilemma of controlling breast cancer, the most common registered malignancy, which is increasingly diagnosed alarmingly among middle-aged females at quite advanced stages [4], [5], [6], [7], [8], [23]. It has been well established that racial disparities contribute to the various morphologies and presentations of breast cancer among patients in different ethnic groups reflecting the reported discrepancy in incidence, prognosis and survival from the disease [24], [25], [26], [27]. The molecular diversity of breast cancer illustrated in genomic analytic studies promoted the development of targeted therapies to the genetic alterations that drive certain identified cancer subtypes. The registered frequencies of ER+, PR+ and HER2+ breast cancers in the present work were 68.5%, 66.2% and 29.6% respectively. Whereas the corresponding figures from western studies are significantly higher for ER+ and PR+ breast cancers, they are statistically lower for HER2+ tumours contributing to better outcomes of the disease in well-developed regions of the world [5], [24], [25], [26], [27], [28], [29].

As observed in earlier studies from Iraq [5], [15], [16], [17], [18] and the literature [9], [10], [11], [12], [13], [14], [24], [25], [26], [27], [28], [29], [30], [31], [32], [33] the major registered breast cancer subtype was Luminal A (47.3%) that comprises positive hormone receptors in the absence of HER2 overexpression; stimulating the favorable biological behavior of the disease in the affected patients. While the displayed rate is close [29] and almost higher than those documented in surveys from Arab countries [30], [31], yet it is significantly lower than those reported in Western studies [5], [24], [25], [26], [27], [28]. Focusing on molecular characteristics and management implications of breast cancer in Arab populations, it was noted that disparities in these regions were not only confined to the clinicopathological features but existed as well at the molecular levels as reflected in the relevant genomic expression profiles [5], [27], [29], [30], [31]. A recent study on the comparative behaviour of breast cancer among Iraqi and British patients pointed out to heterogeneity of the underlying tumour biology that reflected genetic susceptibility. The authors concluded

that the significantly younger ages and advanced stages at the presentation of breast cancer among Iraqi patients, associated with the higher rates of TN and HER2 enriched phenotypes, recommended comprehensive assessment of the surrogate subtypes to ensure effective approaches in the management of the disease in Iraq [5]. Such higher rates of aggressive breast cancer subtypes among patients from low-middle income countries compared to those living in high-income societies were illustrated in earlier surveys [27], [29], [30], [31], [32], [33]. The second prevalent breast cancer subtype observed in the present work (14.6%) was the TN which is characterised by the negative expressions of ER, PR and HER2.

Interestingly that frequency was close to the rate recorded in the Annual Report to the Nations on the incidence of breast cancer subtypes in the US [28]; highlighting the significantly low prevalence of hormone receptor-negative tumours among the American patients. Consistent with the findings displayed earlier by other investigators, patients bearing this subtype in our study were significantly younger than those exhibiting the other phenotypes and presented with larger size tumours at the time of diagnosis [11], [12], [13]. However, they were less likely to have poorly differentiated tumours and positive lymph node involvement than group IV; the HER2-enriched tumour counterparts. Whereas previous reports showed that TN breast cancer tumours with more positive axillary lymph node, higher clinical stages and histological grades leading to aggressive clinical behaviour [34], [35], others demonstrated that TN encompasses a wide spectrum of entities possessing different biological and clinical attitudes including low-grade disease with indolent behaviour and favourable outcome [36], [37].

In general, patients exhibiting the HER2 enriched variant, which constituted 11.5% of total subtypes in the present work, displayed the highest frequency of poorly differentiated cancers and metastatic lymph nodes. It has been recorded in the literature that between 15-25% of breast cancers possess overexpression of HER2 and yield unfavourable clinical outcome [28], [38], [39]. The registered rates of HER2 + subtypes in the Arab series are quite higher [29], [40]; pointing out to the elevated prevalence of less differentiated tumours in the region [29], [30], [31], [40], [41]. Such regional and ethnic differences in the grades of the tumour are most probably related to genetic, biological and environmental factors. TP Luminal B subtype formed about 13.6% in the present study; close to the findings reported in previous studies from Iraq [5], [6], [15], [16], [18], and the neighboring countries [41], [42]. In a recent survey performed on a cohort of Iraqi patients presenting with breast cancer, no significant differences were noted in the clinicopathological presentations of patients with this phenotype compared to the others apart from the variation in the distribution of tumour types; where infiltrative ductal carcinomas were more common [16]. That was in contrast to the findings observed in another study which showed that invasive ductal carcinomas were preponderant among the TN subtypes [36]. Similar to other studies [43], the data of this work did not reveal any statistical differences in the distribution of the histological types among the examined groups, though the rate of lobular carcinoma was more common among patients with Luminal A.

Concerning the breast cancer stage at presentation, our results revealed a significant difference in the distribution among the various groups ($p < 0.05$); with the highest frequency of advanced stages (III and IV) being observed among those harbouring the HER2 enriched subtypes. It has been displayed in the literature that hormone-receptor-positive breast cancer expressions are often associated with earlier stages at presentation and that HER2+ impact is reflected by poorly differentiated tumours and advanced stages [39].

A recently published Iraqi study correlating the stage of breast cancer at the time of diagnosis with the clinicopathological characteristics of the affected patients demonstrated that 64.4% and 67.2% exhibiting Luminal A and Luminal B subtypes respectively were diagnosed at Stages I and II whereas 68% and 62% of those harbouring the TN and HER2+ respectively presented at advanced stages (III and IV) [6]. Comparative retrospective evaluation of the clinical features and survival outcomes of 1134 invasive breast cancer subtypes showed that the overall five-year survival was almost similar in TN and HER2 subtypes, nevertheless, subjects with HER2 presented at later stages with more frequent local recurrences [44].

In conclusion, patients within the TN group were statistically younger and exhibited larger tumour sizes than others. On the other hand, poorly differentiated tumours and metastatic lymph node involvement were more commonly encountered among patients with HER2 subtype who presented with significantly more advanced stages at the time of diagnosis. Adopting molecular subtype classification of breast cancer, as a cost-effective, reliable clinical investigation in countries with limited resources, is recommended to provide a feasible tool for assessing the response to therapy and to serve as a valuable prognostic marker in the management of aggressive forms of the disease among younger patients.

Author Contribution

Prof. Dr Nada Alwan, designed the study, analysed the results, wrote the manuscript and presented the final version. Mr Furat Nidhal supported

in providing relevant information, data entry and data analysis.

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Basic Science

Age Stratification in Genetic Variation of Lipoprotein Lipase in Metabolic Syndrome Javanese Ethnics of Indonesia

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Abstract

BACKGROUND: Metabolic syndrome (Met-S) that caused by heredity and Lipoprotein Lipase (LPL). LPL is involved in the metabolism of serum lipids. Variations in LPL alter enzyme activity, and the most common variations are LPL +495 T > G and LPL Pvu II C > T.

AIM: This study aimed to identify the role of LPL +495 T > G and LPL PvuII C > T gene variations in subjects with Met-S in Javanese ethnic based on age stratification.

METHODS: We recruited 160 participants of Javanese ethnicity consisting of 80 cases and 80 control subjects. Met-S was diagnosed according to the criteria of NCEP ATP III. Peripheral blood samples were collected to determine biochemical parameters. Screening for both polymorphisms was made by PCR-RFLP.

RESULTS: Results found that genotype and allele frequencies for LPL +495 T > G were not significantly different between Met-S and controls with and without age stratification. In LPL PvuII C > T based on age stratification, there were significant differences between TT vs CC, recessive and dominant models in Met-S and control. In the age group > 45 years CC genotypes and TC+CC had increased risk of Met-S compared to TT genotypes. In summary, there was no significant association between LPL +495 T > G gene variation with Met-S.

CONCLUSION: In LPL PvuII gene variation, TC + CC is the risk genotype of Met-S in the age group > 45 years.

Introduction

Metabolic syndrome (Met-S) is a common metabolic disorder and increases the risk for type 2 diabetes mellitus, cardiovascular disease and non-cardiac vascular diseases including stroke, carotid artery disease, peripheral artery disease, chronic kidney disease, atherosclerotic renal artery stenosis, and abdominal aortic aneurysms [1]. The National Cholesterol Education Program Adult Treatment Panel – III (NCEP ATP-III) guidelines and many others have defined Met-S as a combination of three or more of the following components: increased waist circumference (WC), elevated triglycerides (TG), reduced high-density lipoprotein – cholesterol (HDL-C), elevated blood pressure (BP) and elevated fasting plasma glucose (FPG) [2].

Heritability can cause Met-S and with estimates ranging from 10 to 30% [3]. Lipoprotein Lipase (LPL) is involved in the metabolism of lipids. Mature LPL is a 448 amino acid glycoprotein with chromosomal location 8p22, consisting of 10 exons and 9 introns [4]. LPL hydrolyses triglycerides in circulation from chylomicrons and very-low-density lipoproteins and generates fatty acids for storage in the adipose tissue or oxidation in the skeletal muscle [5], [6]. Several mutations and variations have been described in the *LPL* gene in humans, most of which lead to decreased LPL-activity. Variation in LPL altering enzyme activity may confer susceptibility for or have a protective effect against triglyceride level increase, Met-S and coronary arterial diseases (CAD) [7].

Many reports described variations and missense mutations in LPL, which are clustered in several restriction fragment length polymorphisms (RFLPs). An LPL+495 T > G and PvuII C > T are common variants of the LPL gene and may be associated with subtle alterations in plasma lipids [8]. Variation of LPL+495 T > G which results from a Thymine (T) to Guanine (G) substitution and PvuII C > T which results from a Cytosine (C) to Thymine (T) substitution located on intron 6, are located 1.57 kb from the Splicing Acceptor (SA) site. This polymorphism is the product of a change of cytosine for thymine [9].

This study aimed to identify the role of LPL *+495 T > G* and LPL PvuII C > T gene variations in subjects with Met-S in Javanese ethnic based on age stratification.

Methods

Design and participants

This study used a case-control design. Met-S and control participants were Javanese ethnics matched by gender and age, consisting of 80 cases and 80 control subjects (34 males and 46 females, respectively), who were recruited from a local regency in Yogyakarta, Indonesia. Inclusion criteria for the Met-S group were: subjects 20-65 years, diagnosed Met-S according to criteria of NCEP ATP III consisting of WC ≥ 90 cm for men and ≥ 85 cm for women, BP ≥ 130 / 85 mmHg or in the treatment, TG level ≥ 150 mg/dL or in the treatment, HDL-C level < 40 mg/dL for men and < 50 mg/dL for women or under treatment and FPG ≥ 100 mg/dL or treatment [1], [10]. Inclusion criteria for control subject were: 20-65 years and not diagnosed with Met-S according to criteria of NCEP ATP III. The study was approved by the Medical and Health Ethics Committee (MHERC) of the Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada-Dr. Sardjito General Hospital with registration number KE/FK/0761/EC/2018. All participants in this study signed an inform consent form.

Medical history and anthropometry measurements

Demographic data for all participant included age, ethnicity, history of disease, cigarette and alcohol consumption. Anthropometry measurements included weight (kg), height (cm) and waist circumference (cm). Weight was measured using a digital scale by Karada-Scan (Karada Scan HBF 375, by Omron) in light clothing without shoes and socks. Height was measured using stadiometers in standing position barefoot with feet together and arm by the side. WC was measured with tape on the midline between the inferior cost of the rib and the superior of the iliac crest. BMI was calculated from height and weight (kg/m2).

Blood pressure (mmHg) was measured twice by mercury sphygmomanometer in the brachial artery above the intercostal fossa after resting for at least 3 minutes. The mean of the two measurements was used for systolic and diastolic blood pressures [11].

Biochemical measurements

Biochemical analyses using peripheral blood was collected in EDTA blood tubes after 8 hours of fasting. FPG concentrations, total cholesterol (TC), TG, and HDL-C were measured enzymatically with an automated analyser (Cobas c111^{R} analyser with the protocol of Glucose HK, HDL cholesterol Gen4, Triglycerides, Cholesterol Gen2 from Roche diagnostic$^{R;}$ Germany) with standard protocol.

DNA Extraction

DNA was extracted from buffy coat using the FavorPrep™ blood genomic DNA extraction mini Kit (Favorgen) and stored at -20°C. The concentration of DNA extraction used nanodrop to check the purification of DNA with the ratio of absorbance at 260 nm and 280 nm. A ratio of ~ 1.8 is generally accepted as “pure” for DNA.

Genotyping and LPL Gene Polymorphism Analysis Digestion

Genotyping of the *LPL +495 T > G* and PuvII C > T were performed by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism assays (PCR-RFLP). PCR amplification was performed in a Thermal Cycler 1000 instrument (Bio-Rad) using 30 mL of total volume. Amplification follow an initial denaturation at 95°C for 7 minutes, followed by 35 cycles of denaturation at 95°C for 1 minute, annealing at 54°C (HindIII) and 58°C (PvuII), respectively for 1 minute, and extension at 72°C for 1 minute and final extension at 72°C for 7 minutes. The primer set is shown in Table 1.

Table 1: Primer set

Primers	Enzyme and palindromic sequence	Restriction (bp)	allele
+495T>G	*HindIII*		
F : 5'-GATGCTACCTGGATAATCAAAG-3'	5'-A"AGCTT-3'	138 and 214	T
R : 5'- CAGCTAGACATTGCTAGTGT-3'	5'-TTCGA"A-3'	352	G
Pvu II C>T	*PvuII*		
F : 5'- GCTTAATTCTCAATTCAATGTC-3	5'-CAG"CTG-3'	188	C
R : 5'- TTTAGACTCTTGTCCAGGT-3	5'-GTC"GAC-3'	150 and 38	T

In LPL +495 T > G, the PCR product was digested with HindIII enzyme using 5 U of the enzyme at 37°C for 4 hours. In LPL PvuII C > T, using PvuII

enzyme 5 U at 38°C for 1 hour. Hind III and Pvu II enzyme were purchased from New England BioLabs (NEB).

Statistical analysis

Data analysis was performed for normality using Kolmogorov-Smirnov tests. Differences in baseline characteristics between Met-S and control used independent T-test if data had a normal distribution and were expressed as mean ± standard deviation and Mann Whitney test if not normally distributed with median (min-max) by 95% confidence interval (CI). The Hardy – Weinberg equilibrium was tested by a goodness-of-fit with the χ^2 test to compare the observed genotype frequencies with the expected ones among the Met-S subjects. The frequencies of the alleles and genotypes among different subgroups were compared by the chi-square test. Then, if bivariate analysis results were significant, they were estimated by adjusted Odds Ratio (OR) and their 95% CI from logistic regression analyses with the adjustment for BMI and hypercholesterolemia.

Results

Baseline characteristics between Met-S and control subjects are significantly different in Body Mass Index (BMI), Waist Circumference (WC), Blood Pressure (BP) and metabolic profiles (Table 2). The total numbers of subjects in this study were 160, consisting of 80 patients diagnosed with Met-S (case group) and 80 subjects as controls. The selection of case and control subjects in this study were matched by age and sex. Table 1 shows the WC, BMI, systolic and diastolic blood pressure, TG and TC were significantly higher in the Met-S group ($p < 0.001$) as compared to controls.

Table 2: Demographic Characteristics of the Subjects in Met-S and control

Baseline characteristic	Met-S (n = 80)	Control (n = 80)	p
Aged	44.73 (22.06-63.93)	44.80 (20.46-62.49)	0.883**
Gender			
Man	34 (50)	34 (50)	1.000***
Woman	46 (50)	46 (50)	
BMI (kg/m²)	29.97 ± 4.74	24.29 ± 3.62	< 0.001*
WC (cm)	96.03 ± 10.39	81.71 ± (9.35)	< 0.001*
SBP (mmHg)	130.00 (100.00-190.00)	110.00 (90.00-155.00)	< 0.001**
DBP (mmHg)	85.00 (60.00-145.00)	75.00 (60.00-95.00)	< 0.001**
FPG (mg/dL)	69.20 (44.05-227.60)	69.45 (43.20-171.00)	0.167**
Triglyceride (mg/dL)	183.72 ± 94.74	101.92 ± 41.09	< 0.001*
TC (mg/dL)	182.80 (88.10-380.20)	155.15 (95.30-246.60)	< 0.001**
HDL-C (mg/dL)	35.00 (24.40-51.10)	41.90 (22.70-76.70)	< 0.001**

*Independent sample t-test, data presented in the mean (± SD); **Nonparametric using Mann-Whitney test, data is presented in median (min-max); ***chi-square test; p value is significant < 0.05; BMI = Body Mass Index; DBP = Diastolic blood pressure; FPG = Fasting Plasma Glucose; HDL-C = High-Density Lopoprotein Cholesterol; SBP = Systolic blood pressure; TC = Total Cholesterol; WC = Waist Circumference.

The LPL *+495 T > G* genotypes were determined by Polymerase Chain Reaction (PCR) and digestion using HindIII restriction enzyme (Figure 1).

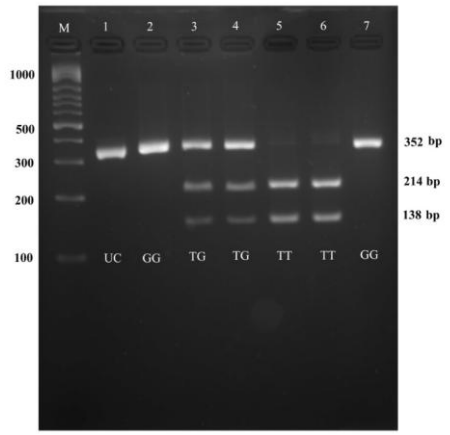

Figure 1: Results of PCR-RFLP gene variation of LPL + 495T > G; M as a marker with 100 bp; lane 1, showed PCR product 352 bp; lane 2-7 showed GG genotype with 1 band at 352 bp; Lane 3-4 showed TG genotypes and consisting of 3 bands each 352 bp, 214 bp and 138 bp; Lane 5-6 showed TT genotypes with 138 bp and 214 bp

The LPL PvuII *C > T* genotypes were determined by PCR and digestion using PvuII restriction enzyme (Figure 2).

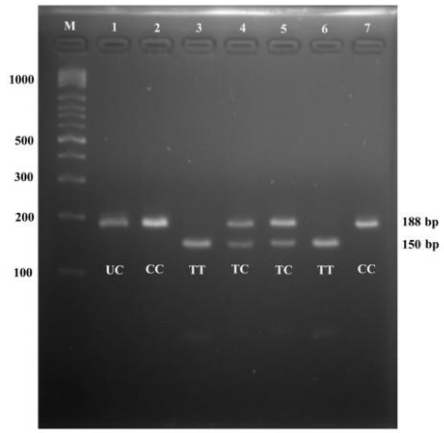

Figure 2: PCR-RFLP results of LPL PvuII C > T gene variation; M as a marker with 100 bp; lane 1, showed pPCR product 188 bp; lane 2-7 showed CC genotype with 1 band at 188 bp; Lane 4-5 showed TC genotypes and consisting of 3 bands each 188 bp, 150 bp and 38 bp; Lane 3-6 showed TT genotypes with 2 bands 150 bp and 38 bp.

The population was in Hardy-Weinberg Equilibrium ($p > 0.05$), for LPL +495 T > G and PvuII C > T gene variation (Table 3).

Table 3: Hardy-Weinberg Equilibrium in LPL +495 T > G and Pvu II C > T

Gene variation	Observed value	Expected value	χ^2 (DF)	*P*-value
LPL *+495T > G*				
TT	98	101	1.84	0.175
TG	58	52		
GG	4	7		
PvuIIC > T				
TT	77	76	0.06	0.816
TC	67	68		
CC	16	15		

DF = 1; χ2 = Chi squared test.

Genotype and allele frequencies for LPL +495 T > G were not significantly different between Met-S and controls (Table 4). The comparison between the additive, recessive and dominant genotype frequencies between the Met-S group and the control group showed no statistically significant differences.

Table 4: Genotype and Allele Distribution of LPL + 495T> G Genes in Met-S and Control Subjects

LPL *+495 T > G*	Met-S N (%)	Control N (%)	*P*	OR (CI 95%)
Genotype				
Additive Model				
TT	51 (63.8)	47 (58.8)	Ref	
TG	26 (32.5)	32 (40.0)	0.384*	0.749 (0.390-1.437)
GG	3 (3.8)	1 (1.2)	0.355**	2.765 (0.278-27.51)
Recessive / Dominant Models				
TT	51 (63.8)	47 (58.8)	Ref	
TG+GG	29 (36.2)	33 (41.2)	0.516*	0.810(0.428-1.531)
GG	3 (3.8)	1 (1.2)	Ref	
TG+TT	77 (96.2)	79 (98.8)	0.310**	0.325(0.033-3.192)
Allele				
T	128 (81.5)	126 (79.2)	Ref	
G	29 (18.5)	33 (20.8)	0.609*	0.865(0.496-1.509)

*Chi-square test; **Fisher exact; significant p value < 0.05; CI: Confidence Interval; OR: Odds Ratio; Ref: Reference.

Age stratification in LPL +495 T > G, showed no significant differences between genotype additive, recessive and dominant models in the Met-S group and controls (Table 5).

Table 5: Age Stratification in the distribution of Genotype and Allele in LPL +495 T > G Gene Variations in Age-related Met-S

LPL *+495 T > G*	Aged ≤ 45 years				Age > 45 years			
	Met-S n (%)	Control n (%)	*P*	OR (CI, 95%)	Met-S n (%)	Control n (%)	*P*	OR (CI, 95%)
Genotype								
Additive Model								
TT	27 (65.9)	22 (53.7)	Ref		24 (61.5)	25 (64.1)	Ref	
TG	11 (26.8)	18 (43.9)	0.143*	0.5 (0.2-1.27)	15 (38.5)	14 (35.9)	0.815*	1.12 (0.45-2.8)
GG	3 (7.3)	1 (2.4)	0.355**	2.44 (0.24-25.1)	0	0	0	0
Recessive / Dominant Models								
TT	27 (65.9)	22 (53.7)	Ref		24 (61.5)	25 (64.1)	Ref	
GG+TG	14 (34.1)	19 (46.3)	0.260*	0.60 (0.25-1.46)	15 (38.5)	14 (35.9)	0.815*	1.12 (0.46-2.8)
GG	3 (7.3)	1 (2.4)	Ref		0	0	-	-
TG + TT	38 (92.7)	40 (97.6)	0.308**	0.317 (0.03-3.18)	39 (100)	39 (100)		
Allele								
T	65 (82.3)	62 (76.5)	Ref		63 (80.8)	64 (82.1)	Ref	
G	14 (17.7)	19 (23.5)	0.370*	0.703 (0.32-1.52)	15 (19.2)	14 (17.9)	0.837*	1.09 (0.49-2.40)

*Chi-square test; **Fisher exact; significant p value < 0.05; CI: Confidence Interval; OR: Odds Ratio; Ref: Reference.

In the ≤ 45 years age group, the distribution of TT genotypes was a greater compared to TG and GG genotypes frequency in the Met-S group although not statistically significantly different. Comparison of dominant and recessive models also found no statistically significant differences between the Met-S and control group. In group > 45 years, no GG genotypes were found in either Met-S or control subjects. Also, the distribution of genotypes and alleles of LPL + 495T > G gene variation with Met-S in subjects aged > 45 years there had no statistically significant differences.

Genotype and allele frequencies for LPL PvuII C > T were not significantly different between Met-S and controls (Table 6). The most frequent genotype was TT in both study populations, with 38% in the Met-S group and 39% in controls. In LPL PvuII C > T, the genotype TC had the most frequent in the Met-S group compared to controls but it was not significantly different.

Table 6: Genotype and Allele Distribution of LPL PvuII C>T Genes in Met-S and Control Subjects

LPL *PvuII* C>T	Met-S n (%)	Control n (%)	P	OR (CI, 95%)
Genotype				
Additive Model				
TT	38 (47.5)	39 (48.8)	Ref	
TC	34 (42.5)	33 (41.2)	0.867*	1.057 (0.549-2.036)
CC	8 (10.0)	8 (10.0)	0.962*	1.026 (0.350-3.013)
Recessive/Dominant Models				
TT	38 (47.5)	39 (48.8)	Ref	
TC+CC	42 (52.5)	41 (51.2)	0.874*	1.051 (0.565-1.955)
CC	8 (10.0)	8 (10.0)	Ref	
TC+TT	72 (90.0)	71 (90.0)	1.000*	1.000 (0.356-2.809)
Allele				
T	110 (72.4)	111 (73.0)	Ref	
C	42 (27.6)	41 (27.0)	0.898*	1.034 (0.624-1.712)

*Chi-square test; **Fisher exact; significant p value < 0.05; CI: Confidence Interval; OR: Odds Ratio; Ref: Reference.

Age stratification in LPL PvuII C > T (Table 7) showed there were significant differences between TT vs CC, recessive and dominant models in Met-S and control. The distribution of TT genotypes and T on LPL PvuII > T gene variations age group ≤ 45 years had a greater frequency in the Met-S group compared to TC and CC genotypes although no statistically significant differences were found. In the age group > 45 years, the distribution of TC genotypes in LPL PvuI C > T gene variation was higher in the Met-S group compared to TT and CC genotypes. CC genotypes and TC + CC had increased risk of Met-S compared to TT genotypes.

Table 7: Age Stratification in the distribution of Genotype and Allele in LPL PvuII C > T Gene Variations in Age-related Met-S

LPL PvuII C > T	Aged ≤ 45 years				Age > 45 years			
	Met-S n (%)	Control n (%)	*P*	OR (CI, 95%)	Met-S n (%)	Control n (%)	*P*	OR (CI, 95%)
Genotype								
Additive Model								
TT	24 (58.5)	16 (39.0)	Ref	1.00	14 (35.9)	23 (59.0)	Ref	1.00
TC	14 (34.1)	17 (41.5)	0.214*	0.55 (0.21-1.42)	20 (51.3)	16 (41.0)	0.129*	2.054 (0.81-5.23)
CC	3 (7.3)	8 (19.5)	0.054*	0.25 (0.06-1.09)	5 (12.8)	0	0.032#	17.83
Recessive/Dominant Models								
TT	24 (58.5)	16 (39.0)	Ref		14 (35.9)	23 (59.0)	Ref	
TC + CC	17 (41.5)	25 (61.0)	0.077*	0.453 (0.19-1.1)	25 (64.1)	16 (41.0)	0.070#	2.567 (1.03-6.40)
CC	3 (7.3)	8 (19.5)	Ref		5 (12.8)	0	Ref	
TC + TT	38 (92.7)	33 (80.5)	0.105*	3.07 (0.75-12.5)	34 (87.2)	39 (100)	0.064#	0.079
Allele								
T	62 (78.5)	49 (66.2)	Ref	1.00	48 (65.8)	62 (79.5)	Ref	1.00
C	17 (21.5)	25 (33.8)	0.089*	0.54 (0.26-1.11)	25 (34.2)	16 (20.5)	0.058*	2.02 (0.97-4.2)

*Chi-square test; **Fisher exact; significant p value < 0.05; CI: Confidence Interval; OR: Odds Ratio; Ref: Reference.

In the age group > 45 years, multivariate analysis showed a value of *p* > 0.05 in the Hosmer

and Lemeshow test (Table 8). Simultaneously, obesity and genetic variation could contribute to causing Met-S as much as 41.6%. Obese patients had 13.55 times increase risk of Met-S compare to the non-obese subject and the result was statistically significant. Subjects with TC + CC genotypes had 3.62 times higher risk of Met-S compared to TT genotype.

Table 8: Multivariate logistic regression analysis of the relationship between Pvu II C > T gene variation and Met-S at age > 45 years

Variable	B	SE	*P*	Adjusted OR (95%, CI)
Obesity	2.606	0.590	< 0.001	13.55 (4.27-43.03)
Genotype TT	Ref			
TC + CC	1.287	0.588	0.029	3.62 (1.14-11.48)

Discussion

A study of the relationship gene variation in the LPL shown different result and the regulation of lipid concentration is complex [12]. Met-S is associated with genetics and can be influenced by the environment. Several genetic studies show different results, and this is likely because Met-S is not only caused by one type of gene but is also caused by the interaction of several genes and is influenced by environmental factors. Also, population genetic studies shown ethnic differences are one of the factors that influence differences in genotype frequency found in both LPL + 495T > G and PvuII > T gene variations. For example, food intake that may be very different between ethnic groups modulates the genetic influence on lipid metabolism [13]. Variations of the LPL + 495T > G and PvuII C > T, which are located respectively in introns 8 and intron 6, have host elements that regulate gene transcription and translation [14]. Although introns do not encode amino acids, they play an important role in processing mRNA precursors and the incorporation of exons as protein-coding [12].

LPL +495 T > G gene variation, and the frequency of TT genotypes in some countries are more prevalent in most case groups compared to control groups such as Egypt (45%) [15], Saudi Arabia (50.8%) [8], Iran (58.8%) [16], India (48.5%) [17], Iran (56, 5%) [18] and in this study (63.8%). In this study, TT frequencies were more common in controls compared to cases. Lipoprotein Lipase Pvu II C > T gene variation in this research was the same as research conducted in the populations in Turkey, Saudi Arabia, Korea and Egypt [6], [8], [19], [20]. [19]. also reported that TC genotypes had a higher frequency in patients with coronary artery disease compared to the control group.

In the age group > 45 years, in the dominant model, TC + CC genotypes can increase the risk of Met-S by 2.567 (95% CI: 1.03-6.40). After multivariate analysis, it was found that TC + CC genotypes increased the risk of Met-S by 3.62 times compared to TT genotypes. A similar study was done by Pereira *et al.,* in stroke patients in Colombia with a median age of 64 years without analysing food intake data and physical activity found that TT genotypes and T alleles were protective factors for stroke events [14]. This is different from the research conducted by *Shin et al*. which found no association between food intake and physical activity with variations in the LPL PvuII C > T gene in the population in Korea. In the study, TT genotypes showed an increase in triglyceride levels and a decrease in HDL and TT + TC genotypes with 1.5 times increased risk of Met-S compared to CC genotypes. Decreased LPL due to gene variation causes inhibition of lipolysis. Inhibition of lipolysis causes increases in VLDL and chylomicron. Furthermore, the degradation of triglycerides in adipose tissue results in an increase in free fatty acids in the blood. Increased triglycerides are caused by a decrease in the utilisation of lipids by tissue and then can cause Met-S [6]. The differences in this research with previous studies include the lack of a large number of samples, ethnic differences and several environmental factors such as physical activity and food intake that are not controlled for the research subjects. The number of samples used in the study will affect the strength of the statistical results so that the study of the relationship of gene variation to an illness requires a greater number of samples. Also, more control is needed on environmental factors that can affect the results.

There was no association between LPL +495 T > G gene variation with Met-S. In LPL PvuII gene variation, TC + CC is the risk genotype of Met-S in the age group > 45 years but no significantly different in the age group ≤ 45 years.

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Basic Science

Preparation of In Situ Cross-Linked N-Maleoyl Chitosan-Oxidized Sodium Alginate Hydrogels for Drug Delivery Applications

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Abstract

AIM: This study was aimed to prepare in situ cross-linked N-maleoyl chitosan – oxidised sodium alginate (MCS – OSA) hydrogel loaded with metronidazole (MTZ) for drug delivery applications.

METHODS: The hydrogel was prepared by in situ cross-linking via Schiff base reaction between amine (-NH2) groups from MCS and aldehyde (-CHO) groups from OSA at the different ratio, and the MTZ was loaded into the hydrogels along with the gelatin processes.

RESULTS: The highest drug entrapment efficiency (DEE) was exhibited by MTZ-H3 (5: 5) with DEE of 99.20% and a gel fraction of 97.52%. FTIR results revealed that Schiff base reaction was occurred by the absorption peak of –C = N- groups at 1628 cm^{-1} and indicated that there is insignificant alteration at different ratio of MCS and OSA. The best sustained of in vitro release profiles of MTZ was shown by MTZ-H3, which is 74.92% and 75.65% at pH 1.2 and 7.4 for 12 h of release, respectively.

CONCLUSION: The optimised ratio between MCS and OSA to prepare in situ cross-linked hydrogels were found to be 5:5 according to the results of DEE and in vitro drug release profiles of MTZ and the MTZ loaded MCS-OSA hydrogels have a great potential which can be applied in biomedical applications.

Introduction

Polysaccharide-based polymer hydrogels such as cellulose, chitosan, starch, alginate, dextrin and it's derivatives have attracted attention of researchers for drug delivery applications which can be controlled and applied at specific sites owing to the ease of preparation, good encapsulation properties of variation drugs, good biocompatibility and responsive to external stimulant [1], [2], [3], [4], [5]**.** Smart and controllable based drug delivery hydrogels considered as a preferable alternative which can improve in decreasing the dosage and side effects compared to the single unit release of tablet [6], [7]. However, the applications of chitosan in controllable drug delivery systems are limited due to its solubility [8], [9], [10]. Hence, to enhance the solubility of chitosan in water and different pH of aqueous solutions, modification by amine ($-NH_2$) and hydroxyl (-OH) functional groups were conducted as reported in Mohamed and Fahmy (2012) [11] and Wang et al., (2016) [12] works, such as acylation of chitosan for N-maleoyl chitosan (MCS) preparation.

MCS can be synthesized via acylation of maleoyl group to N-terminal in glucosamine chitosan unit. Recently, the utilisation of chitosan derivative such as MCS in polymer-based drug delivery has drawn the attention of researchers besides using pristine chitosan. Some advantages of MCS are non-toxic, degradable, good hydrophilicity and biocompatibility which also soluble in any medium at different pH. The solubility of MCS depends on the degree of substitution of maleoyl in water and

physiological pH [13], [14], [15]. Therefore, in this research, MCS was assumed to be cross-linked with natural bifunctional cross-linking agents (e.g. dialdehyde alginate) and *in situ* hydrogel can be obtained.

Oxidised sodium alginate can be prepared by oxidation reaction using oxidising agents (periodic acid or sodium periodate) to oxidise 2,3-O-dihydroxyl alginate for dialdehyde alginate preparation [12]. The OSA has been widely used as natural macromolecule cross-linking agent for hydrogels preparation due to the minimum or non-toxic properties which can replace the relatively toxic chemical cross-linking (e.g. formaldehyde, acetaldehyde and glutaraldehyde) [16]. Also, OSA has good biodegradable and intrinsic biochemical characteristics such as good solubility in a variation of pH, containing aldehyde functional groups, abundant nature, and ease of covalently cross-linking.

Several studies have reported synthesising *in situ*-forming hydrogels via Schiff base covalent cross-linking without any chemical cross-linking agents between modified-chitosan derivative and dialdehyde alginate. For example, *in situ*-forming hydrogels via Schiff base covalent cross-linking between $–NH_2$ groups from N, O-carboxymethyl chitosan and –CHO groups from OSA containing silver nanoparticles (AgNPs) for antibacterial and bioactive compound delivery applications has been published by Fan et al., (2011) [17]. The N, O-carboxymethyl chitosan/oxidised alginate hydrogels containing BSA [18], curcumin and nano curcumin [19], RGD-grafted oxidised sodium alginate–N-succinyl chitosan-based for bone tissue engineering [20], hydroxypropyl chitosan and sodium alginate dialdehyde for the reconstruction of the corneal endothelium [2].

In this study, MTZ loaded MCS-OSA hydrogel is prepared through *in situ* Schiff base reaction between amine ($-NH_2$) groups from MCS and aldehyde (-CHO) groups from OSA which has not been reported before and the schematic reaction is illustrated in Figure 1. Moreover, the effects of the different ratio between MCS and OSA on physicochemical properties, drug entrapment efficiency (DEE) and *in vitro* drug release profiles of MTZ including the kinetic modelling at pH 1.2 and 7.4 were evaluated.

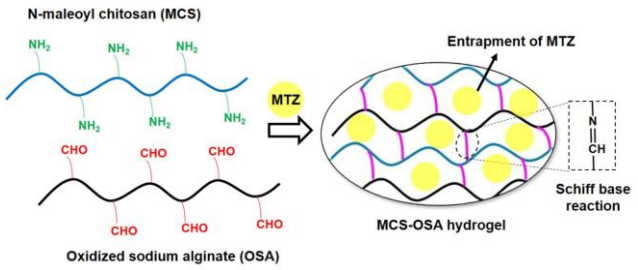

Figure 1: Schematic of in situ drug loading in MCS-OSA hydrogels via Schiff base cross-linking reaction

Material and Method

Materials

The materials used in this experiment were N-maleoyl chitosan (MCS) (degree of substitution = 0.66) and oxidized sodium alginate (OSA) (aldehyde contents = 7.43 mmol/g) which were synthesized according to our previous research [21], [22], HCl, NaCl, phosphate buffer solution (PBS) (pH 7.4) and metronidazole (MTZ) was a gift from PT. Kimia Farma Plant Bandung Indonesia. All chemicals were used as received without further treatment.

Preparation of MCS-OSA hydrogels

MCS and OSA were dissolved separately in 0.1 M PBS solution (pH 7.4) with a desired concentration of 2% (w / v). Then, each solution of MCS and OSA were mixed at room temperature at a different ratio which is abbreviated as H1 (9: 1), H2 (7: 3), H3 (5: 5), H4 (3: 7), and H5 (1: 9). Before the characterisation, the obtained hydrogels were washed several times by distilled water, followed by drying for further characterisation [23], [24], [25].

Preparation of in situ MTZ loading in MCS-OSA hydrogels

MTZ-MCS-OSA hydrogel was fabricated by dissolving an amount of 25 mg MTZ in a total volume of 10 ml mixture solution of MCS-OSA at room temperature. Initially, MTZ was mixed well in MCS solution before mixing between MCS and OSA at a different ratio. Then, the obtained MTZ-MCS-OSA hydrogels were dried for further analyses and oxidised [23], [24], [25].

FTIR analysis

Samples were analysed as a powder mixed with KBr powder by Fourier transform infrared (FTIR) method. Spectra were collected in the wavenumber of 4000-400 cm^{-1}, a scan number of 64 and a resolution of 4 cm^{-1} using a Shimadzu IR Tracer-100 spectrometer.

The surface morphology of hydrogels was observed with a ZEISS EVO MA10 (Germany) at an accelerating voltage of 10 kV and magnification of 1000 x. Before the scanning process, the hydrogels were dried to prevent the shrinkage of samples and then coated by a thin layer of platinum through the sputtering method.

XRD analysis

The crystallinity of prepared hydrogels was determined using a Bruker D2 Phaser X-ray

diffractometer at 2Θ = 10-70° (scanning rate = 6°/min) in Cu Kα radiation (λ =0.151418 nm) with a working voltage of 30 kV and current of 10 mA.

Physiochemical properties of MCS-OSA hydrogels

In this study, physicochemical properties comprise of gel-forming time, gel fraction and swelling ratio (both pH 1.2 and 7.4) of the H1-H5 hydrogels.

Gel forming time

Gel forming time was observed visually at room temperature while the mixture solution of MCS and OSA losing the fluidity from viscous solution to elastic (rubber) or solid phase. The time when the solution turns to solid than was recorded as gel-forming time [23].

Gel fraction

The samples (Wo) were immersed in 50 mL distilled water for 24 h until equilibrium swelling was achieved, to remove the soluble parts MCS-OSA in the hydrogels. Afterwards, the hydrogels were dried at 50°C in the oven and reweighed (We). The gel fraction (GF) was performed in triplicate and calculated by the following equation [23]:

$$GF(\%)= \frac{We}{Wo} \times 100\% \quad (1)$$

Swelling ratio

The swelling experiments were conducted in solution pH 1.2 and pH 7.4 separately. The hydrogels were immersed in 50 ml of each solution at 37°C, then weighed (Ws) at a certain time interval (1-360 min) until equilibrium swelling was achieved. The swelling ratio (SR) was conducted in triplicate and determined by the following equation [23], [26]:

$$SR(\%)= \frac{Ws-We}{We} \times 100\% \quad (2)$$

Determination of drug entrapment efficiency (DEE)

The drug entrapment efficiency (DEE) of MTZ-MCS-OSA hydrogels was determined by the extraction method. 50 mg of the MTZ-MCS-OSA hydrogels was dispersed in 0.1 M PBS solution (pH 7.4) and stirred at 37 ± 0.5°C for 24 h. Then, the solution was filtered and diluted for UV-Vis spectrophotometer analysis at a wavelength of 320.6 nm. The determination of DEE was conducted in triplicate to ensure their reproducibility of results and data are presented as mean ± standard deviation (SD). The drug entrapment efficiency (DEE) was calculated by the following equation [24], [25].

$$DEE\ (\%)= \frac{EDL}{TDL} \times 100\%$$

Where EDL and TDL denote the experimental drug loading and theoretical drug loading of MTZ in the hydrogels, respectively. Then, the entrapment of MTZ in MCS-OSA hydrogels was abbreviated as MTZ-Hnhydrogels, which Hn denotes as H1 to H5 hydrogels.

In vitro release profiles of drug-loaded hydrogels

In vitro release profiles of MTZ-MCS-OSA hydrogels (H1-H5) were determined in two different pH environment which are 1.2 (0.1 N HCl solution) and 7.4 (0.1 M PBS solution) [24], [25] by using USP paddle method dissolution with a speed of 50 rpm and total volume of 900 ml at temperature of 37 ± 0.5°C [27]. After certain time intervals between 0 and 12 h, aliquots (5 ml) of the sample solution were taken from the release medium, and an equivalent amount of fresh 0.1 N HCl solution was added to maintain a constant volume. The taken solution was then diluted and the concentration of MTZ was analysed by UV-Vis spectrophotometer at the wavelength of 277 nm for pH 1.2. The same procedure was also used to carry out to determine the *in vitro* release profiles of MTZ-MCS-OSA hydrogels (H1-H5) in pH 7.4 solution and the wavelength of UV-Vis spectrophotometer analysis was at 320.6 nm. The drug release experiments were conducted in triplicate to ensure their reproducibility of results and data are presented as mean ± standard deviation (SD).

Statistical analysis

All measured data are expressed as mean ± standard deviation (S.D.) and performed using Origin software, version 8.6 and Kruskal-Wallis test with a value of $p < 0.05$ which was considered to indicate the statistical significance.

Results

FTIR Analysis

As shown in Figure 2, the absorption peak at the wavelength of 3448.72 cm^{-1} presented in the spectra of MCS attribute to stretching vibration of –OH and $–NH_2$ which is overlapping, meanwhile weak absorption at 2931.80 cm^{-1} wavelength correspond to stretching vibration of C-H [23],[28]. The vibration of C = O from maleic anhydride acid in the chitosan backbone indicated by the absorption peak at 1712.79

$cm^{-1}$and also confirmed by C = O vibration of amide groups at 1558.48 cm^{-1}. Moreover, at 825.53 cm^{-1} is attributed to C = C groups in maleoyl [13], [29]. Hence, the characterisation by FTIR revealed that the synthesised compound is N-maleoyl chitosan (MCS).

On the other hand, FTIR spectra of OSA, the free –OH, intramolecular and non-oxidized intermolecular bonding are evidenced by broaden peak at 3471.87 cm^{-1}. Afterwards, the absorption peaks at 2939.52 and 1405.18 cm^{-1} are stretching vibration of aldehyde (-CHO) groups [23]. At the wavelength of 1627.92 cm^{-1} is referred to C = O stretching vibration of aldehyde functional groups which resulted from the oxidation of –OH. Moreover, the formation of hemiacetal (aldehyde) was proved by the absorption band of C-O-C (cyclic ether) at 1033.85 cm^{-1} [24]. The absorption peaks at 794.67 and 732.95 cm^{-1} are referred to C-H groups which contributed to bond-breaking of C-C in OSA.

All FTIR spectrums of hydrogels (H1-H5) showed the insignificant difference as represented in Figure 2. The stretching vibration of –OH at 3464.15 cm^{-1} revealed the intermolecular hydrogen bonds [25]. The presence of characteristic peak of hemiacetal formation at 864.11 cm^{-1} is due to stretching vibration of –C-N- a bond which also considered as the coupling reaction between –CHO from OSA and $–NH_2$ from MCS [25]. In addition, the absorption peak at 1627.92 cm^{-1} confirmed the formation of –C = N- groups (Schiff base or imine) [25], [30], [31].

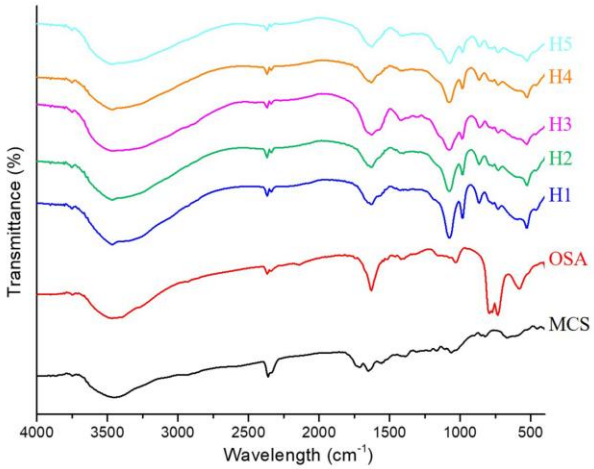

Figure 2: FTIR spectrum of MCS, OSA and MCS-OSA hydrogels at different ratio (H1-H5)

SEM analysis

The images of SEM (magnification: 1000 x) were depicted in Figure 3 and indicated that Schiff base cross-linking exhibited the presence of rough and dense surface of all MCS-OSA hydrogels (H1-H5). However, the surface morphology of all hydrogels (H1-H5) showed an insignificant difference.

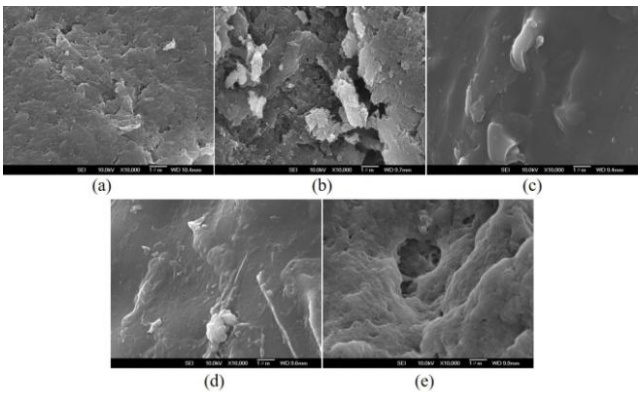

Figure 3: SEM images of MCS-OSA hydrogels: A) H1; B) H2; C) H3; D) H4; and E) H5 at magnification of 1000 x

XRD analysis

The diffraction patterns indicated the amorphous structure of the hydrogels. All of the hydrogels exhibited a broad peak at 2□= 21° which is attributed to the networks of hydrogels as shown in Figure 4 and the results showed insignificant alteration at the different ratio between MCS and OSA.

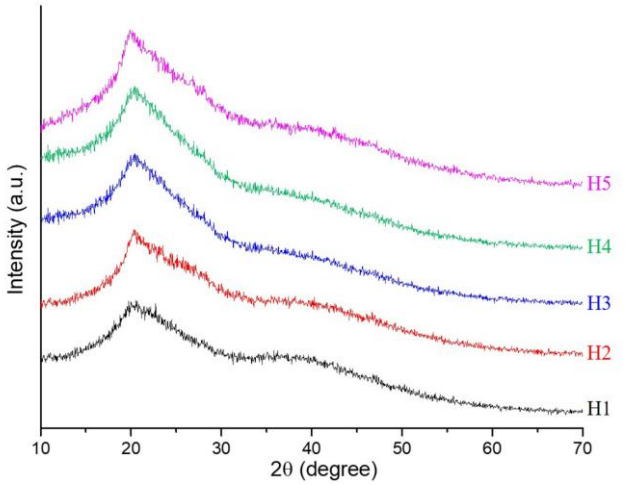

Figure 4: XRD patterns for MCS-OSA hydrogels at different ratio (H1-H5)

Gel forming time

Gel forming time (gelation time) of H1-H5 were observed and determined at room temperature when both mixtures of MCS and OSA underwent cross-linking (gelation) to form hydrogels. The gel-forming time for H1-H5 varied between 4.51 and 23.52 min as represented in Table 1.

With increasing ratio of MCS from H1 to H3, the ability to cross-link to create hydrogel network was faster because at high concentration of MCS, more -NH_2 groups reacted with –CHO groups from OCS. Moreover, H4 and H5 showed drastically slow gelation time due to the saturation of aldehyde groups at a high ratio of OCS and led to the hindrance of cross-

linking [17]. Similar results were also obtained in the study reported by Kamoun (2016) [23] and described that the faster gelation time of succinyl chitosan-dialdehyde starch (SCS-DAS) hydrogel was obtained at a higher amount of SCS.

Table 1: The result of gelation time of MCS-OSA hydrogels at different ratio (H1-H5)

Samples	Gel forming time (min)
H1	4.51 ± 0.02
H2	5.18 ± 0.02
H3	7.49 ± 0.02
H4	19.44 ± 0.02
H5	23.52 ± 0.03

*(n = 3, ± SD).

Gel fraction

The gel fraction increased from 81.72-97.52% for H1-H3 and decreased to 55.13% and 12.58% for H4 and H5, as shown in Table 2. As mentioned earlier, for H4 and H5, the saturation of aldehyde groups was occurred and hindered the cross-linking therefore decrease the gel fraction. Besides, the results of the gel fraction are linearly related to the cross-linking density of hydrogel and indicated that the highest cross-linking density was possessed by H3.

Table 2: The result of gel fraction of MCS-OSA hydrogels at different ratio (H1-H5)

Samples	Gel fraction (%)
H1	81.72 ± 0.06
H2	85.03 ± 0.02
H3	97.52 ± 0.04
H4	55.13 ± 0.05
H5	12.58 ± 0.04

*(n = 3, ± SD).

The swelling ratio of hydrogels

The swelling ratio (SR) of the hydrogels at pH 1.2 (0.1 N HCl solution) and pH 7.4 (0.1 M PBS solution) was shown in Figure 5(a) and (b), respectively which plotted as a function of time. At pH 1.2 (0.1 N HCl solution), the swelling ratio of H1, H2 and H3 hydrogels increased from 86.5% to 120.68% and 141.42%. In correlation with gel fraction, high gel fraction resulted in high cross-linking density and limited the mobility of polymer chains, therefore, decrease the swelling properties. At the same time, the hydrophilic groups, -COOH of MCS also can contribute to hydrating more water molecules.

Meanwhile, after adding OSA, the C = N bonds (Schiff base reaction) was formed for the gelation between –CHO from MCS and $–NH_2$ from OSA which enhance the hydrophobicity. Therefore, at high concentration of OSA, the hydrophobicity of hydrogels increased and fewer hydrogen bonds of hydrogel with H_2O was formed.

On the other hand, H4 possessed low gel fraction to compare to H1, H2 and H3. Thus the swelling properties of H4 increased [17]. Moreover, the swelling ratio of H4 at the interval time of 120-360 minutes of immersion showed a drastically decline from 168.98% to 119.71% which is due to the unstable and easily degraded networks [24]. Furthermore, such a phenomenon was also occurred for H5 and has been degraded at a swelling time of 90 minutes.

On the other hand, a similar trend was observed between the results of swelling ratio in pH 1.2 and pH 7.4. Nevertheless, the swelling ratio at pH 7.4 increased drastically compared to pH 1.2, and this could be owing to the higher electrostatic repulsion of interpolymer and interpolymer chain which resulted in more expanding networks of hydrogel [25]. Besides, at higher pH, the swelling ability was higher for the polymer containing anionic (-COOH) groups [28]. Afterwards, the phenomenon of degradation and unstable network was also obtained for H4 and H5 at pH 7.4.

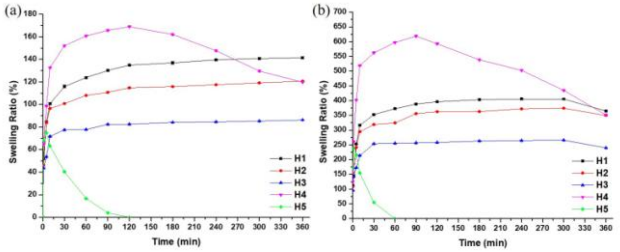

Figure 5: Swelling ratio profiles of MCS-OSA hydrogels at: A) pH 1.2; B) pH 7.4 (n = 3, ± SD).

Determination of drug entrapment efficiency (DEE)

The results of drug entrapment efficiency (DEE) of MTZ-MCS-OSA hydrogels (H1-H5), including the experimental drug loading (EDL) are summarised in Table 3 and represented in Figure 6. The results of drug entrapment efficiency (DEE) of hydrogels varied between 84.76% and 99.22%, where the highest DEE was possessed by MTZ-H3 hydrogels. Since the MTZ-MCS-OSA hydrogels were prepared via *in situ* cross-linking (Schiff base), during the cross-linking processes, the MTZ is entrapped in the networks of the hydrogel. Therefore, higher gel fraction exhibited the highest DEE.

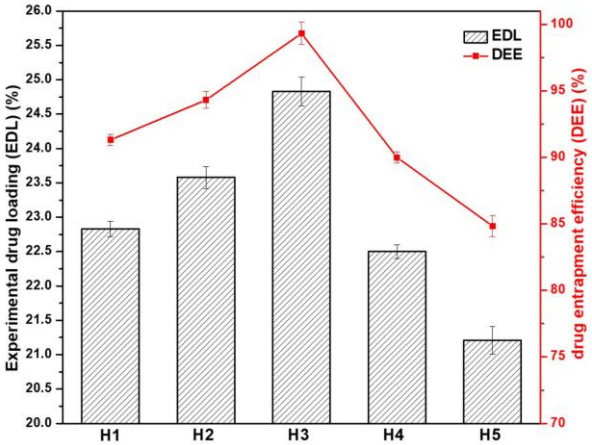

Figure 6: Drug entrapment efficiency (DEE) of MTZ-MCS-OSA hydrogel (n = 3, ± SD)

In vitro drug release and kinetic modelling

The *in vitro* release profiles of MTZ-MCS-OSA hydrogels (H1-H5) in both pH 1.2 (0.1 N HCl solution) and pH 7.4 (0.1 M PBS solution) at 37 ± 0.5°C are presented in Figure 7 (a) and (b), respectively. The cumulative release of MTZ-MCS-OSA hydrogels (H1-H5) at pH 1.2 for 60 min was determined to be 68.68, 63.37, 22.57, 81.66 and 87.50%, respectively. The burst release (burst effect) was exhibited by all hydrogels except MTZ-H3 hydrogel and indicated the release profiles of MTZ-MCS-OSA hydrogels were depended significantly on the composition of MCS and OSA [23], [33], [34], [35].

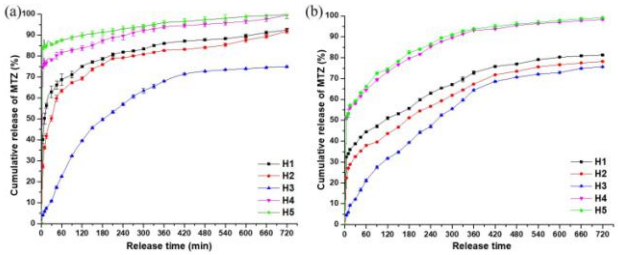

Figure 7: The release profiles of MTZ-MCS-OSA hydrogels at: (a) pH 1.2; (b) pH 7.4 (n = 3, ± SD)

On the other hand, a more delayed pattern of drug release profiles was obtained for MTZ-H3 hydrogel, and the cumulative release for 1 h in pH 1.2 medium was 22.57%. Similarly, the drug release was found to be sufficiently sustained for 12 h of release, with cumulative release of 74.92%, while the cumulative release of MTZ-MCS-OSA (H1, H2, H4 and H5) varied between 91-99% which also indicated that MTZ-H3 hydrogel possessed retentive properties for drug delivery system.

Table 3: The release parameter values obtained by fitting *in vitro* release data to Higuchi and Korsmeyer-Peppas release models at pH 1.2

Release systems	Cumulative release (%)			Higuchi		Korsmeyer-Peppas		
	2h	6h	12h	$k_H(h^{-0.5})$	R^2	$K_p(h^{-0.5})$	n	R^2
MTZ-H1	75.06	86.03	92.44	4.54	-	38.07	0.14	0.99
MTZ-H2	69.38	82.64	91.86	4.35	0.29	27.02	0.19	0.97
MTZ-H3	39.59	67.98	74.92	3.27	0.95	4.29	0.45	0.95
MTZ-H4	83.83	93.85	99.49	5.01	-	64.12	0.06	0.99
MTZ-H5	89.90	95.87	99.81	5.18	-	75.69	0.04	0.99

*The negative R^2 values were obtained from the Higuchi model fitting to release data of MTZ in MCS-OSA hydrogels.

The synthesized *in situ* drug-loaded MCS-OSA hydrogels released MTZ at a higher rate in pH 1.2 (0.1 N HCl solution) compared to the release in pH 7.4 (0.1 M PBS solution). It is because the solubility of MTZ is higher at pH 1.2 (64.8 mg/mL at room temperature) than in pH values between 2.5 and 8.0 (10 mg/ml) [36], [37], [38].

To further determine the mechanisms involved in drug release from the hydrogels, the *in vitro* release data in pH 1.2 and 7.4 were fitted using Higuchi [39] and Korsmeyer-Peppas [40] release model by nonlinear least-squares regression analysis using the Origin software as shown in Figure 8.

Table 4: The release parameter values obtained by fitting *in vitro* release data to Higuchi and Korsmeyer-Peppas release models at pH 7.4

Release systems	Cumulative release (%)			Higuchi		Korsmeyer-Peppas		
	2h	6h	12h	$k_H(h^{-0.5})$	R^2	$K_p(h^{-0.5})$	n	R^2
MTZ-H1	51.00	72.85	81.27	3.70	0.61	17.88	0.23	0.98
MTZ-H2	43.60	67.35	78.19	3.43	0.82	11.78	0.29	0.99
MTZ-H3	31.77	64.37	75.65	3.06	0.98	2.72	0.52	0.98
MTZ-H4	73.25	93.15	98.32	4.78	0.01	35.52	0.16	0.99
MTZ-H5	74.53	93.72	99.14	4.85	-	36.77	0.15	0.99

*The negative R^2 values were obtained from the Higuchi model fitting to release data of MTZ in MCS-OSA hydrogels.

The models including the coefficient of determination (R^2) are presented in Table 3 and Table 4, respectively.

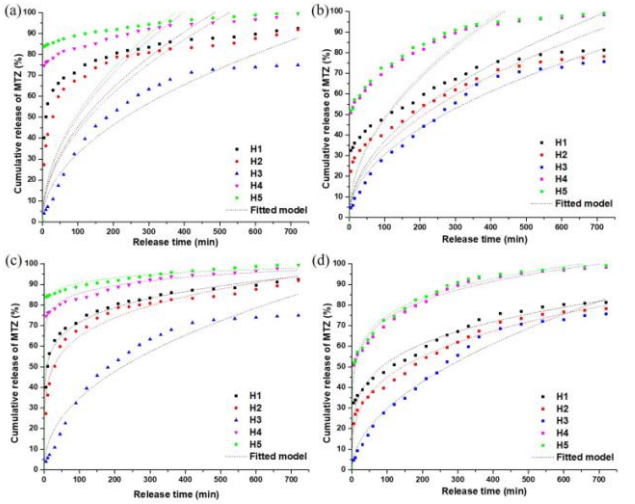

Figure 8: The fitted release profiles of Higuchi model at A) pH 1.2; B) pH 7.4 and Korsmeyer-Peppas model at C) pH 1.2; D) pH 7.4 to release MTZ in MCS-OSA hydrogels (n = 3, ± SD)

Discussion

The results of *in vitro* release data at pH 1.2 indicated that the Higuchi release model only can be used to describe the release profiles of MTZ-H3 hydrogels with R^2 value = 0.95 while poor R^2 value (0.29) was obtained for MTZ-H2 hydrogels and the release profiles of MTZ-H1, MTZ-H4 and MTZ-H5 were failed to be described by Higuchi model, as indicated by negative R^2 values.

Furthermore, Korsmeyer-Peppas release model (pH 7.4) showed good agreement between the experimental data and model predictions with high R^2 values ranging from 0.95-0.99. Likewise, good fitting was also showed by Korsmeyer-Peppas release model with R^2 ranged from 0.98-0.99 of *in vitro* release data at pH 7.4. Besides, the *in vitro* release data at pH 7.4 fitted by Higuchi model showed that the R^2 values of H1, H2 and H3 are 0.61, 0.82 and 0.98, respectively and the model failed to fit H4 and H5 *in vitro* release data, as indicated by negative R^2 values. Moreover, only MTZ-H3 hydrogels in pH 7.4 release medium showed exponent n factor n values of 0.52

which the mechanism was governed by anomalous (non-Fickian) transport (0.45 < n < 1.00). Other than that, the n values of all MTZ-MCS-OSA hydrogels at both pH 1.2 and 7.4 were less than or equal to 0.45 (n ≤ 0.45) and confirmed the drug release mechanism is Fickian diffusion [41].

In conclusion, *in situ* MTZ-loaded MCS-OSA hydrogel has been successfully synthesized by utilizing MCS and OSA at different ratio (H1-H5) via Schiff base cross-linking reaction and the time of gelation varied between 4.51 and 23.52 min. On the other hand, increasing ratio of OSA from H1 to H3 showed increasing in gel fraction and decreases for further increasing (H4 and H5). On the contrary with gel fraction, the swelling ratio increased linearly with increasing ratio of MCS. In brief, MTZ-H3 hydrogels possessed the highest gel fraction and DEE as well. Moreover, the drug release mechanism of all release systems is governed by Fickian diffusion except for MTZ-H3 which is anomalous transport (non-Fickian diffusion). Therefore, this MTZ-MCS-OSA hydrogel potentially can be applied in drug delivery system.

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Interleukin 10 Induces the Expression of Membrane-Bound HLA-G and the Production of Soluble HLA-G on HeLa CCL-2 Cells

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Abstract

BACKGROUND: Interleukin-10 is a cytokine that has a pleiotropic effect on the immune system and inflammation. IL-10 can contribute to the anti-tumour immune response by increasing HLA-G expression.

AIM: This study aimed to determine the effect of IL-10 induction on membrane HLA-G expression and soluble HLA-G production of HeLa CCL-2 cells.

METHODS: HeLa CCL-2 cells were cultured in the well plate and divided into 4 groups consist of 1 control group that was not induced by IL-10 and 3 treatment groups that were induced by IL-10 500 ng/ml, 1000 ng/ml and 2000 ng/ml respectively. All groups were incubated for 48 hours in a 37°C incubator at 5% CO_2 atmospheric pressure. HLA-G measurements were carried out both in cell lysate and cell culture supernatant using ELISA and in membrane-bound using immunofluorescence method. The expression of HLA-G in membrane-bound calculated using the ImageJ application. Data obtained were analysed by ANOVA and LSD test.

RESULTS: In the control group, the HLA-G level in the culture supernatant was higher than in cell lysate ($p = 0.000$), as well as in all treatment groups ($p = 0.000$). There were significant differences between the treatment group ($p = 0.000$) and within the treatment group ($p = 0.000$) at HLA levels. The highest expression of HLA-G in HeLa cell membranes found in cell culture induced by IL-10 concentrations of 500 ng/ml, i.e., 59.28 AU in view. HLA-G membrane expression in the IL-10 1000 ng/ml induced group was significantly different from all treatment groups ($p = 0.000$).

CONCLUSION: HeLa CCL-2 cells express HLA-G on the membrane and release dissolved HLA-G without induction of IL-10 although IL-10 induction augments the presence and the production of HLA-G in HeLa CCl-2 cells.

Introduction

Interleukin-10, also called human cytokine synthesis inhibitory factor, is a cytokine secreted by monocytes which has a pleiotropic effect on the immune system and inflammation. Most leukocytes secrete IL-10 to a certain level. The highest secretion of IL-10 comes from monocytes and their mature forms: macrophages and dendritic cells. Specific granulocytes and agranulocytes: eosinophil and NK cells, and small lymphocytes: T and B cells, also release IL-10 but at lower levels [1].

Human leukocyte antigen (HLA)-G is HLA class 1 molecule consists of membrane-bound isoform (mHLA-G) namely HLA-G1, HLA-G2, HLA-G3 and HLA-G4 and 3 soluble HLA-G (sHLA-G) namely HLAG5, HLA-G6 and HLA-G7 [2]. HLA-G expression is initially observed in extra-villous cytotrophoblasts and is considered to play an important role in fetal-maternal immune tolerance [3]. In addition to extravillous cytotrophoblasts, HLA-G expression is limited to some healthy adult tissues, including the cornea, thymus medulla and Langerhans Island of pancreatic [4]. HLA-G expression can be activated in a variety of pathological conditions such as cancer,

viral infections, organ transplants, autoimmune diseases, and inflammation [5]. HLA-G has the effect of inhibiting the immune response and induces the production of regulator and suppressor cells so that tumour cells can escape immune surveillance [6].

It has been reported that IL-10 can contribute to anti-tumour immune responses by either decreasing regulation of HLA class I expression allowing tumours to exit lysis mediated by CTLs or by increasing HLA-G expression allowing cells to escape lysis by NK cells through interaction with inhibiting killer receptors in NK cells. In cervical cancer, HLA-G and IL-10 expression can be higher in tumour cells than in normal cervix and may be involved in early carcinogenesis. However, the relationship between IL10 and HLA-G in cervical cancer is not yet cleared [7].

Polakova & Russ, in 2000, informs that HeLa cells do not express HLA-G antigens by observing flowcytometry using 87G and 01G antibodies [8]. Flajollet et al., in 2009 using ras-responsive element-binding protein 1 (RREB-1) as an HLA-G transcription suppressor that has been known to suppress HLA-G promoters in HLA-G negative cells (HeLa cells) revealed that HeLa cells were referred to as negative cells HLA-G [9]. Therefore, in this study, we want to study the ability of IL-10 to induce HLA-G expression using the HeLa CCL-2 cells line.

Material and Methods

Chemicals and reagents

The research materials were HeLa CCL-2 cervical cancer cell line (ATCC), recombinant IL-10 (Elabscience), Alpha MEM (Gibco), 2% Penicillin Streptomycin, Fungison (Amphotericin B 0.5%), Fetal bovine serum (ATCC) 12.5% and Horse serum (ATCC) 12.5%, recombinant HLA-G (4H84) (Santa Cruze), and HLA-G Elisa kit (Elabscience).

The tools used laminar airflow Hood/tissue culture cabinet (Nuaire), CO_2 incubator, inverted microscope, centrifuge, 24-well plate, 96 well plates, micropipette, micro-tip, spectrophotometry, ELISA reader, Improved Neubauer Hemocytometer Chamber, fluorescent microscope (Olympus), round coverslip, object-glass, flask 25.

Human cell line culture

The HeLa CCL-2 cell lines were grown in Alpha MEM supplemented with Glutamax- I,4500 mg/L glucose (Invitrogen), 10% FCS (BioWhittaker), 1000 U of Penicillin, and 10 µg/ml Streptomycin. The cells were cultured in flask 25 and maintained at 37°C and 5% CO_2 until confluent. Trypsinization, the number of cells per ml, was counted with a hemocytometer.

HeLa CCL-2 Cell Induction with IL-10

A total of 105 cells/ml was seeded into the 24 wells plate, in which coverslips had been placed. IL-10 with several concentrations of 500 ng/ml, 1000 ng/ml, and 2000 ng/ml was added in HeLa cell culture in triplicate. For analysis of HLA-G secretion in the supernatant and cell lysate, 4 x 104 HeLa cells were also cultured into the 96 wells plate and were induced with IL-10. All plates were incubated for 48 hours in a 37°C incubator with CO_2 pressure 5 atmospheres.

Measurement of HLA-G membrane-bound expression with Double-label immunofluorescence

Cells on the coverslip were fixed for 10 min in absolute methanol at 4°C. Samples were then rehydrated in PBS tween 0.05% 3 times, and subsequently PBS Triton-X 1000 0.1% for 5 min. Cells were incubated for 60 min in blocking buffer (1%: BSA 0.1 g in PBS 10 ml). The HLA-G (4H84) mAbs 1:400 were applied and then incubated and were followed by incubations with biotinylated goat anti-mouse mAb and FITC-labeled streptavidin (1:1000) in the dark. After washing, goat anti-mouse labelled antibody was added for 60 min, followed by DAPI (1:1000) as counterstain. Samples were then mounted on object glass and observed by a fluorescence microscope.

Measurement of HLA-G supernatant and Celllysate with Elisa

Cell culture supernatant: The samples were centrifuged for 20 min at 1000 × g at 2 ~ 8°C and the supernatant were collected.

Cell lysates: The cells were washed gently with a moderate amount of pre-cooled PBS and dissociated the cells using trypsin. The cell suspension was collected into a centrifuge tube and centrifuged for 5 min at 1000 × g. The medium was discarded and washed 3 times with pre-cooled PBS. For each 1 × 10^6 cells, added 150-250 µL of pre-cooled PBS to keep the cells suspended. The freeze-thaw process was repeated several times until the cells are fully lysed and centrifuged for 10 min at 1500 × g at 4°C. The cell fragments were removed; the supernatant was collected to carry out the assay.

Elisa (HLA-G Elisa kit: Elabscience)

The standard working solution added to the first two columns: Each concentration of the solution is added in duplicate, to one well each, side by side (100 µL for each well). The samples were added to the other wells (100 µL for each well). The plate covered

with the sealer provided in the kit and incubated for 90 min at 37°C. The liquid was removed out of each well, do not wash. Immediately 100 µL of Biotinylated Detection Ab working solution added to each well. Cover with the Plate sealer and gently mix up and incubated for 1 hour at 37°C.

The solution was aspirate from each well, and 350 µL of wash buffer was added to each well. Soak for 1 ~ 2 min and the solution was aspirated from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. 100 µL of HRP Conjugate working solution was added to each well was covered with the Plate sealer and incubated for 30 min at 37°C. The solution was aspirate from each well, repeat the wash process for five times. 90 µL of Substrate Reagent was added to each well. Cover with a new plate sealer. Incubate for about 15 min at 37°C. The plate was protected from light. 50 µL of Stop Solution was added to each well. The optical density (OD value) of each well was determined at once with a micro-plate reader set to 450 nm.

Statistical analysis

From the data obtained, a normality test was carried out using the Kolmogorov-Smirnov test. The data obtained will be tested by ANOVA ($p < 0.05$), and differences between groups were further tested by Least Significant Difference (LSD).

Results

In this study, HeLa CCL-2 cell cultures were divided into a control group and treatment groups. In the control group, it was found that supernatant HLA-G level was higher than HLA-G cell lysate level (65.50 ng/ml vs 21.59 ng/ml), as well as in all treatment groups as shown in Table 1. The mean HLA-G level in the supernatant (77.372 ng/ml) was higher than the mean HLA-G level in cell lysate (28.68 ng/ml).

Table 1: Comparison of HLA-G Supernatants with HLA-G CellLysate Level after IL-10 Induction

Sample	N	Mean of HLA-G (ng/ml)
Supernatants Control	3	65.50
IL-10 500 ng/ml	3	76.66
IL-10 1000 ng/ml	3	83.10
IL-10 2000 ng/ml	3	84.24
CellLysate Control	3	21.59
IL-10 500 ng/ml	3	24.32
IL-10 1000 ng/ml	3	32.67
IL-10 2000 ng/ml	3	36.16

There was a significant difference among treatment groups and within the treatment group on the levels of HLA-G secreted in the supernatant or on cell lysate ($p = 0.000$; $p = 0.000$). Comparison of HLA-G supernatant and cell lysate levels, as illustrated in Figure 1.

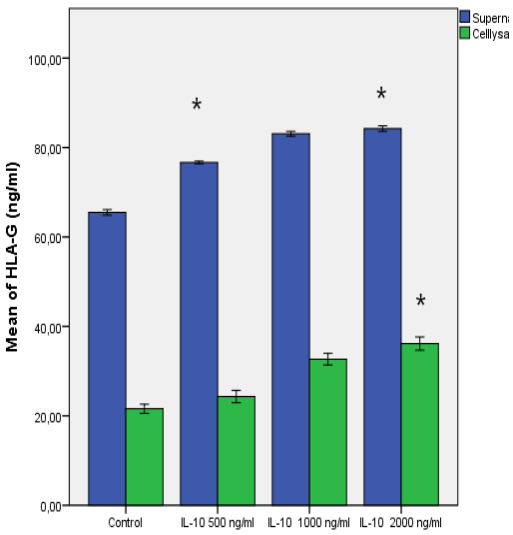

Figure 1: Comparison of HLA-G Supernatant Levels and Cell Lysate CCL-2 HeLa Cells After IL-10 Induction; The highest HLA-G secretion in the supernatant and cell lysate of HeLa cell culture were shown after induction of IL-10 concentration of 2000 ng/ml (84.24 ng/ml and (36.16 ng/ml)); Comparison the HLA-G supernatant and cell lysate on CCL-2 HeLa Cells culture differed significantly between control and treatment groups (sig < 0.05)

Membrane-bound HLA-G expression of HeLa CCL-2 cells was observed using a fluorescent microscope. Illustrations of HLA-G expression that were obtained in each treatment group is shown in Figure 2.

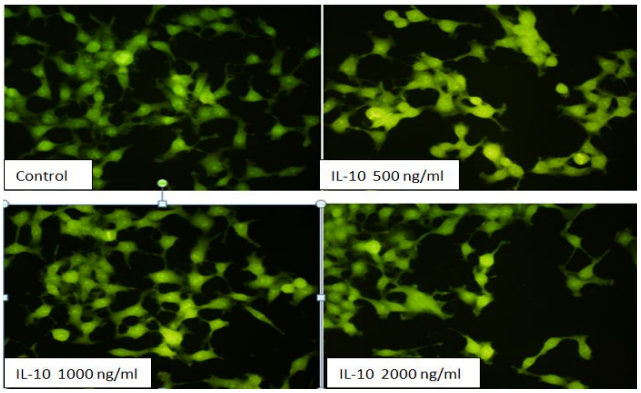

Figure 2: HLA-G Expression in HeLa CCL-2 Cells Culture; Fluorescent images in the treatment group induced by IL-10 500 ng/ml gives a stronger expression of HLA-G than the other treatment groups. By using ImageJ application, the mean HLA-G expression for the control and treatment fields were: 37.26; 59.28; 57.16 and 47.11 AU which were observed under a fluorescent microscope with a 400-x magnification

The averages of HLA-G expression were analysed using the ImageJ application (table 2). The highest expression of HLA-G (59.28 AU) was found on HeLa cell membranes IL-10 500 ng/ml-induced group. After being analysed with ANOVA, HLA-G membrane expression in the non-IL-10-induced group was significantly lower from the IL-10 induced groups, that were 500 ng/ml ($p = 0.014$) and 1000 ng/ml ($p = 0.020$). HLA-G membrane expression in the IL-10 500

ng/ml-induced group not significantly different from the IL-10-induced group 1000 ng/ml (p = 0.708) and 2000 ng/ml (p = 0.083). HLA-G membrane expression in the IL-10 1000 ng/ml-induced group not significantly different from the IL-10-induced group 2000 ng/ml (p = 0.130). Hence, HLA-G membrane expression in the IL-10 500 ng/ml and 1000 ng/ml-induced group was significantly different from non-IL-10 inducing, as shown in Figure 3 and Table 2.

Table 2: HLA-G Expression of CCL-2 HeLa Cell Membrane after IL-10 Induction

Treatment Group	N	Mean of HLA-G expression in view (AU)
Control	5	37.259
IL-10 500 ng/ml	5	59.279
IL-10 1000 ng/ml	5	57.156
IL-10 2000 ng/ml	5	47.108

In the control group, HeLa cells can express HLA-G on the membrane, although this group did not receive IL-10. HLA-G expression in the group treated with IL-10 500 ng/ml had the highest expression (59.279 AU), and significantly different from the control (p = 0.014).

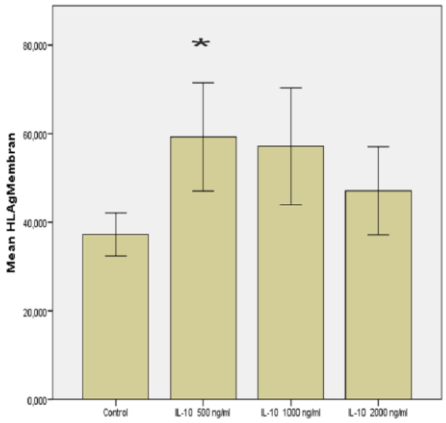

Figure 3: Comparison of HLA-G Expression of HeLa CCL-2 Cell Membrane

Discussion

Parameters of the tumour microenvironment that play a role in modulation of the HLA-G gene include stress conditions such as heat shock and hypoxia; cytokines such as IFNɣ, IL-10, leukaemia inhibitory factor (LIF), and GM-GSF; and hormones such as glucocorticoids and progesterone. The mechanism of action of all these parameters is not yet clear. In this study, IL-10 was used to induce the production of HLA-G protein on HeLa CCL 2 cells as a model of tumour cells. The results of this study demonstrated that the HeLa CCL-2 cells could express and secrete HLA-G with or without IL-10 treatment. The higher the concentration of IL-10 treatment, the higher the HLA-G expression in the membrane and HLA-G secretion to the supernatant, as shown in Table 1 and Figure 1. The previous study by Polakova & Russ, in 2000, Hela cells, along with 61 other cells line did not express HLA-G antigens as observed by flow cytometry using 87G and 01G antibodies. Research several years later, using the RT- PCR method revealed that stable levels of HLA-G mRNA in HeLa cells were very low(~1.5 x 10-5) compared to HLA-G levels in positive. JEG-3 choriocarcinoma cells with HLA-G (HLA-G mRNA level: 1). RREB-1 and DNA methylation at the HLA-G locus is complementary mechanisms [9]. However, this study proved that HeLa cells could express HLA-G before or after IL-10 induction.

The previous study of trophoblastic cells, IL-10 can activate HLA-G transcription in trophoblastic tissue. Northern blotting analysis and quantification of total RNA from trophoblasts showed that IL-10 induction increased the transcription level of HLA-G mRNA at least 7 times compared to the HLA- G mRNA of cells without IL-10. The RNase protection test also showed that HLA-G mRNA levels were stabilised 7.2-fold after induction of IL-10 in trophoblastic explants. To analyse IL-10-induced HLA-G transcription patterns, HLA-G-specific primers are used to strengthen the isolated HLA-G mRNA form. Southern blotting of HLA-G RT-PCR products reveals that induction of IL-10 enhances all transcripts, including G5 and G6, which encode dissolved HLA-G isoforms [11].

In this study, IL-10 treatment induces HLA-G expression on the HeLa cell membrane as well as HLA-G secretion into the HeLa cells culture supernatant. Possible underlying mechanisms can be explained based on previous studies on cervical cancer. It is well-known that progression is associated with a shift from Th1 to Th2 cytokine production. This shift toward a Th2 cytokine profile, characterised by IL-10 secretion, is associated with the progression of premalignant lesions to cancer [13]. Moreover, another study showed peripheral blood monocytes expressed classic HLA class I and proteins containing IL-10 receptors. HLA-G transcripts are analysed by RT- PCR, using primary HLA-G and HLA-G5-specific primers, which encode HLA-G5 soluble proteins. Moderate HLA-G gene transcripts in monocytes, weak HLA-G transcription signals enhanced after induction of IL-10. It is possible that IL-10 may contribute to the activation of HLA-G expression in melanoma cells, thus participating in the escape of tumours from immunosurveillance [11]. HLA-G expression at the surface of tumour cells can participate in the evaluation of anti-tumour immune responses and favour tumour progression [12].

In this study, the results of HLA-G production of cell lysate and HLA-G secretion in HeLa cell culture supernatant were confirmed by microscopic examination with HLA-G antibody (4H84) using a fluorescent microscope to determine the quantity of membrane HLA-G expression in each group

treatment. From the results of microscopic observations of the field of view, the highest results were obtained in the treatment group with IL-10 500 ng/ml induction with a mean of 59,28 AU, which was directly proportional to HLA-G levels in the supernatant and cell lysate. HLA-G expression of the membrane increases after IL-10 500 ng/ml treatment and decreases again at IL-10 2000 ng/ml. These results were consistent with previous studies which that analysed the expression of HLA-G proteins in induced and non-IL-10-induced monocytes using anti-HLA-G-specific 87G mAb. The study showed that the percentage of cells with positive HLA-G and HLA-G cell surface density increased after IL-10- induction [11].

In conclusion, HeLa CCL-2 cells expressed HLA-G on its membrane and release dissolved HLA-G without induction of IL-10, although IL-10 induction augments the presence and the production of HLA-G in HeLa CCl-2 cells.

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Clinical Science

Using Score for Neonatal Acute Physiology Perinatal Extension II (SNAPPE II) in Neonates with Acute Kidney Injury

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Keywords: Neonates; Acute kidney injury; SNAPPE 2 score

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Abstract

BACKGROUND: Acute kidney injury is a severe clinical condition. It is common in neonates in intensive care unit. It is defined as a sudden deterioration in kidney function resulting in derangements in fluid balance, electrolytes, and waste products. The score for neonatal acute physiology perinatal extension in critically sick neonates with kidney injury is a useful tool for assessing the severity of the disease.

AIM: This study aimed to determine the incidence of AKI and the role of SNAPPE 2 score in predicting mortality and morbidity of kidney injury in neonates.

METHODS: The study was designed as a prospective clinical investigation performed in the period of three years, which included 100 neonates (50 with AKI and 50 without AKI) hospitalised in intensive care unit of University Clinic of Children Diseases in Skopje. The severity of the illness of hospitalised newborn infants was estimated with SNAPPE 2 score realised in the first 12 hours of admission to NICU. Medical data records of admitted neonates with AKI were analysed. The material was statistically processed using methods of descriptive statistics.

RESULTS: During the study period, 770 new born's were hospitalised in the intensive care unit due to various pathological conditions and 50 new born's were selected with AKI. The control group consisted of 50 neonates with comparable associated pathological conditions, but without kidney injury. The calculated prevalence of AKI in neonates was 6.4%. Most of the involved neonates in the study in both groups (AKI and non-AKI) were born at term (64% and 54%) with a predominance of male neonates (68% and 60%). The mortality rate was significantly higher in newborns with AKI than in the control group (36% vs 24%) ($p < 0.01$). The mean SNAPPE 2 score value in neonates with AKI was higher than in the control group (58.72 vs 40.0), and the difference was significant ($p = 0.00001$). Difficult score level predominated in half (50%) of newborn infants with AKI, while median score level predominated in control group (42%). There was a significant difference between the mean score value in neonates with AKI and lethal outcome compared to neonates with AKI without lethal outcome (70.73 ± 18.6 vs 40.2 ± 16.6) ($p < 0.0001$).

CONCLUSION: Acute kidney injury is a life-threatening condition with still high mortality rate. The severity of the illness of hospitalised neonates in an intensive care unit is estimated by SNAPPE 2 score. Also, the risk of mortality is estimated too, taking into consideration the fact that higher values of the score are associated with higher mortality. Appropriate treatment of neonates with severe kidney injury improves the outcome and reduces the mortality of the disease.

Introduction

Acute kidney injury (AKI) is a severe clinical condition. It is common in neonates in an intensive care unit (NICU). It is defined as a sudden deterioration in kidney function resulting in derangements in fluid balance, electrolytes, and waste products.

The diagnosis of AKI is based on a rise of serum creatinine (sCr) and urine output of fewer than 1.0 ml/kg/h. Serum creatinine has different value in neonates because of the presence of maternal creatinine, lower glomerular filtration rate (GFR) and differences in maturation [1], [2], [3], [4], [5], [6], [7], [8].

The incidence of kidney injury in children is significantly lower than in adults except in neonates where it occurs in 8 to 24% with mortality rates between 10% and 61% [9], [10].

Predisposing factors associated with acute kidney injury in neonates are certain clinical conditions such as asphyxia, sepsis, prematurity, meconium plaque syndrome, congenital heart diseases, invasive procedures and some nephrotoxic drugs. Appropriate

treatment of associated comorbidities, limited use of nephrotoxic drugs reduces the risk of kidney injury in neonates hospitalised in the intensive care unit [8], [9], [10], [11], [12].

The scoring system in critically sick neonates with kidney injury is used as a tool for predicting morbidity and mortality of the disease. It assesses the severity of the disease concerning the levels of deviation from normal physiology through the evaluation of numerous physical and laboratory tests. The most commonly used scoring system is Score for Neonatal Acute Physiology Perinatal Extension II (SNAPPE 2 score). It can predict the outcomes in critically ill newborns. It was developed by Ridsrtson in 1993 as an index for the severity of the disease. It is performed within the first 24 hours of admission of newborns to NICU and contains 34 clinical tests and vital signs. In 2001 Rittersson simplified the score by creating a second generation of SNAPPE 2 score containing 6 physiological parameters and 3 parameters for assessing perinatal mortality [13].

SNAPPE 2 is a useful tool for assessing the severity of the disease that correlates with neonatal mortality in intensive care units. A higher level than 40 is associated with a higher mortality rate. The parameters associated with perfusion as a mean arterial pressure and acidosis are significantly associated with greater organ dysfunction and higher mortality [14], [15], [16].

This study aimed to determine the incidence of AKI and the role of SNAPPE 2 score in predicting mortality and morbidity of kidney injury in neonates.

Methods

The study was designed as a prospective, clinical, epidemiological investigation performed in three years, which included 100 neonates (50 with AKI and 50 without AKI) hospitalised in NICU of University Clinic of Children Diseases in Skopje.

Criteria for inclusion in the study were: neonates up to 28 days of postnatal age; neonates treated in NICU due to certain pathological condition with or without the development of kidney injury. AKI was defined by elevated serum creatinine (> 130 μmol/L in neonates younger than 33 weeks and > 90 μmol/L in neonates older than 33 weeks) and the presence of oliguria (less than 1.0 ml/kg/h). According to our criteria, all neonates who were older than 28 days of age, who had less than 24 hours of hospitalisation and neonates who had cardio-surgical interventions were excluded from the study.

Medical data records of admitted neonates with AKI were analysed. The neonates were analyzed according to gender, birth weight, and gestational age. The laboratory examinations of serum creatinine values were done in the biochemical laboratory of the Clinic of Children Diseases using Kodak camera dry biochemistry. The severity of the illness of hospitalised neonates was estimated with SNAPPE 2 score realised in the first 12 hours of hospitalisation to NICU. Also, the risk of mortality was estimated, taking into account the fact that higher values of the score are associated with a higher mortality rate. Table 1 shows the examined variables in SNAPPE 2 score.

Table 1: Score systems in NICU

SNAPPE	SNAPPE 2
arterial pressure	mean arterial pressure
cardiac frequency	temperature
respiratory rate	pO2 / FiO2
temperature	Ph. Blood
PCO2	convulsions
PO2 / FiO2	diuresis
PCO2	
leukocytes	
platelets	
urea in serum	
creatinine in serum	
diuresis 24 hours	
indirect bilirubin	
direct bilirubin	
sodium in serum	
potassium in serum	
calcium total	
calcium ionizing	
glycemia	
bicarbonate	
Ph. Blood	
convulsions	
apnea	

The material was statistically analysed using the methods of descriptive statistics. To determine the significance of differences in the parameters, the tests for independent samples were analysed. Statistical significance was determined for the values of $p < 0.05$.

Results

During the study period, 770 newborns were hospitalised in NICU due to various pathological conditions and 50 newborns were selected with AKI. The control group consisted of 50 neonates with comparable associated pathological conditions, but without kidney injury. The calculated prevalence of AKI in neonates was 6.4%. The mean gestational age of newborns with AKI was 37.42 ± 3.1 weeks and 36.26 ± 3.8 weeks in the control group. The mean birth weight of neonates with AKI was 2890.8 ± 898.1 grams, while in the control group was 2699.4 ± 894.6 grams.

Most of the involved neonates in both groups were born at term (64% and 54%) with a predominance of male (68% and 60%). Figure 1 and figure 2 show the distribution of neonates with AKI and non-AKI depending on age and gender.

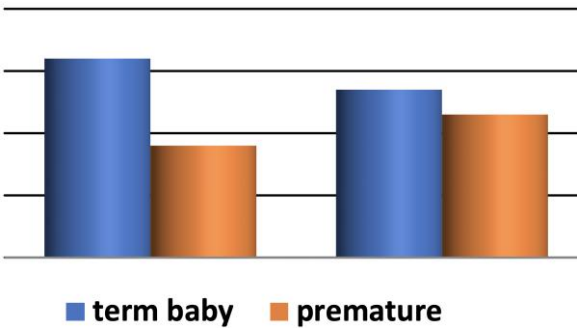

Figure 1: Distribution of newborns with AKI and Non-AKI depending on age

According to the outcomes of the disease, 18 neonates with AKI and 12 neonates of the control group had a lethal outcome. So, the mortality rate in neonates with AKI was 36%, while 24% in the control group. This difference was not statistically significant ($p < 0.01$).

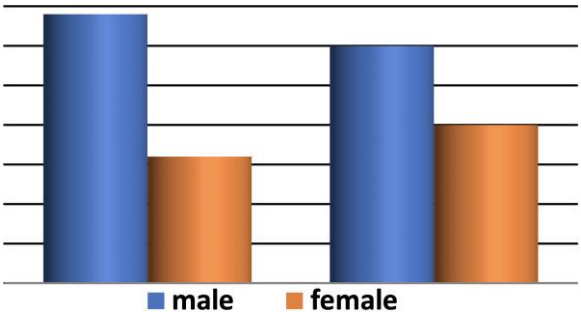

Figure 2: Distribution of newborns with AKI and Non-AKI depending on gender

All neonates were evaluated by SNAPPE 2 score within the first 12 hours of admission in the intensive care unit. The score values were analysed in 4 categories: light (score 1-20), medium (score 21-40), difficult (41-70) and very difficult (over 70). The results showed that the mean score value in neonates with AKI was 58.72 ± 20.4, while 40.0 ± 20.0 in the control group. The difference of 18.72 between AKI group and control group was confirmed as significant ($p = 0.00001$) (Table 2).

Table 2: Distribution of SNAPPE 2 score values in neonates in NICU

	AKI	Non-AKI	
Mean ± SD	58.72 ± 20.4	40.0 ± 20.0	T = 4.6
Min	29	8	P = 0.0000001
Max	97	78	

Table 3 shows the distribution of neonates with AKI depending on score levels compared to the control group. In half (50%) of neonates with AKI predominate difficult score level, while the median score level predominated in 42% neonates of the control group.

Table 3: Distribution of neonates with AKI depending on score levels compared to the control group

Score	AKI / Non-AKI	Mean ± SD	Min-Max
Light	AKI N = 0	0	0
	Non-AKI N = 8	14.0 ± 3.9	8-19
Medium	AKI N = 10	33.8 ± 3.3	29-38
	Non-AKI N = 21	28.7 ± 4.8	22-39
Difficult	AKI N = 25	52.24 ± 7	42-67
	Non-AKI N = 16	43.18 ± 5.8	38-52
Very difficult	AKI N = 15	86.13 ± 8.3	77-97
	Non-AK N = 5	81.6 ± 8.3	72-91

There was a significant difference between the mean score value in neonates with AKI and lethal outcome compared to newborn infants with AKI without lethal outcome (70.73 ± 18.6 vs 40.2 ± 16.6) ($p < 0.0001$) (Figure 3).

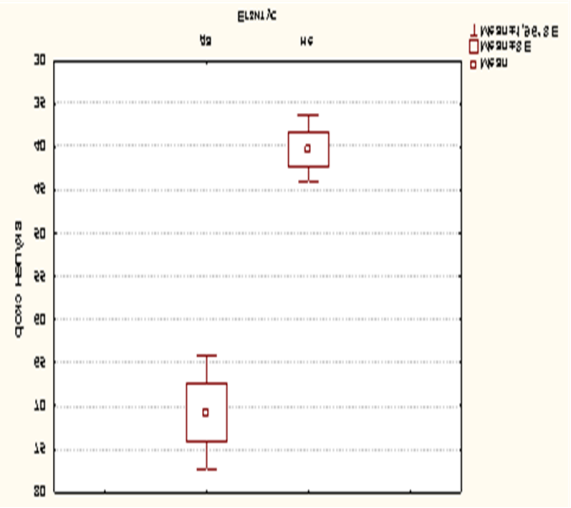

Figure 3: Distribution of mean score values in neonates with AKI and lethal outcome and neonates with AKI without lethal outcome

Figure 4 shows the distribution of score values in neonates compared to control group, depending on lethal outcomes. 66% of neonates with AKI and lethal outcome had a very difficult score level, as opposed to 59% survived neonates with AKI who had a lower score level. Compared to the control group, 54% neonates with lethal outcome had a difficult score level, and 51% of survived neonates had a medium score level.

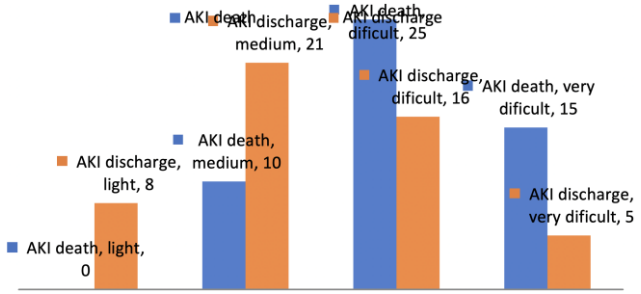

Figure 4: Distribution of score values in neonates with AKI compared to control group, depending on lethal outcomes

Discussion

This study presents a clinical, epidemiological study that evaluated neonates who were treated in intensive care at the Universiti Clinic of Children Diseases. During the period of three years, a high number of neonates with various pathological conditions and AKI were hospitalised. But this study included 50 neonates with documented kidney injury, as well as other 50 neonates, as a control group, with comparable associated pathological conditions, but without kidney injury. The calculated prevalence of kidney injury in neonates was 6.4% [16], [17].

This data correlates with data presented in the literature, where the incidence of AKI in neonates has the highest rate (6-24%) compared to other age groups (infants, children and adults). The occurrence of AKI in neonates is influenced by various factors, such as gestational age, birth weight and co-morbid conditions present during and immediately after birth. A similar finding as in our study has been published in other studies done in various neonatal centres. In the study of *Vachvanichsanong et al.,* the incidence of kidney injury in newborn infants was 6.3%, while in *Bolat et al*., and *Stapleton et al.,* it was 8% and 8.4%, respectively [17], [18], [19], [20], [21], [22], [23], [24], [25], [26], [27], [28], [29].

However, there are opposite findings. Thus, in the study of *Momtaz et al.,* the incidence of kidney injury was 1.5%; in the studies of *Mortazavi et al*., 2.7%; in *Agras et al.,* 3.4%, and in the *Mohkam et al*., 3.2%. We assume that these differences can be due to differences in the criteria for diagnosing AKI in neonates [30].

According to the sex distribution, for all gestational ages, in both groups of neonates (with AKI and control group), the majority of neonates were male (68% and 60%). A different finding was presented in the study of *Momtaz et al.,* where female predominated in 87.7% of cases [21], [22], [23], [24].

According to the distribution of gestational age, the majority of neonates in both groups were term neonates (64% and 54%). The mean gestational age of neonates with AKI was 37.42 ± 3.1 weeks of gestation and the control group 36.26 ± 3.8 weeks of gestation. The mean birth weight of neonates with AKI was 2890.8 ± 898.1 grams and of the control group 2699.4 ± 894.6 grams. In both groups, predominated neonates with a birth weight over 2500 grams (64% and 54%, respectively).

There were no significant differences in the mortality rate in both groups of neonates (36% vs 24%). This finding of 36% of mortality in neonates with AKI correlates to the data presented in the study of *Gharehbaghi et al.* In our study, the mortality was significantly higher in neonates with AKI and congenital heart disease as comorbidity, and they underwent invasive therapeutic procedures (umbilical catheterisation and assisted ventilation). Special attention needs to be paid in the application of invasive procedures in neonates due to the association of these procedures with the risk of AKI. Critically sick neonates are at risk of having kidney injury, as they are commonly exposed to nephrotoxic medications and invasive therapeutic intervention such as assisted ventilation [25], [26], [27], [28].

In our study, neonates with AKI and lethal outcome have had severe SNAPPE 2 score. The high score level was significantly associated with the severity of the disease. Especially, very ill neonates with AKI and other co-morbid conditions were significantly associated with higher-level score and higher mortality. These findings correlate with the data presented in the study of *Mortazavi et al.* The mortality rate was significantly higher in neonates with difficult score level when admitted to the NICI, in whom further co-morbid conditions developed [29], [30], [31]. [32], [33].

In conclusion, acute kidney injury is a life-threatening condition with still high mortality rate. The severity of the illness of hospitalised neonates in the intensive unit is estimated by SNAPPE 2 score. Also, the risk of mortality is estimated too, taking the fact that higher values of the score are associated with higher mortality. Appropriate treatment of neonates with severe kidney injury improves the outcome and reduces the mortality of the disease.

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Clinical Science

Predictions of Hypoxic-Ischemic Encephalopathy by Umbilical Cord Blood Lactate in Newborns with Birth Asphyxia

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Keywords: Hypoxic ischemic encephalopathy; Asphyxia; Umbilical cord; Lactate

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Abstract

AIM: The aim of the study was to investigate the role of umbilical cord blood lactate as early predictors of hypoxic ischemic encephalopathy in newborns with perinatal asphyxia and to evaluate their sensitivity and specificity for the early identification of hypoxic ischemic encephalopathy infants.

METHODS: We performed a descriptive cross sectional study between April 2014 and April 2015 at Hue Central Hospital, Vietnam. 41 asphyxia newborns (Apgar score ≤ 7) were included in the study. Umbilical cord blood is sampled for lactate analysis.

RESULTS: Umbilical cord blood lactate levels were significantly higher among infants born with HIE (mean 8.72 ± 1.75, range 5.12 – 11.96) compared to that with asphyxic infants without HIE (mean 6.86 ± 1.33, range 4.74 – 10.30), p = 0.00. With the optimal cutoff point for umbilical cord blood lactate level of 8.12 mmol/l to susspected of HIE (area under the curve 0.799) had a sensitivity 73.7% (95% CI: 48.8-90.9), specificity 86.4% (95% CI: 65.1-97.1).

CONCLUSION: Umbilical cord blood lactate could be used as early predictors in diagnosis of hypoxic ischemic encephalopathy in newborns with asphyxia.

Introduction

Hypoxic ischemic encephalopathy (HIE) is one of the most serious birth complications affecting full term infants [1]. It occurs in 1.5 to 2.5 per 1000 live births in developed countries. HIE is a brain injury that prevents adequate blood flow to the infant's brain occurring as a result of a hypoxic-ischemic event during the prenatal, intrapartum or postnatal period [2], [3]. By the age of 2 years, up to 60% of infants with HIE will die or have severe disabilities including mental retardation, epilepsy, and cerebral palsy (CP) [2], [4], [5], [6], [7]. The early prediction of hypoxic ischemic encephalopathy is particularly important because of the brief thera-peutic window and possible side effects of neuro protective interventions [8].

Several biomarkers were done for proper assessment of the severity of brain damage. An ideal biomarker for the diagnosis of HIE should be specific, early, rapid, and easily done. The results of these biomarkers should be interpreted in conjunction with the clinical history and physical examination [9], [10].

Lactate is invariably produced in the event of hypoxia and poor tissue perfusion. When a clinical reduction of oxygen and substrate delivery occurs, aerobic metabolism through Krebs cycle cannot be sustained and tissues need anaerobic metabolism to meet the energy requirement. This in turn leads to increase in the production and accumulation of blood lactate [11]. Lactate is produced by anerobic oxidation during an asphyxia insult and continues to be excreted via the kidney for a long period after the insult so that measurement of urinary lactate may reflect the blood lactate level and the degree of metabolic derangement as a result of hypoxia/ischemia [12].

The aim of the study was to investigate the

role of umbilical cord blood lactate as early predictors of HIE in Newborns with Perinatal Asphyxia and to evaluate their sensitivity and specificity for the early identification of HIE infants.

Patients and Methods

Patients

A prospective study including 41 infants with a proven diagnosis of asphyxia who were admitted to neonatal intensive care unit (NICU) in Hue Central Hospital, Vietnam, from April 2014 to April 2015. Apgar score at 1 minute after birth was used to identify perinatal asphyxia in the new born. Those babies with apgar score of ≤ 7 were considered to have had perinatal asphyxial insult.

The asphyxia newborns were categorized into two groups according to the presence of HIE. Suspect neonatal HIE in the baby who is asphyxia at birth and who, in the earliest hours of life, presents with disturbed neurological function including [13]:

- A subnormal level of consciousness or seizures.

- And frequently:

+ Difficulty initiating and maintaining respiration.

+ Depression of tone and reflexes.

Exclusion Criteria: 1) Newborn delivered with major congenital anomalies or chromosomal abnormality and 2) Multiple pregnancies.

Measurements

Umbilical cord blood is sampled by nursing personnel immediately after delivery for all infants deemed to be viable. Umbilical blood samples were drawn from a double-clamped segment of the umbilical cord into 2 ml plastic syringes flushed with a heparin solution. Blood lactate were measured using whole blood in automated benchtop analyzers. The DXC-800 Automated Chemistry Analyser (Beckman Coulter) was used for lactate assays. Obtained data was recorded in a newborn's notes.

Data analysis

We first selected a range of cutoff points for the umbilical cord lactate biomarker to distinguish HIE from asphyxic newborn without HIE. We reported the sensitivity, specificity and negative predictive value (NPV) for HIE of each lactate cutoff point. Next, we generated a receiver-operator curve (ROC) to visually represent the trade-off between sensitivity and specificity. We utilized ROC curve analysis to select the optimal lactate cutoff point to minimize both the number of false positives. A Mann–Whitney U-test was performed to compare lactate levels between children with HIE and without HIE. We used SPSS version 19.0 for all statistical analyses.

Results

41 asphyxia newborns with singleton, liveborn infants with no major anomalies delivering between April 2015 to April 2016 were analysed. Gestational age ranged from 35 to 41 weeks (mean 37.41 ± 0.31 weeks). Umbilical cord blood lactate levels were significantly higher among infants born with HIE (mean 8.72 ± 1.75, range 5.12 – 11.96) compared to that with asphyxic infants without HIE (mean 6.86 ± 1.33, range 4.74 – 10.30), p = 0.001 (Table 1).

Table 1: Umbilical cord blood lactate level in asphyxic newborns with and without HIE

	Non-HIE (n = 22)	HIE (n = 19)
Mean lactate, mmol/l (SD)	6.86 (± 1.33)	8.72 (± 1.75)
Median lactate, mmol/l (interquartile range)	6.60 (5.99-7.70)	8.88 (8.05-9.84)

Each step-wise increase in the umbilical cord blood lactate level cutoff point lowered the sensitivity, but increased the specificity for HIE (Table 2). Next we present a ROC curve for umbilical cord blood lactate level for HIE (Figure 1).

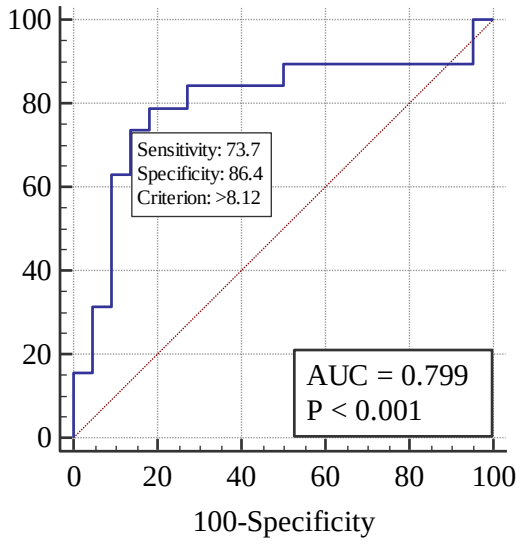

Figure 1: Receiver-operating curve for lactate to distinguish HIE from asphyxic newborn without HIE. Overall accuracy was good, with area under ROC curve of 0.799 (95% CI: 0.645-0.908), p = 0.0001

Using ROC analysis, we selected an optimal cutoff point for umbilical cord blood lactate level of

8.12 mmol/l to susspected of HIE (area under the curve 0.799). Umbilical cord blood lactate level ≥ 8.12 mmol/l had a sensitivity 73.7% (95% CI: 48.8-90.9), specificity 86.4% (95% CI: 65.1-97.1), +ve LR (likelihood ratio) 5.4, -ve LR 0.3 for HIE (Table 2).

Table 2: The diagnostic accuracy of CSF lactate for bacterial meningitis by cutoff point

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	-LR
≥ 4.74	100.00	82.4-100.0	0.00	0.0-15.4	1.00	
> 4.74	100.00	82.4-100.0	4.55	0.1-22.8	1.05	0.00
> 5.31	89.47	66.9-98.7	4.55	0.1-22.8	0.94	2.32
> 6.48	89.47	66.9-98.7	50.00	28.2-71.8	1.79	0.21
> 6.67	84.21	60.4-96.6	50.00	28.2-71.8	1.68	0.32
> 7.12	84.21	60.4-96.6	72.73	49.8-89.3	3.09	0.22
> 7.36	78.95	54.4-93.9	72.73	49.8-89.3	2.89	0.29
> 7.89	78.95	54.4-93.9	81.82	59.7-94.8	4.34	0.26
> 8.02	73.68	48.8-90.9	81.82	59.7-94.8	4.05	0.32
> 8.12	73.68	48.8-90.9	86.36	65.1-97.1	5.40	0.30
> 8.28	63.16	38.4-83.7	86.36	65.1-97.1	4.63	0.43
> 8.29	63.16	38.4-83.7	90.91	70.8-98.9	6.95	0.41
> 9.18	31.58	12.6-56.6	90.91	70.8-98.9	3.47	0.75
> 9.27	31.58	12.6-56.6	95.45	77.2-99.9	6.95	0.72
> 9.88	15.79	3.4-39.6	95.45	77.2-99.9	3.47	0.88
> 10.3	15.79	3.4-39.6	100.00	84.6-100.0		0.84
> 11.96	0.00	0.0-17.6	100.00	84.6-100.0		1.00

Discussion

Perinatal hypoxic-ischemic encephalopathy (HIE) occurs in one to three per 1000 live full-term births [14]. Of affected newborns, 15% – 20% of affected newborns will die in the postnatal period, and an additional 25% will develop severe and permanent neuropsychological sequelae, including mental retardation, visual motor or visual perceptive dysfunction, increased hyperactivity, cerebral palsy, and epilepsy [15]. The outcomes of HIE are devastating and permanent, making it a major burden for the patient, the family, and society.

The early prediction of hypoxic ischemic encephalopathy is particularly important because of the brief therapeutic window and possible side effects of neuro protective interventions [16], [17]. In spite of major advances with sophisticated monitoring technology and knowledge of fetal and neonatal pathology, perinatal asphyxia or more appropriately, HIE remains a serious condition, that leaves a significant handicaps in the survivors [18].

In our study the mean of serum lactate level in HIE group was 8.21 mmol/L while in the non-HIE group the level of lactate was 6.86 mmol/dl which was statistically significant as p value = 0.001. This in agreement with some studies who found that serum lactate was higher in hypoxic group than healthy group. Mohamed Shawky Elfarargy et al., study nucleated red blood cells per 100 white blood cell (NRBC/100 WBC) counts and lactate levels in cord blood as early markers of neonatal HIE, the median of serum lactate level in hypoxic group was 7 mmol/L while in the control group the level of lactate was 1.9 mmol/dl which was statistically significant as p value < 0.0001, and the more grade of HIE the higher serum lactate [19]. Shah et al., [12] carried out a study on 61 term neonates to estimate lactate level as a predictor for short term outcome after intrapartum asphyxia. They found that the median of lactate level 11.09 mmol/dl in asphyxi-ated group met all the criteria of hypoxic ischemic encephalopathy, the study also found that plasma lactate levels lower than 5 mmol/dl were not asso-ciated with severe encephalopathy while plasma lactate levels > 15 mmol/L were associated with moderate to severe HIE in 100% of cases. As in hypoxic patients, a critical reduction in oxygen substrate delivery occurs, aerobic metabolism through Kreb's cycle cannot be sustained, and tissues need anaerobic metabolism to meet their energy requirements. This in turn leads to an in-crease in the production and accumulation of blood lactate [20]. In other study done by Zhang Haiju et al., [8], carried out on 46 asphyxiated new newborns, the level of serum lactate in umbilical cord blood in the first six hours after delivery was 4.3 ± 1.2 SD while in control group, the median of serum lactate was 2.46 ± 0.48 mmol/dl. Many factors may exist that could influence the results, such as the different inclusion criteria, different races and lifestyle.

In our study umbilical cord blood lactate level to diagnose HIE was > 8.12 mmol/L which yield a sensitivity of 73.7%, specificity 86.4%. Overall accuracy was good, with area under ROC curve of 0.799 (95% CI: 0.645-0.908), p = 0.0001. This is in agreement with some studies which concluded that Umbilical lactate can be used in a middle-low resource setting as a measurement of intrapartum hypoxia, with reasonable sensitivity and specificity. Mohamed Shawky Elfarargy et al reported that serum lactate level to diagnose HIE was > 3.6 mmol/L which yield a sensitivity of 87%, specificity 100%, PPV 100% and NPV 88% with a diagnostic accuracy of 93%. Allanson ER et al., from 3 March-12 November 2014, conducted a prospective cohort study of UA lactate levels at Kalafong Hospital, Pretoria, South Africa. A lactate was recorded for 946 deliveries (20.3%). One hundred ninety babies required neonatal resuscitation, with an optimal cutoff for lactate of 5.45 mmol/L (sensitivity 68%, specificity 72%). 124 babies required nursery admission with the optimal cutoff for lactate 4.95 mmol/L (sensitivity 61%, specificity 59%) [21]. According to Simovic A, significantly higher concentrations of lactate (p 8.7 mmol/L with 80 % sensitivity and 82% specificity indicated the development of the hypoxic-ischemic encephalopathy stage II/III, while the lactate level > 9.95 mmol/L was a predictor of death, with 75% sensitivity and 74.4% specificity [22]. Vannucci et al., [23], who showed that, the best cut off of lactate in predicting HIE was 4.25 mmol/L with sensitivity of 94% and specificity of 86%. In previous studies, it was shown that lactate concentration in the term newborns which is in first 24 hours > 7.5 mmol/l with 94 % sensitivity and 67% specifi city may indicate the development of the HIE and that an early increase in lactate in severe asphyxia > 15 mmol/l was a sure indication of serious consequences or death with 100 % sensitivity of and

88 % specificity [12], [20], [24].

In conclusion, umbilical cord blood lactate could be used as early predictors in diagnosis of hypoxic ischemic encephalopathy being very easy, cheap and non-invasive measure. Combined Apgar score and umbilical cord blood lactate in diagnosis of HIE gives us better sensitivity and specificity than lactate alone, also it could be used in prognosis of the newborns with hypoxic ischemic encephalopathy and for detecting the outcome.

Ethics Approval and Consent to Participate

The present study was approved by the Hue Central Hospital Ethical Committee. Informed consent was waived. Consent for publication Not applicable.

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Clinical Science

Cardiovascular Comorbidity in Patients with Chronic Obstructive Pulmonary Disease: Echocardiography Changes and Their Relation to the Level of Airflow Limitation

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Abstract

AIM: To compare the frequency of echocardiographic changes in patients with chronic obstructive pulmonary disease (COPD) and non-COPD controls and to assess their relation to the level of airflow limitation.

METHODS: Study population included 120 subjects divided into two groups. Group 1 included 60 patients with COPD (52 male and 8 females, aged 40 to 80 years) initially diagnosed according to the actual recommendations. Group 2 included 60 subjects in whom COPD was excluded serving as a control. The study protocol consisted of completion of a questionnaire, pulmonary evaluation (dyspnea severity assessment, baseline and post-bronchodilator spirometry, gas analyses, and chest X-ray) and two dimensional (2D) Doppler echocardiography.

RESULTS: We found significantly higher mean right ventricle end-diastolic dimension (RVEDd) in COPD patients as compared to its dimension in controls (28.0 ± 4.8 mm vs. 24.4 ± 4.3 mm; P = 0.0000). Pulmonary hypertension (PH) was more frequent in COPD patients than in controls (33.3% vs. 0%; P = 0.0004) showing a linear relationship with the severity of airflow limitation. The mean value of left ventricular ejection fraction (LVEF%) was significantly lower in COPD patients than its mean value in controls (57.4 ± 6.9% vs 64.8 ± 2.7%; P = 0.0000) with no correlation with severity of airflow limitation.

CONCLUSION: Frequency of echocardiographic changes in COPD patients was significantly higher as compared to their frequency in controls in the most cases being significantly associated with the severity of airflow limitation. Echocardiography enables early, noninvasive, and accurate diagnosis of cardiac changes in COPD patients giving time for early intervention.

Introduction

Chronic obstructive pulmonary disease (COPD) is accompanied by comorbidities which have a significant impact on its prognosis. Furthermore, cardiovascular comorbidities are considered as a major cause for hospitalization and mortality in COPD patients [1], [2], [3], [4], [5], [6]. COPD affects pulmonary blood vessels, right ventricle and left ventricle leading to right ventricular dysfunction, left ventricular dysfunction, pulmonary hypertension (PH) and cor pulmonale [7].

COPD patients have two to three-fold increased risk for hospitalisation due to cardiovascular morbidity compared to patients without COPD [8]. Also, 20-30% of all patients with chronic heart failure have COPD [9], [10]. Shortness of breath and reduced effort tolerance is present in both diseases, so cardiac failure in COPD often remains unrecognised and symptoms are attributed to COPD exacerbations. The reason is that two dimensional (2D) Doppler echocardiography as a diagnostic method for heart failure is not implemented in primary care and is not a standard diagnostic procedure for pulmonologists controlling these patients [9], [11]. Mortality from cardiovascular diseases (CVD) is about 30% and in patients with mild and moderate COPD, the most common cause of hospitalisation and mortality is cardiovascular disease. Lung Health Study showed that reduction of 10% of forced expiratory volume in one second (FEV_1) value in the patients with mild and moderate COPD increases the risk of fatal cardiovascular events up to 30% and of non-fatal coronary events up to 20% [12]. As it was mentioned in the Towards a Revolution in COPD Health (TORCH) study, in patients with severe COPD, CVD

was less significant for morbidity and mortality whereas respiratory failure was the predominant factor [13]. Prevalence of pulmonary hypertension (PH) in COPD patients is estimated to 20-30% [4]. PH in COPD patients usually is mild to moderate with mean systolic pulmonary arterial pressure (sPAP) in the stable disease of 20-35 mmHg. Only 5-10% of patients with severe COPD have severe PH [14], [15]. As it was found in the National Emphysema Treatment Trial, 90.8% of COPD patients had sPAP higher than 20 mmHg and less than 5% had sPAP higher than 35 mmHg [16]. sPAP in COPD patients usually increases slowly, approximately 0.4-0.6 mmHg per year [17], [18].

We aimed to compare the frequency of echocardiographic changes in patients with chronic obstructive pulmonary disease (COPD) and non-COPD controls and to assess their relation to the level of airflow limitation.

Material and Methods

Study design and setting

Frequency and severity of echocardiographic changes registered by 2D Doppler echocardiography in initially diagnosed COPD patients and non-COPD controls were compared in cross-sectional analysis. The study was conducted at the General Hospital "8-th September", Skopje, in the period January – May 2018 as a continuum of our investigation of the impact of cardiovascular comorbidities on COPD (19). The study was approved by the Ethics Committee of the Medical Faculty at University "Sts.Cyril and Methodius", Skopje (03-2237/5/21.05.2018) [19].

Study population

The study population consisted of 120 subjects divided into two groups. Group 1 included 60 patients with newly diagnosed COPD following the actual Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria. Also, 60 subjects in whom COPD was excluded served as a control (Group 2). Non-COPD subjects were matched to COPD patients by sex, age, body mass index (BMI), and smoking status. Written informed consent was given by all subjects before entering the study.

Inclusion and exclusion criteria for Group 1 and Group 2 are explained in our article on carotid artery disease, and lower extremities artery disease in patients with COPD published previously [19].

Study protocol

The study workup included completion of a questionnaire, as well as pulmonary and echocardiographic evaluation.

Questionnaire

The interviewer-led questionnaire consisted of four parts, including questions on demographics, smoking history, respiratory and other symptoms in the last 12 months, as well as medical history and medication use.

The Body Mass Index (BMI) as a measure of body fat based on height and weight that applies to adult population was calculated by BMI calculator [20].

Study subjects were classified by their smoking status according to the World Health Organization (WHO) recommendations [21].

Respiratory symptoms in the last 12 months, i.e. cough, phlegm, dyspnea, wheezing, and chest tightness, were documented by the European Community for Coal and Steel questionnaire (ECCS-87), and the European Community Respiratory Health Survey (ECRHS) questionnaire [22], [23].

Pulmonary evaluation

The pulmonary evaluation included: dyspnea severity assessment, pre- and post-bronchodilator spirometry, arterial gas analysis, and chest X-ray.

Assessment of the degree of dyspnea was done using the British Medical Council Dyspnea Scale [24].

Pre-bronchodilator (baseline) spirometry included measures of forced vital capacity (FVC), FEV_1, FEV_1/FVC, and maximal expiratory flow at 75%, 50%, 25%, and 25-75% of FVC (MEF_{75}, MEF_{50}, MEF_{25}, and MEF_{25-75}, respectively) by electronic spirometer Spirobank G USB Spirometer (Medical International Research, Roma, Italy) with recording the best result from three measurements the FEV_1 values of which were within 5% of each other. The results of measurements were expressed as percentages of the predicted values following the actual recommendations of the European Respiratory Society (ERS) and ATS. Post-bronchodilator spirometry was performed 20 minutes after administration of 400 μg salbutamol by metered-dose inhaler through the spacer. Fixed airflow narrowing was considered if post-bronchodilator FEV_1/FVC value remained less than 0.70. The degree of FEV_1 reversibility was expressed as % FEV_1 reversibility ([post-bronchodilator FEV_1 – pre-bronchodilator FEV_1]/pre-bronchodilator FEV_1 x 100). Significant FEV_1 improvement (a change of more than 12% and more than 200 mL) in the presence of fixed airflow limitation did not negate a diagnosis of COPD [25].

The diagnosis of COPD was established according to the actual GOLD recommendations, i.e.,

COPD was considered by the presence of persistent airflow limitation, i.e. a post-bronchodilator FEV_1/FVC value less than 0.70, in the subjects who had dyspnea, chronic cough or sputum production, and a history of exposure to risk factors for the disease (noxious particles and gases, i.e. tobacco smoke, smoke from home cooking and heating fuels, and/or occupational dusts and chemicals). Also, based on the level of the airflow limitation, the COPD severity was classified as mild, moderate, severe, and very severe (GOLD 1, GOLD 2, GOLD 3, and GOLD 4, respectively) [26].

Values of the arterial blood gases were measured using the SIEMENS RAPIDPOINT 405 System (Siemens Healthineers, Australia).

Cardiovascular evaluation

Resting 2D Doppler Echocardiography was performed by a cardiologist using General Electric Vivid 7, according to the recommendations of the American Heart Association (AHA). Measured parameters included: left ventricular end-diastolic dimension (LVEDd), left ventricular end-systolic dimension (LVEDs), left atrial dimension (LA), left ventricular ejection fraction (LVEF %) by Teischoltz, interventricular septum (IVS), right ventricular end-diastolic dimension (RVEDd), right atrial dimension (RA), estimation of left ventricular diastolic dysfunction, measurement of systolic pressure in pulmonary artery (sPAP), wall abnormalities in right ventricle, and mitral, aortic, tricuspid and pulmonary valvular evaluation. Right ventricular dilation is present when RVEDd exceeded the normal range of 0.9-2.6 cm. Right ventricular systolic dysfunction was present when it was hypokinetic. E/A, i.e. diastolic filling of the left ventricle, was classified initially based on the peak mitral flow velocity of the early rapid filling wave (E) and the peak velocity of the late filling wave caused by atrial contraction (A). In normal subjects, LV elastic recoil is vigorous because of normal myocardial relaxation. Therefore, more filling is completed during early diastolic, so LVEDd is present when E/A value is less than 1.3 (age group 45-49 years), less than 1.2 (age group 50-59 years), less than 1.0 (age group 60-69 years), and less than 0.8 (age group aged equal or more than 70 years) (30). Pulmonary hypertension was defined as a sPAP value equal to or higher than 30 mmHg. According to the severity degree, PH is classified as mild (sPAP = 30-50 mmHg), moderate (sPAP = 50-70 mmHg) and severe (sPAP > 70 mmHg) [27], [28].

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 17.0 for Windows. Continuous variables were expressed as mean values with standard deviation (SD), and the nominal variables as numbers and percentages. Analyses of the data included testing the differences in prevalence, comparison of the means, and testing the association mentioned above. A *P*-value of less than 0.05 was considered as statistically significant.

Results

Characteristics of the study subjects were similar in both EG and CG with the exception of the mean values of spirometric parameters which were significantly lower in COPD patients than in controls.

Table 1: Characteristics of the study subjects

Characteristic	Group 1 (n = 60)	Group 2 (n = 60)
Sex		
M	52 (86.7%)	47 (78.3%)
F	8 (13.3%)	13 (21.6%)
Mean age (yrs)		
M	65.9 ± 7.5	64.3 ± 8.1
F	67.9 ± 6.1	65.3 ± 7.4
Smoking status		
Active smokers	5 (58.3%)	37 (61.6%)
Ex-smokers	25 (41.7%)	23 (38.3%)
Pack-year smoked	66.1 ± 25.8	64.8 ± 21.5
Mean BMI value	25.8 ± 4.9	25.9 ± 3.1
Mean baseline values		
FVC (% pred)	78.8 ± 12.3	113.1 ± 19.7
FEV_1 ((% pred)	47.5 ± 17.9	90.4 ± 15.2
FEV_1/FVC ratio	0.6 ± 0.07	0.8 ± 0.04

M: male; F: female; yrs: years; BMI: body mass index; FVC: forced vital capacity; FEV1: forced expiratory volume in one second; % pred: percentage of the predicted value.

Distribution of the COPD patients by severity of the disease based on the degree of airflow limitation in mild, moderate, severe, and very severe form was 6.7% (4/60) patients), 35% (21/60), 36.7% (22/60), and 21.7% (13/60), respectively.

Table 2: Echocardiographic changes in COPD patients classified by the level of airflow limitation

Echocardiography characteristic of all COPD patients (n = 60)	GOLD 1 FEV1 ≥ 80% pred. (n = 4)	GOLD 2 FEV1 = 50%-79% pred. (n = 21)	GOLD 3 FEV1 = 30%-49% pred. (n = 22)	GOLD 4 FEV1 < 30 % pred. (n = 13)
Normal findings	0 (0%)	0 (0%)	2 (9.5%)	2 (50%)
Mild PH	0 (0%)	3 (14.3%)	6 (27.3%)	4 (30.8%)
Moderate PH	0 (0%)	1 (4.8%)	1 (4.5%)	2 (15.4%)
Severe PH	0 (0%)	1 (4.8%)	1 (4.5%)	1 (7.7%)
Mild TR	2 (50%)	10 (47.6%)	12 (54.5%)	6 (46.1%)
Moderate TR	0 (0%)	0 (0%)	3 (13.6%)	4 (30.8%)
Severe TR	0 (0%)	1 (4.8%)	2 (9.1%)	0 (0%)
Mild PR	0 (0%)	2 (9.5%)	1 (4.5%)	1 (7.7%)
RVEDd > 26mm	1 (25%)	10 (47.6%)	13 (59.1%)	7 (53.9%)
Enlargement of RA	0 (0%)	1 (4.8%)	2 (9.1%)	2 (15.4%)
Mild MR	2 (50%)	9 (42.9%)	10 (45.4%)	6 (46.1%)
Mild AR	0 (0%)	3 (14.3%)	6 (27.3%)	3 (23.1%)
Mild MS	0 (0%)	1 (4.8%)	1 (4.5%)	0 (0%)
IVS > 12mm	2 (50%)	6 (28.6%)	14 (63.6%)	7 (53.9%)
LVEDd >56mm	0 (0%)	3 (14.3%)	3 (13.6%)	0 (0%)
LA > 40mm	2 (50%)	9 (42.9%)	8 (36.4%)	5 (38.5%)
Concentric hypertrophy of LV with diastolic dysfunction of the type-impaired relaxation	1 (25%)	3 (14.3%)	12 (54.5%)	3 (23.1%)
Impaired global systolic function of LV	0 (0%)	3 (14.3%)	2 (9.1%)	0 (0%)
Abnormal left ventricular kinetics	0 (0%)	4 (19.0%)	3 (13.6%)	0 (0%)

COPD: chronic obstructive pulmonary disease; GOLD: Global Initiative for Chronic Obstructive Lung Disease; PH: pulmonary hypertension; TR: tricuspid regurgitation; PR: pulmonary regurgitation; RVEDd: right ventricular end-diastolic dimension; RA: right atrial; MR: mitral regurgitation; AR: aortic regurgitation; MS: mitral stenosis; IVS: interventricular septum; LVEDd: left ventricular end-diastolic dimension; LA: left atrial; LV: left ventricle.

Registered echocardiographic changes in the subjects of Group 1 classified according to the

severity of airflow limitation are presented in Table 2. The most frequent abnormality of the right heart was increased RVEDd, whereas dilated left atrium and left ventricular hypertrophy were the most frequent left heart abnormalities. Also, TR was the most frequent valvular abnormality.

Comparison of echocardiographic findings indicated a significant difference between right heart parameters in the Group 1 as compared to their values in the Group 2. Also, except interventricular septum, there was a significant difference between the two groups (Table 3).

Table 3: Comparison of certain echocardiographic parameters in the two groups

Echocardiographic parameter	Group 1 (n = 60)	Group 2 (n = 60)	P value
LA (19.0-40.0 mm)	39.0 ± 5.3	36.4 ± 3.5	*P* = 0.0019
LV-s < 39 mm	33.6 ± 6.4	30.7 ± 2.4	*P* = 0.0013
RVEDd (7.0-26 mm)	28.0 ± 4.8	24.4 ± 4.3	*P* = 0.0000
LVEDd (35.0-56.0 mm)	50.05 ± 5.8	46.2 ± 4.1	*P* = 0.0000
IVS (7.0-12 mm)	11.1 ± 1.9	11.4 ± 1.2	*P* = 0.1032
LVEF%	57.4 ± 6.9	64.8 ± 2.7	*P* = 0.0000

LA: left atrial dimension; LV-s: left ventricular systolic dimension; RVEDd: right ventricular end-diastolic dimension; LVEDd: left ventricular end-diastolic dimension; IVS: interventricular septum; LVEF%: left ventricle ejection fraction.

As it is mentioned above, the mean value of the LVEF% in non-COPD controls was significantly higher compared to its value in COPD patients but there was no correlation with COPD severity (Table 4).

Table 4: LVEF% value about COPD severity

COPD severity	LVEF% value
GOLD 1	61.3%
GOLD 2	56.1%
GOLD 3	57.0%
GOLD 4	55.6%

COPD: chronic obstructive pulmonary disease; LVEF%: left ventricle ejection fraction; GOLD: Global Initiative for Chronic Obstructive Lung Disease.

Frequency of PH was significantly higher in COPD patients compared to its frequency in non-COPD controls (33.3% *vs.* 0%; *P* = 0.0004*).* Frequency of PH proportionally increased with FEV_1 decline (Table 5).

Table 5: Frequency of PH about the level of airflow limitation

COPD severity	Frequency of PH
GOLD 1	0%
GOLD 2	23.8%
GOLD 3	41.0%
GOLD 4	46.1%

COPD: chronic obstructive pulmonary disease; PH: pulmonary hypertension; GOLD: Global Initiative for Chronic Obstructive Lung Disease.

Discussion

Comorbidities such as cardiac disease, peripheral vascular disease, hypertension, metabolic syndrome, diabetes mellitus, osteoporosis, and psychological disorders are commonly presented in patients with COPD with great variability in reported prevalence. Besides, cardiovascular comorbidities are considered as an important cause of mortality in COPD patients [5], [6].

The present study aimed to compare the frequency of echocardiographic changes registered by resting 2D transthoracic Doppler echocardiography in COPD patients and non-COPD controls and to assess their relation to the level of airflow limitation. We performed a cross-sectional study including 120 subjects divided into two groups. The first group included 60 initially diagnosed with COPD, whereas the second group included an equal number of subjects in whom COPD was excluded complementary by their demographic characteristics to the patients with COPD. Airflow limitation in more than half of the newly diagnosed COPD patients was assessed as severe or very severe indicating delayed recognising of the disease and late-onset of adequate treatment.

Resting 2D Doppler echocardiography was done by the same cardiologist and valvular anatomy and function, left, and right ventricle size and cardiac function were assessed.

Values of the RV parameters were significantly higher in COPD patients. There was a high prevalence of PH, right atrial enlargement, RV systolic dysfunction and tricuspid regurgitation in COPD patients, and their severity increased with COPD severity. Cor pulmonale was found in 16.7% of our COPD patients which correlates with 17.5% in Gupta et al., a study [29].

TR peak gradient as a marker for indirect evidence of PH was studied in all patients. TR in the present study was found in 44.4% of the COPD patients, that is less than its frequency in the studies performed by Higham et al., (77%) and by Kassim et al., (70%) [30], [31]. The difference can be explained by several factors like the type of equipment used, the experience of the operator, the quality of the image obtained or the body habitus. Impairment of RV function and alteration of pulmonary blood vessels complicate the clinical course of COPD and correlates inversely with the survival of these patients [7].

LV systolic function was significantly higher in non-COPD controls as compared to the COPD patients. Frequency of the LV systolic dysfunction was 5.5% in the COPD patients, that was similar to its frequency reported in the Gupta study (7.5%). Abnormal LV performance in the patients with COPD may be due to hypoxemia, acidosis, concurrent coronary artery disease, and ventricular interdependence as the RV and LV share a common septum [29].

Two major factors implicated in the mortality of COPD are the severity of PH and the development of cor pulmonale. Cor pulmonale reduces the survival of up to 30% [32]. Although the true prevalence of PH in COPD is still unknown, an elevation of pulmonary

arterial pressure is reported in 20-90% of patients when measured by right heart catheterisation [29]. The level of PH has a prognostic value for COPD patients that is demonstrated in several studies. In one of them, the 5-year survival rates were 50% in patients with mild PH (20-30 mmHg), 30% in those with moderate to severe PH (30-50 mmHg), and 0% in the small group of patients with severe PH (> 50 mmHg) [30]. As a conclusion, the high level of PH is associated with poor prognosis in COPD patients [7].

PH was not detected in any subject from Group 2, whereas its frequency among COPD patients was 33%. PH frequency reported in the studies conducted by Kassim et al., and Rabab et al., was 36% and 55.6%, respectively [31], [32]. Distribution of PH in patients with moderate, severe, and very severe COPD in the present study was 23.8%, 41% and 46.1%, respectively. Increased PH prevalence following increasing level of airflow limitation is also reported in the study conducted by Gupta & Mann and Rabab i.e. the reported prevalence of PH in patients with mild, moderate, severe, and very severe COPD was 16.7%, 54.5%, 60.0% and 83.3%, respectively [29], [33].

The findings of this study are subjects of at least three limitations. Firstly, the relatively small size of the study groups may have implications on data obtained and its interpretation. Secondly, the irregular distribution of the patients with COPD by the level of airflow limitation may also impact data obtained and its interpretation. Besides, the distribution of the study subjects by sex is also unequal. On the other side, the strength of the study is echocardiographic assessment in the initially diagnosed COPD patients.

In conclusion, in a cross-sectional study on the frequency of echocardiographic changes in newly diagnosed COPD patients and their relation to the level of disease severity we found a significantly higher prevalence of echocardiographic changes in COPD patients than in non-COPD controls which were significantly related to the level of airflow limitation. Our findings indicated that echocardiographic assessment should be a constitutive part of a periodic screening of all patients with COPD to implement a combined therapeutic strategy which should reduce morbidity and mortality in these patients.

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Clinical Science

Changes of Left Ventricular Systolic Function in Patients Undergoing Coronary Artery Bypass Grafting

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Abstract

AIM: This prospective study was designed to evaluate the changes in left ventricular (LV) systolic function after coronary artery bypass grafting (CABG) in patients with both normal and abnormal pre-operative systolic function.

METHODS: During the period from October 2017 to October 2018, forty-seven consecutive patients undergoing CABG were enrolled in this prospective study. Transthoracic echocardiography was performed within 1 week before CABG as well as 4 to 6 months after surgery. All measurements were made by a single experienced investigator.

RESULTS: While the mean LV ejection fraction (LVEF) showed neither improvement nor significant reduction in the whole group of patients following CABG (from 54.21 ± 15.36 to 53.66 ± 11.56%, p = 0.677), significant improvement in LVEF was detected in the subgroup of patients with pre-operative LV dysfunction (from 40.05 ± 8.65 to 45.85 ± 9.04%, p = 0.008). On the other hand, there was a significant decline in LEFT in the subgroup of patients with normal pre-operative LEFT (from 64.70 ± 9.72 to 59.44 ± 9.75%, p = 0.008). As for the other parameters of systolic function, significant decrease in LV end-diastolic volume index (LVEDVI) (p = 0.001), LV end-systolic volume index (LVESVI) (p = 0.0001), wall motion score index (WMSI) (p = 0.013) and LVmass index in male patients (p = 0.011) was shown only in patients with decreased LVEF after CABG. Patients with improved postoperative LVEF (53.2% of all patients) had significantly lower baseline LVEF (p = 0.0001), higher LVESVI (0.009) and higher WMSI (p = 0.006) vs patients with worsened postoperative LVEF (38.3% of all patients). Postoperative improvement of LVEF was correlated with stabile angina, lack of preoperative myocardial infarction and smoking, higher baseline WMSI, higher LV internal diameters and indexed volumes in diastole and systole and lower baseline LVEF. In stepwise linear regression analysis the value of baseline LVEF appeared as independent predictor of improved LVEF after CABG (B = 0,836%; 95% CI 0.655-1.017; p = 0.0001).

CONCLUSION: Our study showed that LVEF, internal baseline diameters and indexed volumes of LV in diastole and systole are important determinants of postoperative change in LVEF. In patients with preoperative depressed myocardial function, there is an improvement in systolic function, whereas in patients with preserved preoperative myocardial function, the decline in postoperative LVEF was detected.

Introduction

Patients with multivessel coronary artery disease (CAD), especially those with stenosis of the left main (LM) coronary artery and suitable coronary anatomy benefit from coronary artery bypass grafting (CABG) [1], [2], [3]. The goal of CABG is not only to allay symptoms and improve survival [4] but also to optimise cardiovascular function and prevent progressive remodelling. Coronary artery disease (CAD) lead's to left ventricular (LV) dysfunction as a result of myocardial scarring, stunning, or hibernation [5]. The impact of CABG on regional and global LV systolic function has been studied but with conflicting results, most probably because of patient selection. Most of the studies that evaluated the effect of CABG in patients with severe LV dysfunction showed significant improvement in LV ejection fraction (LVEF) and LV systolic parameters after revascularisation [3], [6], [7].

Moreover, those patients with ischemic symptoms and the most severe LV dysfunction appear to benefit most from surgical revascularisation. On the other hand, only a few retrospective studies evaluate the changes in LV systolic function after CABG in patients with preserved baseline LVEF. In these patients despite the apparent improvement in

cardiac function conventional echocardiography did not change significantly even showed a decline in baseline LVEF [8], [9]. Despite advances in cardiac imaging, we believe that 2-dimensional (2D) echocardiography is still most suitable for detection of myocardial function recovery after CABG to highlight the controversies. This prospective study was designed to evaluate the changes in LV systolic parameters after CABG in patients with both normal and abnormal pre-operative systolic function.

Methods

Study patients

During the period from October 2017 to October 2018, forty-seven consecutive patients undergoing CABG were enrolled in this prospective study. All procedures were done on-pump with cardiopulmonary bypass (CPB). In every case, the left internal thoracic artery (LITA) was used to bypass the left anterior descending artery (LAD). None of the patients had associated surgical procedures such as valve replacement or surgery of the ascending aorta. This study was approved by the Medical Ethics Committee of Medical School, University Ss. “Cyril and Methodius”, Skopje, and all patients provided informed consent.

2-D Echocardiography parameters

Transthoracic echocardiography was performed within 1 week before CABG as well as 4 to 6 months after surgery. All measurements were made by a single experienced investigator. Standard assessments of LV dimensions, wall thickness, LV mass, LV volumes as well as LV systolic function were performed in standard views using 2D echocardiography and Tissue Doppler imaging (TDI) on commercially available equipment (Vivid 7; GE, USA) according to the professional association recommendations [10].

Statistical analysis

Categorical parameters were summarised as percentages and continuous parameters as mean ± SD. Comparisons of preoperative vs postoperative data were performed using a Wilcoxon Signed Rank test for related samples. Continuous variables were compared using the nonparametric Mann-Whitney test for independent samples and categorical parameters were compared using Pearson’s chi-square test. Assessment of correlations was done using Pearson’s correlation analysis. Stepwise regression analysis was performed to define the independent significant predictive variable of postoperative LV ejection fraction. All data analysis was performed using SPSS version 25.0 (IBM SPSS, Inc., Chicago, Illinois), and p-value ≤ 0.05 was considered significant.

Results

Patient characteristics

The patients were divided into two subgroups: those with normal preoperative LVEF (LVEF > 50% n = 27) and those with abnormal LVEF (LVEF ≤ 50% n = 20). The baseline demographic and clinical characteristics of the patients as a whole and divided in subgroups, including coronary anatomy, and the postoperative course are shown in Table 1 and were similar in both subgroups.

Forty-three percent of patients had decreased LVEF (≤ 50%) at baseline. These patients had significantly lower body mass index, higher Euro SCORE 2 and more likely to have chronic kidney disease. There was no statistically significant difference in age, gender, prior myocardial infarction, SINTAX score and other comorbidities between the groups. Distribution of 3-vessel CAD and significant LM stenosis was also similar between the two groups. The number of bypassed vessels was not significantly different between the subgroup of patients with normal, and abnormal LVEF. The majority of patients received three bypass grafts (median 3, range 2—5), and in all patient, a left internal thoracic artery to the left anterior descending coronary artery bypass graft was used (Table 1).

Table 1: Baseline characteristics in the study population as a whole and comparison of demographic, clinical and operative characteristics of 47 patients divided according to the preoperative LVEF

Parameter	All patients N = 47	LEFT > 50% N = 27	LVEF ≤ 50% N = 20	p
Age (years)	65.55 ± 8.25	64.93 ± 7.74	66.40 ± 9.02	0.628
Gender (n/%)				
Male	35 / 74.5	18 / 66.7	17 / 85.0	0.154
Female	12 / 25.5	9 / 33.3	3 / 15	
BMI (kg/m2)	27.40 ± 4.38	28.99 ± 4.68	25.25 ± 2.86	0,004
Euro SCORE	2.17 ± 0.60	1.66 ± 0.91	2.61 ± 1.97	0.058
Angina, stable (n/%)	26 / 55.3	18 / 66.7	8 / 40	0.064
Previous MI (n/%)	25 / 53.2	12 / 44.4	13 / 65.0	0.135
Previous PCI (n/%)	15 / 31.9	7 / 25.0	8 / 40.0	0.306
Urgent CABG (n/%)	14 / 29.8	9 / 33.3	5 / 25.0	0.748
Preoperative AF (n/%)	2 / 4.3	1 / 3.7	1 / 5.0	0.828
COPD (n/%)	8 / 17.0	4 / 14.8	4 / 20.0	0.640
PVD (n/%)	6 / 12.8	2 / 7.4	4 / 20.0	0.201
CKD (n/%)	9 / 19.1	2 / 7.4	7 / 35	0.017
Smoking (n/%)	17 / 36.2	7 / 25.9	10 / 50,0	0.089
Hypertension (n/%)	47 / 100	27 / 100	20 / 100	-
Dyslipidemia (n/%)	46 / 97.9	26 / 96.3	20 / 100	0.384
Diabetes mellitus (n/%)	23 / 48.9	13 / 48.1	10 / 50.0	0.900
SYNTAX score	31.53 ± 6.58	31.48 ± 5.99	31.60 ± 7.46	0.612
Left main disease	19 / 40.4	11 / 40,7	8 / 40.0	0.599
LAD proximal disease	38 / 80.9	20 / 74.1	18 / 90.0	0.170
1 vessel disease	-	-	-	-
2 vessel disease	9 / 19.1	6 / 22.2	3 / 15.0	0.407
3 vessel disease	38 / 80.9	21 / 77.8	17 / 85.0	
Number of grafts	2.77 ± 0.72	2.85 ± 0.77	2.65 ± 0.67	0.523
Number of grafts per patient (n/%)				
1	1 / 1.2	-	1 / 5.0	0.597
2	15 / 31.9	9 / 33.3	6 / 30.0	
3	26 / 55.3	14 / 51.9	12 / 60.0	
4	4 / 8.5	3 / 11.1	1 / 5.0	
5	1 / 1.2	1 / 3.7	-	
CPB time (min)	108.91 ± 29.73	108.37 ± 28.37	109.65 ± 32.20	0.763
Ischemic time (min)	66.09 ± 20.03	65.52 ± 18.92	66.85 ± 21.92	0.698

CABG = coronary artery bypass graft surgery; MI = body mass index; ACS = acute coronary syndrome; MI = myocardial infarction; PCI = percutaneous coronary intervention; AF = atrial fibrillation; COPD = chronic obstructive pulmonary disease; PAD = peripheral vascular disease; CKD = chronic kidney disease; SYNTAX = SYNergy between percutaneous intervention with TAXus drug-eluting stents and cardiac surgery; CPB = Cardio Pulmonary Bypass; LAD = Left Anterior Descending.

Left ventricular myocardial function before and after CABG

Echocardiographic systolic parameters in the study group as a whole and in the subgroups of patients with normal and decreased LVEF before and after CABG are shown in Table 2.

In the study group as a whole there was statistically significant reduction in LVEDVI (p=0.001), LVESVI (p = 0.003), IVSd (p = 0.037) and WMSI (p = 0.016). There was a significant improvement in MAPSE (p = 0.001). Mean LVEF showed neither improvement nor significant reduction in the whole group of patients (from 54.21 ± 15.36 to 53.66 ± 11.56%, p = 0,677). There were no postoperative changes in other LV measurements including LVIDd, LVIDs, posterior and septal wall thickness, and LVmass index (Table 2).

When we divided our cohort according to the LVEF, significant improvement in LVEF was detected in the subgroup of patients with pre-operative LV dysfunction (from 40.05 ± 8.65 to 45.85 ± 9.04%, p = 0.008), resulting in a mean change in LVEF of 5.80%. On the other hand, there was a statistically significant decline in LVEF in the subgroup of patients with normal pre-operative LVEF (from 64.70 ± 9.72 to 59.44 ± 9.75%, p = 0.008), resulting in a meaningful change in LVEF of −5.26%.

As for the other parameters of systolic function, statistically significant decrease in LVEDVI (p = 0.001), LVESVI (p = 0.0001), WMSI (p = 0.013) and LVmass index in male patients (p = 0.011) was shown only in patients with decreased LVEF after CABG (Table 2).

Table 2: Comparison of echocardiographic parameters of LV systolic function before and after CABG in patients divided according to the preoperative LEFT

Parameters	All patients	p	LVEF > 50% N = 27	p	LVEF ≤ 50% N = 20	p
LVEF (%)						
Before CABG	54.21 ± 15.36	0,677	64.70 ± 9.72	0.008	40.05 ± 8.65	0.008
After CABG	53.66 ± 11.56		59.44 ± 9.75		45.85 ± 9.04	
LVIDd (mm)						
Before CABG	51.79 ± 8.76	0.981	47.52 ± 7.07	0.666	57.55 ± 7.51	0.678
After CABG	51.83 ± 8.34		48.07 ± 6.26		56.90 ± 8.23	
LVIDs (mm)						
Before CABG	33.96 ± 10.52	0.052	28.44 ± 7.50	0.241	41.40 ± 9.45	0.106
After CABG	32.26 ± 9.72		27.48 ± 7.24		38.70 ± 8.99	
IVSd (mm)						
Before CABG	13.06 ± 2.25	0.037	13.41 ± 2.27	0.111	12.60 ± 2.18	0.175
After CABG	12.34 ± 2.66		12.74 ± 2.55		11.80 ± 2.78	
PWd (mm)						
Before CABG	11.21 ± 2.04	0.345	11.56 ± 1.98	0.418	10.75 ± 2.07	0.681
After CABG	10.79 ± 1.98		11.07 ± 1.79		10.40 ± 2.21	
LVEDVI (ml/m2)						
Before CABG	64.54 ± 31.73	0.001	48.44 ± 19.88	0.107	86.29 ± 32.10	0.001
After CABG	54.41 ± 22.22		43.65 ± 14.62		68.94 ± 22.71	
LVESVI (ml/m2)						
Before CABG	33.89 ± 27.19	0.003	17.52 ± 10.60	0.614	55.99 ± 27.24	0.0001
After CABG	26.81 ± 17.24		18.12 ± 8.73		38.54 ± 19.05	
SVI (ml/m2)						
Before CABG	38.08 ± 9.99	0.804	38.97 ± 10.03	0.313	36.89 ± 10.07	0.455
After CABG	39.18 ± 10.56		38.30 ± 10.46		40.37 ± 10.85	
MAPSEavarage (mm)						
Before CABG	12.94 ± 2.26	0.001	13.81 ± 2.32	0.035	11.78 ± 1.58	0.008
After CABG	14.08 ± 2.08		14.98 ± 1.98		12.88 ± 1.57	
s'TDI (cm/s)						
Before CABG	6.04 ± 1.44	0.608	6.34 ± 1.67	0.695	5.65 ± 0.96	0.281
After CABG	6.12 ± 1.21		6.20 ± 1.32		6.02 ± 1.11	
WMSI						
Before CABG	1.34 ± 0.35	0.016	1.17 ± 0.25	0.615	1.57 ± 0.33	0.013
After CABG	1.23 ± 0.23		1.12 ± 0.14		1.37 ± 0.25	
LVmass index (g/m2)						
Male						
Before CABG	143.43 ± 39.26	0.017	129.04 ± 33.39	0.420	158.67 ± 40.14	0.011
After CABG	122.31 ± 24.14		122.87 ± 26.23		143.89 ± 32.41	
Female						
Before CABG	133.08 ± 30.85	0.347	113.84 ± 20.52	0.767	147.72 ± 15.15	0.109
After CABG	115.29 ± 26.82		107.95 ± 25.80		137.31 ± 17.97	

CABG = coronary artery bypass graft surgery; CI = cardiac index; IVSd = septal wall thickness; LVEDVI = left ventricular end-diastolic volume indexed to body surface area; LEFT = left ventricular ejection fraction; LVESVI = left ventricular end-systolic volume indexed to body surface area; LVIDd = left ventricular end-diastolic dimension; LVIDs = left ventricular end-systolic dimension; MAPSE = mitral annular plane systolic excursion; PW = posterior wall thickness; s'TDI = peak systolic mitral annular velocity by TDI; SVI = systolic volume indexed to body surface area; WMSI = wall motion score index; *p < 0,05 for comparison between groups.

The only parameter that significantly improved in both groups after CABG was MAPSE (p = 0,035, and p = 008 in patients with preserved and reduced LVEF respectively). Except for MAPSE, none of the systolic echocardiographic parameters improved in the subgroup of patients with preserved LVEF (Table 2).

Parameters related to LVEF change post CABG surgery

In our study, out of 47 patients, 4 patients (8.5%) had unchanged LVEF (+ / -5%) after successful CABG operation, 25 patients (53.2%) had increased LVEF (> 5%) and 18 patients (38.3%) had decreased in the postoperative LVEF (> 5%). Comparison of the three groups (Table 3) showed an only significant difference between patients with improved and decreased postoperative LVEF. Thus, patients with improved postoperative LVEF had significantly lower baseline LVEF (p = 0.0001), higher LVESVI (0.009) and higher WMSI (p = 0.006) *vs* patients with worsened postoperative LEFT.

Table 3: Baseline echocardiographic parameters of all patients about perioperative change in left ventricular ejection fraction

Parameter	Unchanged EF N = 4	Improved EF N = 25	Worsened EF N = 18	p
LVIDd (mm)	48.0 ± 3.9	53.7 ± 9.3	49.8 ± 8.3	0.243
LVIDs (mm)	29.0 ± 4.2	37.1 ± 11.6	30.7 ± 8.6	0.089
IVSd (mm)	13.2 ± 2.9	12.9 ± 2.4	13.1 ± 1.8	0.945
PWd (mm)	10.2 ± 1.5	10.8 ± 2.3	11.9 ± 1.5	0.173
LVEDVI (ml/m2)	64.1 ± 34.1	74.0 ± 33.6	51.4 ± 24.7	0.069
LVESVI (ml/m2)	25.7 ± 11.3	44.9 ± 30.9	20.3 ± 15.5	0.009 *Improved vs. Worsened* 0.0001
LVEF (%)	56.7 ± 5.1	46.6 ± 14.9	64.1 ± 11.2	*Improved vs. Worsened*
SVI (ml/m2)	36.5 ± 6.0	37.9 ± 10.2	38.6 ± 10.7	0.930
CI (L/min/m2)	2.7 ± 0.8	2.5 ± 0.7	2.6 ± 0.7	0.888
MAPSEavarage (mm)	14.1 ± 2.6	12.4 ± 1.9	13.2 ± 2.5	0.277
s'TDI (cm/s)	5.4 ± 0.9	5.9 ± 1.5	6.2 ± 1.4	0.544
WMSI	1.3 ± 0.2	1.4 ± 0.4	1.3 ± 0.3	0.006 *Improved vs. Worsened*

CABG = coronary artery bypass graft surgery; CI = cardiac index; IVSd = septal wall thickness; LVEDVI = left ventricular end-diastolic volume indexed to body surface area; LEFT = left ventricular ejection fraction; LVESVI = left ventricular end-systolic volume indexed to body surface area; LVIDd = left ventricular end-diastolic dimension; LVIDs = left ventricular end-systolic dimension; MAPSE = mitral annular plane systolic excursion; PW = posterior wall thickness; s'TDI = peak systolic mitral annular velocity by TDI; SVI = systolic volume indexed to body surface area; WMSI = wall motion score index; *p < 0.05 for comparison between groups.

Postoperative improvement of LVEF was correlated with stabile angina, lack of preoperative myocardial infarction and smoking, higher baseline WMSI, higher LV internal diameters and indexed volumes in diastole and systole and lower baseline LVEF (Table 4).

Table 4: Correlation between the change of LVEF and preoperative parameters

Parameters	ΔLVEF
Angina (%)	R = 0.386; p = 0.007
Previous MI (%)	R = -0.288; p = 0.049
Smoking (%)	R = -0.319; p = 0.029
LVIDd (mm)	R = -0.294; p = 0.045
LVIDs (mm)	R = -0.404; p = 0.005
LVEDVI (ml/m2)	R = -0.467; p = 0.001
LVESVI (ml/m2)	R = -0.557; p = 0.0001
LVEF	R = 0.652; p = 0,0001
WMSI	R = -0.480; p = 0.001

To determine the independent predictors of improvement of LVEF after CABG, we performed multiple stepwise linear regression analysis with covariates that showed a significant relation to it. The results demonstrated that the value of baseline LVEF appeared as an independent predictor of improved LVEF after CABG (Table 5, Figure 1).

Table 5: Stepwise regression analysis of LVEF after CABG as the dependent variable and clinical and echocardiographic parameters as independent variables in cases for which LVEF improved

Coefficients[a,b]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	14,624	4,281		3,416	,002	5,769	23,479
	LVEF before CABG	,836	,088	,894	9,545	,000	,655	1,017

a. Dependent Variable: LVEF post CABG

b. Selecting only cases for which LVEF improved = improved

Thus, for every 1% absolute decrease in pre-operative LVEF, there is postoperative improvement of LVEF of 0,836% (95% CI 0.655-1.017; p = 0.0001).

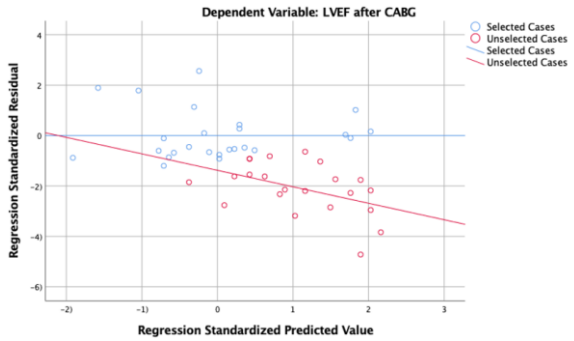

Figure 1: Graphical presentation of regression standardised predicted value for LVEF after CABG as the dependent variable in cases for which LVEF improved

Discussion

CABG surgery can improve the myocardial blood supply in the hibernating regions of the heart. This results in increased contractility and better performance of the myocardium [11], [12]. The 2D biplane echocardiography is a widely used method to obtain pre and postoperative systolic parameters of the right and left ventricle in patients undergoing CABG surgery.

In this study, the parameters of LV ejection fraction, LV internal dimensions and LV indexed volumes in systole and diastole, cardiac index, mitral annular plane systolic excursion, wall motion score index and LV mass index obtained with conventional 2D echocardiography were used to assess the global systolic function in patients with CAD undergoing CABG.

We showed deterioration in LVEF after CABG in patients with normal baseline LVEF. Except for MAPSE, other systolic parameters did not change significantly even after successful CABG treatment in patients with preserved baseline LVEF. On the other hand, an improvement in LV systolic function was observed in patients with decreased pre-operative LVEF. There were significant improvement in LVEF, LV systolic and diastolic indexed volumes, WMSI and LV mass index in this subgroup of patients.

Prior studies have similarly found an improvement in LVEF and other systolic parameters in patients with pre-operative LV systolic dysfunction. In the largest prospective, randomised, controlled trial, the STICH trial, Michler et al., [13] in a post hoc subgroup analysis showed a significant improvement in LV size and function in the subgroup of patients with higher baseline LV end-systolic dimensions. Our study also showed improvement in LVEF in the subgroup of patients with preoperative LV systolic dysfunction and higher baseline internal diameters as well as indexed volumes of LV in diastole and systole.

While many studies evaluate changes of perioperative systolic parameters in patients with reduced LVEF, only a few studies examine changes in LV systolic function in patients with preserved baseline LVEF. In a small prospective study, Diller et al. demonstrated improvement in LV diastolic function and did not find a significant reduction in LVEF immediately after CABG [14]. In the largest study to assess pre and post-operative echocardiograms in a population including both normal and reduced pre-operative LV function, Koene et al., [8] showed a decrease in LVEF with CABG in patients with normal baseline LV systolic function. In this study, the magnitude of decrease in LVEF was 3% mean and ranged from -33% to 15%. Our study is in agreement with these findings demonstrating a decrease in LVEF in patients with preserved baseline LVEF resulting in a mean decrease in LVEF of 5.26%. This postoperative decrease in LVEF might result from myocardial stunning [15], reperfusion injury [16] and early postoperative graft failure [17].

In our study, a total of 18 patients (38.3%) had decreased in the postoperative LVEF (> 5%). This suggests that CABG itself contributes to postoperative myocardial dysfunction. Although these patients were angina free 4-6 mounts after CABG, the relative decline in LVEF suggests that myocardial recovery might take longer time. We strongly believe that these results are worthy of further investigation to understand the effect of CABG on myocardial function. Another issue that should be investigated is whether the lack of improvement of LVEF post-CABG portends a worse outcome.

The major limitations in our study are that we used only conventional 2-D echocardiography imaging to assess pre and postoperative systolic LV function. Other technologies such as magnetic resonance

imaging, positron emission tomography and speckle tracking imaging might have yielded other results, but 2-D echocardiography is a widely used method for quantifying perioperative LV function. This study has the advantage of being prospective and all consecutive patients that met inclusion criteria were enrolled in the study but we believe that their number is too small and is thus hypothesis-generating rather than definitive. Another disadvantage is that paired echocardiograms were done a maximum of 6 months after surgery, time that might be too short for complete myocardial recovery after surgery. In our study, all patients were done on the pump with crystalloid cardioplegia and this might affect postoperative LV function in a certain percentage of patients.

In conclusion, our study showed that LVEF, internal baseline diameters and indexed volumes of LV in diastole and systole are important determinants of postoperative change in LVEF. In patients with the preoperative depressed myocardial function, we should expect improvement in systolic function, whereas in patients with preserved myocardial function, decline in postoperative LVEF should be anticipated, despite successful CABG. The present study suggests further investigations in order to understand the effect of CABG on myocardial function.

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Medication Errors and Their Relationship with Care Complexity and Work Dynamics

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Abstract

BACKGROUND: Medication errors are currently known as the most common medical errors. Research shows that work environment and organisation management, in addition to the role of nurses, contribute to the occurrence of an error.

AIM: Therefore, the present study was conducted to determine the rate of nurses' medication errors and its relation to the care complexity and work dynamics in the Taleghani Pediatric Hospital of Gorgan in 2017.

MATERIAL AND METHODS: This was a descriptive-correlational and cross-sectional study. Sampling was done through census method (N = 100). The data collection tools consisted of four questionnaires of demographic information, Salyer work dynamics, Medication Administration Errors, and Velasquez Nursing Care Complexity. Data were analysed in SPSS V.16 software using descriptive and inferential statistical methods including independent t-test and Pearson's correlation.

RESULTS: Medication calculation errors, wrong dose and wrong medication were the most common non-injectable medication errors, respectively. Drug incompatibility, wrong infusion rate and medication calculation errors were the most common injectable medication errors, respectively. There was a positive correlation between medication calculation errors (P = 0.02, r = 0.23), wrong solvent (P = 0.04, r = 0.21), and drug incompatibility (P = 0.01, r = 0.25) with amount of work dynamics. Also, there was a positive correlation between medication calculation errors (P = 0.03, r = 0.22) and wrong medication (P = 0.00, r = 0.31) with the nursing care complexity.

CONCLUSION: Regarding the irrefutable impact of working conditions on the occurrence of errors, it appears that the study and complete recognition of nurses' working conditions and their adjustment would lead to a reduction in medication errors.

Introduction

Medication errors are currently known as the most common medical errors [1]. The US National Coordinating Council for Medication Error Reporting and Prevention (NCC MERP) defines medication errors as follows: "A medication error is any preventable event that may cause or lead to inappropriate medication use or patient harm while the medication is in the control of the healthcare professional, patient, or consumer"[2]. Studies show that medication errors occur three times more frequently in admitted children than adults, and most of these errors are harmful [3]. Because of the wide variation in body mass, children need a unique dosage calculation based on their weight, body surface, age, and clinical status, which increases the risk of medication errors [4]. Reports from the National Patient Safety Agency (NPSA) in the UK found that medication errors that occur during drug treatments include 16% prescription writing errors, 18% prescription preparation errors and 50% medication administration errors. These errors are estimated at 3-37% prescription errors, 5-15% prescription preparation errors and 72-75% medication administration errors for children [5].

In studies on the type of medication errors, the wrong dose was the most common type of medication error in children [6], [7] while using the wrong medication was the most common medication

error in adults [8]. These findings have been approved by studies conducted in Iran [9], [10], [11], [12]. Based on developed modules of medication errors, errors arise from the interaction among several different factors, including the administrative environment (monitoring), leadership and organisational commitment, management policies and procedures, the complexity of tasks, work culture, and physical environment [13], [14], [15]. Recent studies revealed that the wrong medication was the most common error of injectable medications and 51.5% of the errors were related to the work environment [16]. It was also identified that better nursing work conditions lead to a lower frequency of medication errors [17] and providing proper organisational conditions and work environment can help nurses provide high-quality care based on professional standards. Therefore, work environment conditions can facilitate or hinder nursing [18]. Work dynamics and care complexity were considered as work environment factors in preceding studies [13], [15].

Nurses' activity in the hospital environment is physically and psychologically difficult and can lead to burnout, stress, and error. Busy work environments can contribute to stress in employees, which increases the risk of medication errors [14]. On the other hand, effective and safe medication management for children ensures good health and provides social and economic benefits [19]. Therefore, one of the important aspects of effective and safe medication management is the evaluation of medication errors and their properties to identify and implement preventive strategies [20], [21].

Work environment and its variables can vary in different care centers and consequently the amount and type of medication errors can also vary, this study aimed to determine the rate of medication errors of nurses and its relationship with care complexity and work dynamics in Taleghani Pediatric Hospital of Gorgan in order to provide the grounds for understanding the work environment of nurses in pediatric units and to take an effective step toward defining and implementing preventive strategies of medication errors in pediatric units.

Material and Methods

This study was a descriptive-correlational, cross-sectional survey. The study population consisted of all nurses working in different units of Taleghani Pediatric Hospital (N = 100). Sampling was done through census method, and all nurses who had inclusion criteria participated in the study. The inclusion criteria were: The tendency and consent of nurses to enter the study, employment at the time of the studies, having at least six months of work experience in the pediatric units, having at least a bachelor's degree, and working in different working shifts. Data were collected by self-report and questionnaires. Data collection tools were a demographic questionnaire (age, sex, educational level, type of employment, work experience, ward, number of working shifts), Salyer Work Dynamics Scale, Velasques Nursing Care Complexity Scale and Medication Administration Error Questionnaire.

The 7-item Salyer's was used to measure the work dynamics of nurses. This tool is scored base on a 6-point Likert scale (strongly disagree to agree strongly) with a total score ranging between 7 and 42. A low score indicates a lack of dynamics and a high score indicates high dynamics. The content, structure and face validity of the scale have been approved in the studies. In Iran, a study by Pazoukian et al. (2014) reported its high reliability (Cronbach's alpha coefficient = 0.81) and stability over time [22]. In the present study, the Cronbach's alpha coefficient was 0.80.

Care complexity scale was used to measure this variable in the nurses' work environment. This scale was developed by Dona Maria Velasques in 2005 and had 15 items scored based on a 4-point Likert scale (never to always) with a total score ranging from 15 to 60. In Iran, Pazoukian et al., (2015) reported its reliability with Cronbach's alpha of 0.61, and its correlation coefficient using test-retest as r = 0.88, indicating that the scale has acceptable validity and reliability for use in Iran's health system [23]. In the present study, the Cronbach's alpha coefficient for consistency was 0.77.

The Medication Administration Error questionnaire was used to determine the amount and type of medication errors. The first part provides information on the number of medication errors occurring in the past 6 months and the working shift with the highest number of mistakes. The second part of the questionnaire consists of the questions related to the type of medication errors categorized into two groups of non-injectable medication errors (9 items) and injectable medication errors (12 items) scored by the percentage of medication errors (0-25, 25-50, 50-75 and 75-100) with total score ranging between 0 and 100. The content validity and reliability of the scale have been approved in different studies [11], [12]. In the present study, the questionnaire's Cronbach's alpha coefficient was 0.88.

The researchers started collecting data after obtaining the approval of the Ethics Committee of the Golestan University of Medical Sciences (the ethics code of IR.GOUMS.REC.1395.207) and necessary permits, and coordinating with the research setting authorities. After briefing the participants about the research goals and their rights, and answering their questions, written informed consent was obtained from them. The participants were assured that their information would be confidential. Data were analysed in SPSS v. 16 using descriptive and inferential

statistics. That is, nominal qualitative variables were described by frequency distribution and quantitative variables by the mean and standard deviation. Pearson's correlation and student t-test were used to analysing the data, taking into account a significance level of less than 0.05.

Results

A total of 91 nurses from different parts of the Taleghani Pediatric Hospital were enrolled. Demographic characteristics of the study participants are presented in Table 1.

Table 1: Frequency distribution of demographic characteristics of the nurses in Taleghani Pediatric Hospital in 2017

Variable	Classification	Frequency	Percent
Age (years)	< 35	42	48.27
	> 35	45	51.73
Marital status	Single	18	20
	Married	72	80
Educational level	Bachelor's degree	90	98.9
	Master's degree	1	1.1
Employment Status	Permanent (official-permanent contract)	48	53.93
	Temporary (obliged service, temporary contract, through service companies)	41	46.07
Working shifts	Fixed	14	16.5
	Rotating	71	83.5

The most common types of medication errors for non-injectable medications were respectively medication calculation errors (37.25 ± 09.37), wrong dose (34.22 ± 89.01), wrong medication (30.15 ± 49.72) and wrong time (30.15 ± 22.59). Furthermore, the most common types of medication errors for injectable medications were respectively drug incompatibility (43.29 ± 41.09), wrong infusion rate (43.26 ± 13.37), medication calculation errors (38.25 ± 46.08), wrong dose (38.24 ± 46.80) and wrong solvent (37.24 ± 91.54) (Table 2).

Table 2: The mean and standard deviation of types of errors in injectable and non-injectable medication in the Taleghani Pediatric Hospital in 2017

Non-injectable medication errors	Mean and standard deviation (percent)	Injectable medication errors	Mean and standard deviation (percent)
Wrong administration technique	27.75 ± 16.85	Wrong administration technique	28.85 ± 16.21
Wrong Time	30.22 ± 15.59	Wrong Time	29.67 ± 12.86
Wrong patient	27.20 ± 9.60	Wrong patient	29.40 ± 12.68
Wrong medication	30.49 ± 15.72	Wrong medication	31.32 ± 13.74
Wrong Dose	34.89 ± 22.01	Wrong dose	38.46 ± 24.80
Medication calculation errors	37.09±25.37	Medication calculation errors	38.46±25.08
Administration without a physician's order	25.27±4.55	Administration without a physician's order	28.02±15.29
Administration after a physician's order to discontinue the medication	29.12 ± 14.07	Administration after a physician's order to discontinue the medication	28.85 ± 13.90
Medication administration to a newborn with a known allergy	28.02 ± 26.57	Medication administration to a newborn with a known allergy	25.55 ± 6.43
-	-	Wrong solvent	37.91 ± 24.54
-	-	Wrong infusion rate	43.13 ± 26.37
-	-	Drug incompatibility	43.41 ± 29.06

The results showed no significant relationship between demographic characteristics of study participants and the number and type of medication errors. However, there was a significant correlation between types of shift work (fixed and rotating) and the wrong medication error (non-injectable) (P-value = 0.009). That is, this error occurred more in the rotating shift schedule.

The mean number of medication errors by nurses during the past six months was less than once (0.84). Furthermore, most nurses (59.68%) reported that most of the medication errors occurred in the morning, evening and morning-evening shifts, respectively, as the administration without a physician's order (P-value = 0.03) for injectable medications, and wrong dose (P-value = 0.05) for non-injectable medications had a significant relationship with these working shifts.

The results revealed a relatively high level of work dynamics (28.94 ± 6.24) and the care complexity (43.75 ± 5.004) in the study units.

A positive correlation exised in the relationship between work dynamics and medication calculation errors (r = 0.23, P = 0.02) wrong solvent (r = 0.21, P = 0.04) and drug incompatibility (r = 0.25, P = 0.01). Also, a positive correlation showed in the relationship between care complexity and the medication calculation errors (r = 0.22, P = 0.03) and wrong medication (r = 0.31, P = 0.00).

Discussion

Today, patients' safety is one of the most important goals of health systems and based on that goal, reducing the incidence of adverse events and medical errors has received a lot of attention as it is the most important one.

According to the results of the study, medication calculation errors, wrong dose, wrong medication and wrong time were the most common types of non-injectable medication errors by nurses, respectively. In line with these results, Ramezani et al. also showed that the most common types of non-injectable medication errors in the neonatal unit and NICU were medication calculation errors and wrong dose, respectively [24].

In the present study, drug incompatibility, wrong infusion rate, medication calculation errors, wrong dose, and wrong solvent were the most common types of injectable medication errors by nurses, respectively. Consistent with the results of the present study, several studies reported wrong dose, wrong infusion rate and wrong solvent as the most common injectable medication errors [25], [26], [27].

In the present study revealed that the wrong

medication error (non-injectable)occurred more in the rotating shift schedule. Also, the results of the study showed that the rate of the administration without a physician's order for injectable medications and wrong dose for non-injectable medications significantly increased in the morning, evening and morning-evening working shifts, respectively. In line with these results, Bagheri Nesami et al., reported that the highest non-injectable medication errors (34.5%) occurred at evening shift, (33.1%) at morning shift (32.4%) and night shift [16]. In a study by Mohammadi et al., in Kermanshah teaching centres, most medication errors occurred at morning shift [28]. Unlike the results of the present study, Yousefi et al., showed that the mean number of medication errors was higher at night shift than that of the morning shift, and there was no difference between the morning shift and the rotating shift [29]. Seki et al. did not report significant differences in the occurrence of medication errors between the three working shifts [30]. It appears that the reason for these inconsistent results can be due to different policies in different hospitals about the ratio of nurses to patients, the duration of each shift, the number of personnel in each unit, and how the forces are assigned to each shift concerning background and work experience.

The results of the present study showed that an increase of work dynamics in the study units is accompanied by an increase in the number of medication calculation errors, wrong solvent, and drug incompatibility. Contrary to these results, Chang & Mark showed that in an appropriate work environment with a high dynamic, a low degree of distraction and confusion, the probability of medication errors is relatively lower [31]. Therefore, the occurrence of these medication errors in an environment that is highly dynamic and desirable can be due to other reasons, such as lack of pharmacological knowledge, inappropriate communication among the members of the treatment team and low work experience. Developed medication errors model in recent studies show that error-producing conditions include work environment, team, and individual factors, in which, care complexity and work dynamic are the work environment factors, the physician-nurse relationship is the team factor, and age and nursing work experience are the personal factors [32]. Also, this study released the greater care complexity in the study units resulted in a greater number of medication calculation errors and wrong medication. In line with these findings, the results of Chang and Mark study indicated that care complexity was positively associated with medication errors [31]. Jolaee et al. also reported a statistically significant relationship between the occurrence of medication errors and the working conditions of nurses such that the mean number of medication errors can be reduced by changing the working conditions from unfavourable to favourable conditions [33]. Other studies also reported inadequate staff in the unit, working load and fatigue as the main reasons for medication errors [24], [29], [34]. Inappropriate working conditions such as high workload, disorder, lack of staff, distraction, inappropriate patient-nurse ratio, and delayed execution of patient's medication orders can lead to increased medication errors [30], [35]. Complicated conditions make nurses spend more time taking care of the patient, and this, even with skilled staff, can lead to defects in the care process [36].

Limitations: The self-reporting nature of the questionnaire was a limitation. In this regard, researchers tried to gather real data by creating a friendly atmosphere and mutual trust, and by describing the objectives of the study. Also, the cross-sectional nature of the study cannot determine the causal relationships between the variables, so the interpretation of the findings of the study should be made with caution.

In conclusion, regarding the irrefutable impact of working conditions on the occurrence of errors, it appears that the study and complete recognition of nurses' working conditions and their adjustment would provide a basis for reducing medication errors. Nursing managers and health care providers should identify the causes of medication errors and implement strategies to reduce them. Medication errors occur more often in the children and infant units as the patients in these wards are more vulnerable and need more care. Therefore, paying attention to medication errors and the process of medication administration is highly important. It is suggested that studies be conducted on the occurrence of nursing medication errors in other units and their related factors. Therefore, an efficient error reporting and recording system may reduce medication errors by minimising reporting barriers.

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Experienced Psychosocial Problems of Women with Spouses of Substance Abusers: A Qualitative Study

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Abstract

BACKGROUND: Substance dependence is one of the most important social issues in the world today, which affects not only the life of an addicted individual, but also the relatives of the substance abuser individual, and in particular their spouses and children are also exposed to multiple injuries.

AIM: This study aimed to investigate the experiences of the women with the substance abuser spouse.

MATERIAL AND METHODS: This study is a qualitative study. Sampling was done purposefully. The data were subjected to 25 participants with in-depth and semi-structured interviews (15 wives and 7 therapists and 3 officials dealing with family members of the substance abuser) and analysed using the qualitative approach of the conventional content analysis (Granheim's method).

RESULTS: Out of the results of the interviews, two main categories were extracted, including A) family psychological breakdown and B) Disadvantaged social status. The main category of family psychological breakdown including 5 subcategories: Psychological disadvantages, losing borders in the family, insecure space house for the family, turbulent family and Concern on spouses leaving. Category of disadvantaged social status is also including 3 subcategories: trying to preserve the family's image, social stigma, and social isolation of the family.

CONCLUSION: The families, especially the women with the substance abuser spouse, are severely vulnerable in various psychological and social dimensions. Therefore, the attention of the health cares to these families is important.

Introduction

Substance-dependence is one of the main problems of today's societies that destroy the lives of millions, spending the high cost of the society on fighting, treating and harming it. Today, the problem of addiction goes beyond health care and has become a social crisis all over the world [1] .The World Health Organization in 2010 has estimated substance-dependent people to be about 230 million people around the world, accounting for about 5% of the world's population [2]. In Iran in 2017, the exact number of the addicts has reached two million, eight hundred and eight people and people aged 15-64 are the most substance abusers [3].

Substance-dependence generally causes serious harm to oneself, family and society, and also creates a high cost for the family and the country [4]. In the family system, the negative effects of the abuse are especially on the spouses, parents, and children of the substance abuser [5], and the family members may feel angry, frustrated, anxious, fearful, worried, depressed, ashamed and guilty [6]. When an individual in the family begins to change habits, practices, interactions, the family members are unwittingly attracted to him. Also, the family members are often negatively affected by the addict lifestyle and

feel unhappy, angry, frustrated and confused without knowing what happened. They worry why their loved one is acting differently, withdrawing, and does not his responsibility well. Therefore, it causes tension in the family and eventually, anxiety and depression in the family members [7].

Spouse of men with addiction is suffering about themselves, their lives, and the future of their family members. Their anxiety is the consequence of their mental occupation of the disintegration of their lives. They are anxious and depressed because of the pressures of homework, economic problems caused by addicted husband unemployment, and lack of intimate relationships. They are anxious and depressed because of the pressure of their housework, and the economic problems caused by the addicted spouse's unemployment and the lack of an intimate relationship with each other. Studies have generally shown that higher levels of alcohol and drug use and higher levels of substance-related problems increase the risk for partner aggression [8]. There is a relationship between the men's addiction and marital violence, and the substance provides a source of conflict and violence for the family members due to changes in the system [9].

Due to the stigma associated with substance abuse, most problems with substance abuse are hidden in the family. This not only worsens the experience of tension in the families but may also prevent the assistance and cooperation that the family can provide to improve the person with addiction [7]. People's attitude towards the spouses of the addicted men is a blatant and humiliating one, and people consider them the cause of the addiction of their husbands [10]. Therefore, these women are not only a victim of the addicted person in the family but also suffer greatly from the community. This often leads to feelings of guilt, shame, depression, anxiety, isolation, and suicidal tendencies. On the other hand, man's addiction makes the whole family more isolated and social relationships diminish. Relatives often do not come to their homes because of the poor economic situation and the unemployment of their husbands or the fear of their husband and son being addicted [11].

Some studies (Moriarty *et al.,* 2011; Salehi *et al.,* 2012) have shown that the family members of alcohol or substance consumers have more psychological and social problems [12], [13]. Also, in these people, the medical cost increases compared to the family members without having a person drinking alcohol or drugs [14]. Not only are the lives of the addicted individual, but also the relatives of the addict at risk of multiple injuries to this social phenomenon. So far, there has been no research on the psychological and social problems of women with substance abuser spouses, although there has been a lot of work on the addiction, all of these studies have been about violence and addiction itself and addicts, and again, the problem is that most of the studies conducted in this area have done little with the methodology. Only a few variables are used in this methodology [9], and few studies of the exact and profound aspects of life with a substance-dependent partner have been considered from their attitude.

Given that such information is not available about the effect of spouse's addiction on women's lives, and our main goal is to clarify the profound aspects of this issue and to do this, the questionnaire may not elicit the desired information. In other words, one cannot get a complete picture of what is happening through quantities of study. For this reason, this study is aimed to explore the experiences of women with substance abuser spouses in a qualitative way.

Methods

This study is a qualitative content analysis study with a conventional content analysis approach aimed to explore the experiences of women with spouses with substance use. This conducted in 2018. This way, information is obtained directly from the study participants, without imposing predetermined category or previous theoretical views, and the knowledge generated is based on their unique viewpoints, and actual text data and codes and category are extracted directly and inductively from raw data [15].

The research environment included government and private drug treatment centres in Gorgan, the provincial well-being centre, the provincial health centre and the Counter-Narcotics Headquarters Consulting Center. These centres are the most important ones for providing services to the families of people with substance abuse. The participants included women with substance abuser spouses, therapists and officials dealing with family members of the substance abuser, and inclusion criteria included being completely alert, and being able to communicate and share experiences verbally

A sampling of the present study was purposeful. In purposeful sampling, the selection of the participants with two criteria was sufficient to have sufficient knowledge about the phenomenon under study and to obtain good information [16]. Thus, for sampling, researcher referred to the addiction-related centres and interviewed people who met the criteria for entry into the study, Consider also the distribution of age, education level, number of children, type of substance abuse, duration of spouse's abuse, spouse’s job, and spouse's leaving history for maximum variation.

Data collection continued to the phase of data saturation. This means that the new participants did not make any new issue, and the information provided by them seemed to be repeated [17], which data were

collected for a period of 6 months. In this study, the data were saturated with 25 in-depth and semi-structured interviews with 15 spouses and 7therapists and 3 officials dealing with family members of the substance abuser.

Data Collection tool in this study was semi-structured interviews. The interviews were conducted in a private room in a secluded room and before the interview, the purpose of the interview, confidentiality of data, and the right to leave the interview at any time from the interview were individually explained to each participant. To create an open space and establish the right relationship, the interviewer first introduced himself and finally thanked the attendee for the interview. Oral consent was obtained from the participants.

The interview lasted from 25 to 100 minutes. During the interviews, spouses and staff were routinely asked: 'What has it affected your life since your spouse became addicted? Express your experiences. What has been the impact on your social relationships? (For spouses). Express your experiences of women's lives with spouses of the substance abusers (for therapists and officials).' Then, we used pointed questions (such as can you provide an example? Or can you explain more? Or what do you mean by this?) According to interviews' responses to add to the depth of the data.

The researcher allowed the participants to record the interview, and if they were not allowed to record, Interviews were written**.**

Data Analysis

Immediately after the interview, the participant's comments were reviewed and, if necessary, completed and the interviews were attempted on the same day for typing and analysing. At the same time as data collection, the analysis was also carried out. The content analysis method was used to analyse the data from individual interviews and to extract key codes. As a result, the problems of the women with the spouses of the substance abusers were categorised.

Analysis was done using the 5-step method for analysis of qualitative data described by Griesheim and Landman, as follows: 1. determining content of the analysis or unit of analysis; 2. determining meaning units; 3. condensed meaning units were abstracted and coded; 4. codes were compared based on similarities and differences and classified and subcategories5. Themes of the categories were specified and reflected the latent content of the text (main category).

The researcher immediately listened to the interviews several times after recording each interview, and after finding a general overview of them, the interviews were implemented and written in a word-for-word way, and handwritten notes were reviewed several times. Then the texts were read row by row, and the sentences and phrases were determined, and then the semantic units were extracted in the form of primary codes. In the following, the codes were categorised according to the semantic and conceptual similarity and compressed as little as possible, and the subcategory was formed, which were more comprehensive and abstract. The categorisation of the subcategories led to the formation of the main category [18].

The four criteria of credibility, dependability, conformability, and transferability, as described by Lincoln and Goba, were used for applying trustworthiness to the findings [19]. For this purpose, the researcher attempted to improve the credibility by remaining engaged with the participants and data collection process for a prolonged duration using various information sources, including spouses, nurses, doctors, psychiatrists and officers who were involved in the service centers of this group, also, after encoding each interview, a full version of the code, including the keywords and codes obtained to assess the compatibility of the codes with the participants' experiences, was shared with some of the participants (check member).

Discussion of the research team on conflicting findings and the achievement of the final consensus was also one of the other ways to secure the findings. To provide dependability to the findings, the researchers coded the transcripts of the interviews a few days after the initial coding and compared the results with the first coding, confirmed by achieving similar results and data stability and coordination. In order to enhance dependability and conformability, a part of the interview, along with its analysis, was provided to two external observers who were well experienced in both qualitative research and the subject studied. In order to achieve transferability, the results of the research were shared with several therapists and women whose spouses were Substance users and did not participate in the research, and the extent to which they were matched with their experiences.

Ethics

Ethical considerations were also observed in this research. Among them, informed consent from the participants to participate in the research and recording their interviews, the secrecy and confidentiality of the information was observed, and the right to withdraw from the interview was given to the participants. The text and interviews were kept in a safe place. This study was taken from the approved common project of Isfahan University of Medical Sciences and Golestan University of Medical Sciences that was approved by the Medical Ethics Committee of this university.

Results

Twenty-five 25 participants including 15 women with substance use spouses and 7 therapists including 3 nurses, 2 psychologists, 1 physician and 1 psychiatrist and 3 officials dealing with family members of the substance abuser including 1 province mental health official, 1 province well-being director and 1 Head of Counter-Narcotics Headquarters Consulting Center, participated in in-depth and semi-structured interviews. The participants' demographic characteristics are presented in Table 1).

Table 1: Participants' Demographic Characteristics

Participants	Number	Age Mean	Education
Spouses	15	36.6	Illiterate to bachelor
Service Providers and Officials	10	39.7	Bachelor to doctor degrees

Type of substances used by substance abuser was included: opium, drug Juice, crack drug glass drugs, and tramadol. Addiction period of substance abuser was approximately 3 to 20 years. Occupation of substance abuser was unemployed, worker, employee, self-employment and history of leaving of the substance abuse was approximately 0 to 5 times.

The results of 25 in-depth and semi-structured interviews of participant 121 initial (primary) codes, and 8 subcategories, finally 2 main categories were extracted, including 1) family psychological breakdown and 2) the disadvantaged social status shown in Table 2.

Table 2: Main categories and subcategories of the study of Psychosocial Problems of Women with Spouses of Substance Abusers

Main category/Subcategory
A) Family psychological breakdown
Psychological Disadvantages
Losing borders in the family
Insecure space house for the family
Turbulent family
Concern on spouses leaving
B) Disadvantaged social status
Trying to preserve the family's image
Social stigma
Family social isolation

The main category of the family psychological breakdown

Spouses of substance abusers participating in the study experienced widespread psychosocial fluctuations since their spouses' Substance abuse, which included 5 subcategories: psychological disadvantages, losing borders in the family, insecure space house for the family, turbulent family, concern on spouses leaving. All of these subcategories represent a gap in the psychological and emotional dimension of the family, which were categorised according to semantic similarity and fit into a more abstract category of “psychological breakdown”.

Psychological Disadvantages

Participants in this study stated stress, anxiety, and Depression, which primary codes of “stress”, “anxiety”, and “depression” considering their similarity and consistency in reflecting a Psychological and Emotional problem were placed in a more abstract subcategory of “psychological disadvantages”.

The participants in the study stated that due to their living conditions, they felt worried about their future and their children, that now their spouse is just thinking about their substance abuse, how the future of their children will be in terms of school and marriage. They also had stressed to carry out their activities and often felt as if they had the ability to work, and they were upset by the physical symptoms of stress.

"*Sometimes, I'm always stressed, then what it will happen, and I'm worried, these conditions make me unable to do my daily tasks*" (M-1 Spouse of the Family).

" *I'm worried about my children, that tomorrow they will grow up and one asks them that your father has such condition and that this will make them feel embarrassed*" (M5-Spouse of the Family).

Most participants in the study stated that they were anxious due to the inappropriate behaviours of their husband with them and their children in the family. They stated experiences such as anxiety about a sudden occurrence, a feeling of fear of punishing the children by the father and the feeling of constant and unreasonable concern and the physiological causes of it.

"*I'm always worried, and I'm always concerned, I say to myself what will happen. Because of this, my heart beats up; I sweat my hands and shake my feet*" (M1-Spouse of the Family).

"*I had a patient who always feared that she and her children behave and be beaten by her husband*.” (M20-Psychiatrist Center).

Participants also reported that their husband's addiction had a great impact on them psychologically. Shame, shyness, and social isolation were factors that caused depression in the women with substance abuse spouses.

"*I was once depressed, I felt disappointed, I was sad, I was bored with everything, I had no motivation in life, I cried very soon, I did not bear anything, I went to the psychologist for this reason, I had been a bit overwhelmed for a while*" (M3-Spouse of the Family).

"*The spouse who stated said that she wanted to fire herself or eat a pill and wanted to kill herself. We even had a woman who had fired herself and fired her whole body and hands*" (M21-Center physician).

Losing borders in the family

Most participants stated that the substance abuse had an effect on the behaviour of their husband, causing anger and lowered tolerance and aggressive behaviour (crying out loud, throwing things) which affected their spouses' behaviours. These experienced concepts of participants based on their similarities, assigned to more abstract categories with" losing borders in the family" label.

"*My husband was very brutal in the house, arguing for no reason and this was causing a seizure, he was crying out loud, he was throwing things*" (M-12 Spouse of the Family)

Participants also stated that these behaviours of their husbands led to aggressive behaviours in them.

"*My husband's behaviour has affected me so much; I'm nervous. I'll go to the furnace soon ... When I'm nervous, I'll beat them* " (M7-Spouse of the Family).

"*Here are women who take their husbands' medication; they are very aggressive so that the morale of their husbands also affects them*." (M 19-Center Nurse).

Insecure space house for the family

Participants in this study stated lack of security, uncontrolled behaviour by substance abusers, getting hurt, beat up to death, being attacked with a knife, which was categorised according to semantic similarity and fit into a more abstract category of "Insecure space house for the family".

"*We have an axe in the house when we go to the forest; we will take it, he (my husband) had taken it and wanted to kill us, saying that I had seen you as the sheep and wanted to kill you*" (M-12 Spouse the Family).

"*A woman said that some days my husband imprisoned me in the closet and he went outside and how many days I was jailed there, and I did not feel safe at all, or some days to death he beat me* " (M 16-Center Nurse).

Turbulent family

The participants experienced very severe psychological stress, to be tormented and tire that this subcategory formed.

"*I was so stressed out in this short period of my life that even when my dad I loved so much and lost him; I didn't feel so pressured*." (M15-Spouse of the Family)

"I had been a bit overwhelmed by the severity of my discomfort, and I get worn very soon "(M3-Spouse of the Family).

Concern on spouses leaving

The participants were concerned about the fact that their spouses did not leave successfully and that they increased their medication dosage, instead of reducing their drug use, and they were concerned while meeting with their old friends. These experienced concepts of participants based on their similarities assigned to more abstract categories with "Concern on spouses leaving "label.

"*Then, after a while, they were taking drugs, they increased their drugs a lot, instead of reducing their drug, which made it harder for anyone to take*" (M-16 Center Nursing).

"*The cases that a woman said her husband would leave ten times for the slave to leave, but her husband would not leave*" (M-23 Mental Health Official).

B) The main category of disadvantaged social status includes 3 subcategories: trying to preserve the family's image, social stigma and family social isolation. All of these subcategories were categorised according to semantic similarity and fit into a more abstract category of "disadvantaged social status".

Trying to preserve the family's image

The participants stated that they had to conceal the problem of their spouses' addiction because if anyone knew that their spouse was a substance abuser, they would no longer trust them to work outside the house. And that they were trying not to notice their neighbours or relatives that their husband was addicted and because of the fear of dishonour, they did what their spouses wanted and tolerated their spouse. These experienced concepts of participants were categorised into a more abstract category of "Trying to preserve the family's image".

"*I'm honest; I have to keep my appearance. I care about dressing myself because I work outdoors"* (M 13-Spouse of the Family).

"*My husband tells me to call Moslem (agency driver) to give me my drug. Now you want to make everyone understand that you are taking my drug*" (M 2-Spouse of the Family).

Social stigma

For the vast majority of the women, people's attitude towards the spouses of the addicted men is blamed, and people have a negative attitude and different viewpoint on them, and cases like embarrassment from abnormal behaviours and the physical condition of a spouse are the fingerprints of the people. They expressed a small sense of humiliation and distrust of the people.

These experienced concepts of participants based on their similarities assigned to more abstract

categories with “social stigma "label.

"*Most of the time he is sleepy, or we go somewhere, we get embarrassed, and when we have some guests, he is in a nap. I always tell him that no woman likes to be less likely than another woman because when people realise that their husband is an addict, they ask many questions, they humiliate, they have a different look*"(M-Spouse of the Family).

"*The clients are upset that no one trusts them, and they say that when we borrow money, they are not guaranteed to us, maybe because of the label that always has them*."(M22-province well-being director).

Family social isolation

From the viewpoint of most interviewees, relationships with relatives and acquaintances after men's addiction have declined, and even cut off in some cases. Participants expressed the lack of male presence at parties and the late attendance of men when accompanying them, limiting the woman’s relationship with relatives due to the instability of her husband’s behaviour. These experienced concepts of participants categorised according to semantic similarity and fit into a more abstract category of *"*Family social isolation*"*.

"My husband does not tend to be in contact with others due to his substance abuse. When he does not accompany us, most of the time, I do not like to go along with the children, I'm bored" (M-12 Spouse of the Family).

"Since my husband was sometimes talkative and he said unnecessary words, and others thought that he was in another phase or very frustrated and went in his phase, made me not be in touch with others and get away from others" (M11-Spouse of the Family).

Discussion

The results showed that most women with substance abuser spouse had experienced extensive psychological fluctuations since their spouses used the substances. In this regard, Nikbakht *et al.,* (2016) also stated in a phenomenological study that psychological vulnerability is the consequence of addiction to the family so that these women suffer from a lot of distressing feelings such as anger, sin and frustration. They are affected by these emotions along with many problems. Having an addict, especially spouses, can lead to an experience of life in a world of anxiety, worrying and confusion [9]. Along with this result, Chapsman *et al.* (2011) also stated in Australia that the addict's spouse faces multiple experiences. In the early stages, her spouse's addiction is unbelievable. After being confident in the addiction, the husband enters the resistance stage. She is blaming herself; she is gradually depressed and hates being alive. She hides her husband's addiction from others and always fears to disclose it among friends and relatives and is worried about being rejected by others. And even lose hope for life [20]. Kishor *et al.,* (2013) also found that over 65% of the women with drug use spouses had mental disorders, and early mood and anxiety disorders and about 43% of them had severe mood disorders [21].

The result of this study showed that most women with addicted spouses experienced some degree of "anxiety", "depression" and "stress". Along with this result, Mancheri *et al.,* (2013) also in a descriptive study of 400 addicted people showed that 36.4% of the spouses had moderate anxiety, 36.8% had moderate depression, 36% had low aggression, and 35.8% had a moderate interpersonal sensitivity [7].

When an individual addiction is identified in the family, the family members are affected by the function of the addict, and they experience high levels of stress and anxiety, which affect the lives of the members in all aspects. The family members are responsible for the addict and try to save, protect and control him. This leads to fatigue, anxiety, fear, feelings of guilt and anger in them[7], and because the person using the substance does not do well for his addiction, responsibilities and activities, communication problems and anger among family members are increased [22].

Regarding the worries that exist, Sharifi (2006) states that worrying about the problems of their lives and their future and their children is one of the issues that involve the minds of these people more, and this factor reduces self-confidence in these people [23]. The results showed that most participants were concerned about the failure of their spouses to leave. In this regard, Raibero *et al.,* 2007 in a study found that 75-80% of the self-referral to addiction treatment centres had a history of failed treatment[24]. Hajian *et al.,* 2013 also reported that 72% of the addicts had a history of the failed treatment [25]. This failure rate of the addiction treatment could justify the concern of the participants.

Our participants noted that they had a "turbulent family". In this regard, Penn Hire and colleagues quoted Ahani as saying that families that had a dysfunction or severe Substance use dependency were more turbulent than other families. The association of such families is very turbulent [26].

The disturbance is a kind of negative excitement that encountering a change in the process of life that sometimes affects people with some emotional responses. Humans, when confronted with phenomena that conflict with the culture and the family system, sometimes experience severe psychological pressures [27].

Another result of the study was the social isolation of the family. Together with these results, Malayeri Langroudi Khah et al., (2008) in Tehran showed that one of the social problems of the spouses of the addicted men is "social viewpoint," and stated that most of these women had social repressions and were trying their best to hide the addiction of the spouse. They attend parties without the presence of their husbands, and visiting is more limited to people admitted to the husband, including the addicts and drug dealers. These women think that everyone knows about their spouses' addiction and chooses to stay away from the community [11].

Another common issue that has been acclaimed in most interviews is "social stigma". Along with this result, Nikbakht e*t al.,* also stated in their study that the addiction stigma tended to hurt the personal and social identity of the substance abuser spouse, and the negative attitude of the community toward the addiction would restrict social communication[9].This reduces self-esteem, social isolation, and inefficiency in the Substance user's spouse [28].

In families where the head of the addicted family is an addict, he usually dominates his behaviour and anxiety. Excitement, concern and justification are observed in his words. Mutual respect disappears within the family system, and as a result, the level of incompatibility among the members of the family is increased, and the substance abuser's absences gradually emerge, and the set of these behaviours has a destructive effect on family relationships and relationships, so that the spouse and children do not feel comfortable and secure in the sociability that accompanies him. Thus, in families where the head of the household is an addict, the social relations of the family members are impaired. Because, Violence reigns instead of affection and also in the sociability that he has, Family members feel more ashamed and embarrassed. Instead of feeling comfortable and proud [29].

In conclusion, families, especially women with the Substance user spouse, are subject to severe psychosocial vulnerability and need to have comprehensive understanding and support. Therefore, nurses can develop education programs for people at risk by increasing awareness and changing their behaviour in the process of preventing addiction problems.

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Neonatal Morbidity in Late Preterm Infants Associated with Intrauterine Growth Restriction

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Abstract

AIM: This study aims to compare the neonatal morbidity of Intrauterine growth restricted (IUGR) Late Preterm (LP) babies, to those born Late Preterm but evaluated as Appropriate for Gestational Age (AGA).

METHODS: The study is a 2-year prospective one that used data from the Neonatal Intensive Care Unit (NICU) charts of LP neonates born in our tertiary maternity hospital "Koço Gliozheni" in Tirana. Congenital anomalies and genetical syndromes are excluded. Neonatal morbidity of IUGR Late Preterm is compared to those born Late Preterm but evaluated as AGA. OR and CI, 95% is calculated.

RESULTS: Out of 336 LP babies treated in NICU, 88 resulted with IUGR and 206 AGA used as a control group. We found significantly higher morbidity in the IUGR group for hypoglycemia, polycythemia, feeding intolerance, birth asphyxia and seizures, secondary sepsis have higher morbidity but the difference is not significant. No differences were found for hyperbilirubinemia in both groups. No neonatal deaths were observed in both groups.

CONCLUSION: Our study showed that late preterm IUGR has a significantly higher risk for neonatal morbidity when compared to late preterm AGA babies.

Introduction

Late preterm birth is defined as birth between 34 0/7 weeks and 36 6/7 weeks of gestation [2].

They are the fastest-growing in the preterm group in the last decade. In the United States in 2005, LP births account for more than 70% of all preterm births [15]. The number increased from 10.9% in 1990 in 12.8% in 2007 [3]. Now it is known that in the late preterm infants, the morbidity and mortality are higher than in term neonates. In Albania, there are no official data published about the late preterm birth rate and their morbidity and mortality.

IUGR is the term used to designate a fetus that has not reached its potential growth [1]. Intrauterine growth restriction is one of the causes of late preterm delivery, and it occurs more often in late preterm infants than term ones. IUGR itself is associated with perinatal morbidities and contributes to increased metabolic disease and poor neurodevelopmental outcome.

IUGR is an important cause of high-risk pregnancies and elective preterm deliveries. IUGR is present only in a small percentage of deliveries, but an increased frequency has been observed among women who go into preterm labour followed by premature delivery. Preterm infants and with intrauterine growth restriction are vulnerable to the complications of prematurity and IUGR as well, though there are conflicting findings in the literature about late preterm IUGR and AGA morbidity [7]. It appears normal, unchanged in the IUGR group compared to AGA LP infants [5] suggesting an advantage to the stress of poor growth.

The objective of this study is to compare

neonatal morbidity between late preterm IUGR and AGA infants with the same gestational age to better understand the neonatal outcomes of these infants for both complications of IUGR and prematurity.

Methods

Our study analysed the prospectively gathered data of babies born late preterm in two years from January 2014 – December 2015 in our tertiary maternity hospital "Koço Gliozheni" in Tirana. We analysed the data from medical charts of late preterm infants born in our hospital who entered NICU and compared the morbidity of IUGR LP, with the AGA as a control group. Gestational age at delivery was determined by mothers last menstrual period and or confirmed by early ultrasound examination. The evaluation of IUGR is done by calculating 3 growth indexes (Ponderal Index, that is an index of weight-related to length; the ratio of Head Circumference to Abdominal Circumference (HC/AC); and the difference between Chest Circumference with Abdominal Circumference ≥ 3 cm) after anthropometric measurements in the respective charts. In case 2 or more indexes result not normal the baby is identified as IUGR. AGA are babies whose birth weight is above the 11th to 89th percentile of birth weight in the growth curve of Alexander et al., [14]. Pregnancies with congenital anomalies or with unknown data criteria were excluded. Delivery characteristics included gestational age at birth, route of delivery, birth, Apgar scores. Neonatal data included respiratory morbidity with, (transient tachypnea of the newborn and respiratory distress), neonatal sepsis, sepsis follow up, birth depression with Apgar score < 7 the 5th minute, seizures and metabolic disorders as hyperbilirubinemia, hypoglycemia, polycythemia, and feeding intolerance [8]. Diagnosis and treatments are done by using the NICU protocol for every disorder and the criteria outlined in Standard Textbooks of Neonatology.

Hypoglycemia: defined as a blood glucose level less than 40 mg/dl in the first 24 hours and less than 45 mg/dl after 24 hours.

Hyperbilirubinemia: Clinically visible jaundice requiring phototherapy or exchange transfusion as per hour specific total serum bilirubin nomogram (AAP chart) [19].

Sepsis :Probable sepsis :positive septic screen (two of the five parameters, total white blood count < 5000/mm^3 or > 15000/mm^3, immature to total polymorph ratio ≥ 0.2, absolute neutrophil count less than 1750/mm^3 or > 7200/mm^3, C reactive protein > 1 mg/dl, platelets < 100.000/mm^3), and proven sepsis: Isolation of pathogens from blood or Cerebrospinal fluid [17], [18].

Feeding intolerance: Inability to digest enteral feedings associated with increased gastric residuals, abdominal distension and or emesis, often leading to a disruption of the feeding plan.

Polycythemia: Hematocrit or haemoglobin concentration > 2 SD above the normal value for gestational and postnatal age associated with clinical findings resulting from hyperviscosity [16].

Statistical data were collected into the database. The difference in morbidity between two groups is compared by calculating the OR and confidence interval 95% using the Fisher exact test for statistical analysis. The result is considered significant at $p < 0.05$.

Results

During the 2-year study period, 1334 babies entered the NICU. Of those admissions, 336 or 25% were babies born late preterm i.e. 34 0/7 – 36 6/7 weeks of gestational age. 3 infants are excluded from the study as they were born with a congenital anomaly. IUGR late preterms treated in the NICU were 88 babies and AGA 206 babies.

Gestational age at delivery ranged from 34 to 36.9 weeks, with a median of 35.1 and did not differ between the two groups. Mode of delivery was 78% cesarean section in the IUGR group vs 50% in the AGA group with a significant difference. The length of stay of the newborn in the NICU differed significantly as well between the two groups (Table 1).

Table 1: The length of stay of the newborn in the NICU

	IUGR LP	AGA LP	OR	CI 95%
Median Gestational age	35.1	35.07		
SD	± 0.8	± 0.8		
Mode of delivery (CS)	78% 61	50% 97	2.5	1.49-4.3 p = 0.002
Length of NICU stay	9.45	5.5		
Median weight	1871.9 gr	2500.05 gr		
SD	± 318.5	± 382.1		

LP-late preterm; IUGR-Intrauterine growth-restricted; AGA-appropriate for gestational age.

The neonatal morbidity: we found a slight difference between the two groups as it concerns overall respiratory morbidity (Transient Tachypnea, Respiratory Distress Syndrome) where IUGR LP is less vulnerable to this morbidity compared to AGA LP. No difference was found about, hyperbilirubinemia and sepsis workup. We found a significant difference between the two groups about hypoglycemia, where IUGR LP suffer more than AGA. For polycythemia, feeding problems, birth depression APGAR score < 7, 5th minute of life, seizures and secondary sepsis IUGR LP are more vulnerable, but the difference is not significative (Table 2).

Table 2: There are no deaths registered in either group

	IUGR LP N = 88, 26%	AGA LP N = 206, 61%	OR	CI 95%	p-value
Respir. Morbidity	38 43%	115, 55%	0.6	0.37-0.99	P = 0.05
Hyperbilirubinemia	49 55%	112, 54%	1	0.8-1.2	P = 0.5
Apgar score < 7, 5Th min	6 6.8%	7, 3.3%	2.1	0.68-6.38	P = 0.19
Seizures	3 3.4%	1, 0.4%	7.2	0.74-70.5	P = 0.08
Hypoglycemia	9 10%	3, 1.4%	7.7	2-29.2	P = 0.0013
Polycythemia	3 3.4%	1, 0.4%	7.2	0.74-70.5	P = 0.08
Feeding problems	2 2.2%	3, 1.4%	1.57	0.26-9.6	p=0.6
Neonatal sepsis	3, 3.4%	2, 0.9%	3.6	0.6-21.9	P = 0.16
Sepsis follow up	6, 6.8%	44, 21%	0.27	0.11-0.66	P = 0.002

LP-late preterm; IUGR-Intrauterine growth-restricted; AGA-appropriate for gestational age.

Discussion

Late preterm births are an increasing problem in the world nowadays. They account for 70% of all preterm births. They experience a higher incidence of neonatal morbidity and mortality compared to term neonates [7]. On the other hand, IUGR is a problem that complicates their prematurity situation, contributing to an increased morbidity and mortality observed among late preterms.

Although there is a lack of studies about IUGR late preterms, as in other studies, we found a slight difference for respiratory morbidity, probably this a contribution to their inutero stress that leads to an early pulmonary maturity and the high incidence of CS for AGA LP (50%). We did not observe severe complications as Necrotizing Enterocolitis NEC, Pulmonary Hemorrhage, and death perhaps because these are rare findings in this gestational group [15].

Problems like hypoglycemia, polycythemia and feeding intolerance are common in the IUGR preterm group, and neonatal sepsis is found more in this group rather than AGA because we did not exclude women with preterm premature rupture of membranes from our study [9]. As found in other studies Laptook and Jackson [20], hypoglycemia has an elevated incidence in late preterm infants as a result of deficient neoglycogenesis, hepatic glycogenolysis and hormonal irregularities. Finding seizures more often in IUGR group is linked to metabolic problems like hypoglycemia, but birth asphyxia as well, which is linked to the emergency of Cesarian Section interventions in IUGR Late Preterms in our hospital as a reference hospital in Albania.

Our study is focused on the early morbidity of late preterm infants, and we don't have evidence about their long-term consequences in later life.

In conclusion, IUGR late preterm infants have higher morbidity compared to AGA LP, and IUGR is a major cause for late preterm delivery and CS delivery. A better understanding and evaluation of their problems is very important [12], [13].

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Brain Vitalization Gymnastics Improved Cognitive Function Marked by Increased BDNF, Decreased Serum Interleukin-6 and Decreased S-100β Expression among Elderly in West Denpasar Primary Health Clinic

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Keywords: Brain vitalisation gymnastics (BVG); Elderly cognitive function; BDNF; IL-6; S100β; Brain plasticity markers

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Abstract

BACKGROUND: Brain vitalisation gymnastics (BVG) is a form of physical exercise which attempts to synchronise bodily movements with cognition within the same time frame.

AIM: This study aims to prove BVG can improve cognitive function among the elderly.

METHODS: The impact of BVG was evaluated as opposed to elderly gymnastics (regarded as a control group) for a 4-week study period. Outcomes measured were improvements of cognitive function assessed by MoCA-Ina questionnaire, as well as the difference in serum levels of BDNF, IL-6, and S100β. An experimental pretest-posttest control design was applied to evaluate BDNF and IL-6 levels, while the post-test only designed to evaluate S100β levels. Parametric data were tested for normality before being proceeded into either parametric (independent student' t) or non-parametric (Mann Whitney) test.

RESULTS: BVG significantly improved cognitive function better than elderly gymnastics with MoCA-Ina score of 1.53 ± 1.58 dan 0.11 ± 2.54, respectively ($p \leq 0.047$). BVG group also had increased BDNF levels when compared with control (-6020.58 ± 7857.22 dan 0.11 ± 2.54; $p = 0.027$). Whereas BVG had lower IL-6 levels as opposed to the control group (median pre-test IL-6: 2212, median post-test IL-6: 3197.50; $p = 0.004$). Meanwhile, S100β levels were found lower among BVG when compared with the control group, although statistically insignificant ($p = 0.40$).

CONCLUSION: BVG programme for 4 weeks improved: (1) brain plasticity as shown by increased serum BDNF and S100β levels (although the latter was statistically not-significant), as well as marked decrease of IL-6 levels, (2) cognitive function as proven by an increase of MoCA-Ina score when compared with elderly gymnastics.

Introduction

Ageing causes a variety of biological changes, including the brain. In humans, brain ageing causes cognitive deterioration in many aspects that have been studied and documented. Referring to Harada et al. (2013), the brain's cognitive abilities consist of crystallised and fluid intelligence [1]. Crystallised intelligence includes the ability of individuals in the acquisition of skills and knowledge learned and repeatedly practised, while fluid intelligence is an individual's ability to solve problems and analyse new concepts and not depend on what has been learned before. Brain ageing has minimal impact on crystallised intelligence but has a major effect on the decrease of liquid intelligence. This is demonstrated by the slowing of thinking and motor responsiveness (including fluency of language), decrease of attention especially related to selective attention (i.e. the ability to focus on specific information and ignoring other information that is considered irrelevant, e.g. driving a vehicle), shared attention (e.g. the ability to perform multiple activities simultaneously, e.g. speaking on the phone while cooking), and working memory (e.g. temporary memory capacity while manipulating information,

decreased memory function, especially related to semantic memory, decreased ability to remember new things (memory acquisition) and accessing memory (memory retrieval), decreased visuospatial ability, particularly related to constructive ability (e.g. assembling 3-dimensional objects, such as assembling furniture), and decreasing executive function, e.g. the ability of individuals to engage in independent, purposive, and proportional activities [2], [3], [4], [5], [6]. The executive function is more general and encompasses various cognitive areas, such as the ability to plan, organise, monitor, argue, solve problems, and mental flexibility [1]. Studies show that concept formation, abstraction, and mental flexibility decrease with age [7].

The mechanism underlying the decline in cognitive function due to biological ageing varies. It can be assumed, however, that the impairment of cognitive function is the implication of structural and functional changes in the brain as the ageing process proceeds. For example, the volume of grey substance declines from the age of 20 and atrophy often occurs in the prefrontal cortex, as well as the moderate-scale volume reduction in the hippocampus [8], [9]. The cause of atrophy is the death of neurons that are not matched by meaningful cell replication, but to a greater degree due to the decrease in synaptic density, both in terms of number and size. The synaptic density change involves the decrease of dendritic arborisation complex, shortening of the dendrite, and decreased neuritic spines which are the main location of the excitatory synapse [10], [11]. Also, pathological conditions such as beta-amyloid protein accumulation (Aβ) are found in even healthy elderly brains, which were originally regarded as non-pathological processes, but have recently been recognised as a prognostic factor of future Alzheimer's dementia in subjects with protein accumulation Aβ [12]. Furthermore, there is a decrease in the volume of cerebral white matter which is more significant than that of grey matter, especially in the precentral gyrus, rectus and corpus callosum [13]. Decreased cerebral white matter, such as in the parahippocampal area, can lead to decreased brain communication with other structures such as the hippocampus and is assumed to cause cognitive decline with advancing age.

However, the brain has a special ability to compensate for structural and functional changes, both in physiological states such as ageing as well as on pathological conditions. The brain can adapt to the changing of the internal and external environment of an organism. The corresponding adaptability refers to the regeneration of neurons and synapses, as well as changes (remodelling) of neural pathways [14]. This adaptive skill is known as brain plasticity. Plasticity of the brain in humans are known to persist through old age [15]. For example, ageing individuals are shown to use more brain areas more actively in the process of formation and memory access as a way to compensate for the decline in cognitive function due to advancing age [14]. Brain plasticity reflects the brain's ability to re-organise continuously in the maturation process and respond to changes in stimuli or injury. Various mechanisms of plasticity have been widely studied, including (but not limited to) neurogenesis, synaptogenesis, and angiogenesis [16]. Brain plasticity can be observed at the cellular level in the form of changes in neurons and supporting cells, as well as dendrites and synapses. Functionally, the plasticity of the brain can be subdivided into 3 types, namely the plasticity of neurons (related to brain adaptation at the level of synapses), brain plasticity (changes in brain tissue activity), and individual ability to improve cognitive function after training [17], [18].

Physical activity in various types and levels has been shown to slow the decline in cognitive function and reduce the incidence of dementia [19]. This concept is supported by various types of studies, ranging from observational studies in experimental animals, humans, to biomolecular research. A meta-analysis that studied the relationship between physical activity and cognitive function found that from 11 randomised intervention studies found improvements in cognitive function, particularly in the motor area and auditory attention (strong degree), as well as moderate cognitive speed and visual attention [20]. In addition to physiologic brain ageing, physical activity is also known to improve cognitive function in mild cognitive impairment patients who are the prodromal phase of Alzheimer's dementia [21], [22].

An animal study conducted by Laksmidewi, et al., (2016) proved that regular physical exercise can improve brain plasticity and cognitive function, evident from significant beta-amyloid depletion, significant increase in BDNF serum, decreased serum CRP, and a new finding that astrocytes play an important role in brain plasticity marked by increasing numbers of astrocytes that express BDNF [23]. BDNF is a neurotrophic factor that has a pleiotropic effect on synapse plasticity and plays an important role in short-term memory acquisition and access, formation, consolidation, re-consolidation, maintenance, and long-term memory removal through various molecular signalling pathways including PI3K, PLC-gamma, and ERK1/2, in addition to potassium sodium ion pump regulation, and modulation of NMDA and AMPA receptors [24].

In addition to physiological processes, cognitive function decline can also be found in pathological conditions such as Alzheimer's dementia. Various enzymatic activities in neurons and enzymatic defects within myelin-forming cells oligodendroglia and Schwann cells occur in dementia [25]. One indicator of microglial activation is the secretion of S100β which is a protein that binds to calcium under conditions of metabolic stress and is a biological marker of central nervous system (CNS) damage.

Also, microglia activation results in a state of

neuroinflammation characterised by secretion of proinflammatory cytokines, such as interleukin-6 (IL-6). Excessive secretion of IL-6 is neurotoxic in the CNS and is found in a variety of pathological conditions [26], [27], [28].

Brain Vitality Gymnastics (BVG) is an exercise with repeated stimulation that seeks to harmonise the movement of the body with thinking activity, optimising the ability of memory simultaneously. BVG is associated with cooperation between the function of motion, respiration, and cognition. The movements in BVG can stimulate cooperation between the two cerebral hemispheres [29]. Furthermore, physical exercise can improve cardiac output, increasing the body's oxygen demand, improving neurobiology as well as synthesising brain tissue, improving angiogenesis, neurogenesis, synaptogenesis, neurotransmitter synthesis, and cognitive function [30], [31]. This BVG has also been used by the Health Intelligence Center of the Ministry of Health of the Republic of Indonesia in the elderly group [32].

BVG is known to improve cognitive function in the elderly. In this study, we want to know the effect of BVG on improving cognitive function of elderly and biological markers on brain plasticity (BDNF), CNS damage (S100β), and neuroinflammation (IL-6) compared with elderly gymnastics.

Methods

Study Design and Procedure

This is experimental research with pretest-posttest control design for BDNF and IL-6 groups, and post-test only design for the S100β group. This study was conducted at the Elderly Primary Health Clinic in West Denpasar from May to June 2017.

The elderly were selected based on the inclusion and exclusion criteria, and 38 subjects were divided into two groups of 19, each treated with BVG and elderly gymnastics. All subject within the two groups will be interviewed with a MoCA-Ina assessment questionnaire, blood samples were then taken for BDNF and IL-6 (pre-exercise) levels measurement, and then the elderly were given BVG treatment in the treatment group and the elderly gymnastics for the control group. During the experiment, both BVG and elderly gymnastics were given twice a week for 4 weeks in a row with a duration of 20-30 minutes for each session. After four weeks of BVG training and elderly gymnastics, MoCA-Ina and serum levels of BDNF, IL-6, and S100β were reevaluated (post-exercise). The data obtained were analysed statistically.

Results

In Table 1, the overall age (either control or treatment group) was 67.00 ± 4.66 years. The mean age of the control group was 66.74 ± 4.51, while the treatment group was 66.00 ± 4.93 years. The number of men and women in each group (control and treatment) was relatively balanced, i.e. about 50 percent. All subjects received formal education from junior high school to university, with the highest number of universities 17 (44.7%), senior high school 13 (34.2%) and junior high school 8 (2%). Subject's occupation within the control and treatment group were mostly retired civil servants (PNS), which consists of 9 subjects (23.7%) in the control group and 13 subjects (34.2%) in the treatment group.

Table 1: Baseline Characteristics of Subjects

Parameters	Control (n = 19)	Treatment (n = 19)
Mean age (± SD)	66.74 ± 4.51	66.00 ± 4.93
Sex		
Male	9 (47.4%)	9 (47.4%)
Female	10 (52.6%)	10 (52.6%)
Educational level		
Junior high school	7(36.8%)	1 (5.3%)
Senior high school	4 (21.2%)	9 (47.4%)
Academy/Dipl./Univ.	8 (42.1%)	9 (47.4%)
Occupation		
Civil servant	9 (47.4%)	
Private employee	2 (10.5%)	4 (21.1%)
Entrepreneur	3 (15.8%)	1 (5.3%)
Others	5 (36.3%)	1 (5.3%)

SD = standard deviation.

Table 2 showed that the effect of BVG in cognitive function improvement could be assessed by comparing the mean increase of MoCA-Ina cognitive function score between the treatment group (BVG) and control group (elderly gymnastics) after 4 weeks of gymnastics training. An independent t-test was conducted to determine the significance of BVG compared with elderly gymnastics in improving the cognitive score. The result showed an average increase of MoCA-Ina score by 1.53 ± 1.58 in the BVG group and 0.11 ± 2.54 in the elderly gymnastics group. In this study, it was found that the observed increase of MoCA-Ina score among the treatment group was significantly different (higher) than the control group (p = 0.047).

Table 2: The average increase in MoCA-Ina scores between brain vitalisation gymnastics groups and elderly exercise groups

Groups	Average MoCA-Ina score increase	p-value
BVG	1.53 ± 1.58	0.047*
Elderly gymnastics	0.11 ± 2.54	

BVG = brain vitalization gymnastics; MoCA-Ina = Montreal Cognitive Assessment-Indonesia.

Table 3 showed that the effect of BVG on elevated serum BDNF level in elderly was assessed by comparing the mean of BDNF increase between treatment group (BVG) and control group (elderly gymnastics) after 4 weeks of gymnastics training. In the independent t-test, the average increase in BVG group was -6020.58 ± 7957.22, and the mean of the

elderly gymnastics group increased by 0.11 ± 2.54. In this study, it was found that the elevated serum levels of BDNF treatment group were significantly different from the control group (p = 0.027).

Table 3: Mean elevation of serum BDNF levels between BVG group and elderly exercise group

Groups	Mean BDNF increment	
BVG	-6020.58 ± 7957.22	0.027
Elderly gymnastics	-12028.32 ± 8062.32	

BVG = brain vitalization gymnastics; BDNF= brain derived neurotrophic factor.

Table 4 showed that the effect of BVG on decreasing levels of IL-6 in the elderly was assessed after four weeks of gymnastics training. The median pre-test IL-6 levels were 2212.00, while the median post-test IL-6 levels were 3197.50. There were 12 people with higher IL-6 levels after gymnastics than before gymnastics, and 26 people showed lower levels of IL-6 than before exercise. In the Wilcoxon test, it was found that the difference in serum IL-6 decrease was significantly (p = 0.004).

Table 4: The decrease in serum IL-6 levels between the BVG and the elderly exercise group

	IL-6 levels (pg/mL)			
Parameters	Median	Minimum	Maximum	p-value
Pre-test IL-6 levels	2212.00	566	5741	0.004
Post-test IL-6 levels	3197.50	1013	7669	

IL-6 = interleukine 6.

Table 5 showed that BVG decreased subjects' S100β levels (pg / mL) in the elderly, which was assessed after four weeks of gymnastics training. Mann Whitney test was conducted to compare the S100β's rate of decrease among BVG-treated and elderly gymnastics group. It was found that the decrease of S100β treatment group did not differ significantly with the control group (p = 0.404).

Table 5: The difference between serum S100β levels of BVG group and the elderly exercise group

	S100β levels (pg/mL)			
Parameters	Median	Minimum	Maximum	p-value
BVG	19.63	19.48	21.17	0.404
Elderly gymnastics	19.98	19.48	22.60	

BVG = brain vitalization gymnastics.

Discussion

The concept of brain plasticity is the reorganisation of neural interconnection through a sustained new experience. It is an intrinsic development of adult brain tissue, where ineffective synapses will undergo shrinkage [33], [34], [35]. Animal studies have shown that regular exercise improves brain plasticity as evidenced by significant beta-amyloid depletion, a significant increase in serum BDNF, decreased serum CRP and that astrocytes play an important role in brain plasticity characterised by an increase in the number of astrocytes expressing BDNF [23].

In this study, 38 subjects met the eligibility criteria, which were divided into 19 subjects in the treatment group and 19 subjects were the control group. All subjects were recruited in the area of West Denpasar Puskesmas Bali. In this study, the overall age (either the control group or the treatment group) was 67.00 ± 4.66 years. The mean age of the control group was 66.74 ± 4.51, while the treatment group was 66.00 ± 4.93 years. All subjects of this study run formal education ranging from junior high school to college, with the highest number of academy/diploma/ colleges that is 17 subjects (89.5%). This indicates that the study subjects in both groups had a high educational background, thus contributing to better synapse density and cognitive reserve than subjects with low education [36]. Also, to assess cognitive function using the MoCA-Ina test requires a good level of attention (attention) and has a minimum level of formal education completed primary school. The provision of BVG to increase the cognitive function of MoCA-Ina in elderly was assessed by comparing the mean improvement of cognitive function score between the treatment group (BVG) and control group (elderly gymnastics) after 4 weeks of gymnastics training. The average score improvement of MoCA-Ina in the BVG group was 1.53 ± 1.58 and the MoCA-Ina's mean score improvement in elderly gymnast group was 0.11 ± 2.54. The Montreal Cognitive Assessment (MoCA) is a questionnaire to assess global cognitive functioning including executive and memory functions. Efficient MoCA examination for various causes of cross-age cognitive dysfunction and more sensitive level of education compared with MMSE, particularly significant with frontal lobe abnormalities [37], [38]. In this study, it was found that the effectiveness of MoCA-Ina improvement score between treatment groups increased by 1.53 ± 1.58 which was significantly different compared to the control group of 0.11 ± 2.54 (p = 0.047). It can be concluded that the application of BVG resulted in significantly improved cognitive function compared to the elderly gymnastics group for 4 weeks with regular training.

Brain-Derived Neurotrophic Factor (BDNF) is a neurotrophic factor about Nerve Growth Factor as a marker of brain plasticity. The brain in adulthood has the ability to keep producing new nerve cells known as neurogenesis processes. BDNF is one of the most active markers of plasticity and plays an important role in neural development [39], [40], [41]. As it is known that cognitive function and memory were arranged in the cerebral cortex, hippocampus, and the frontal part of the brain, it is known that BDNF is important for long-term memory associated with Nerve Growth Factor [41], [42]. Four weeks of BVG administration affected elevated serum BDNF levels in the elderly. In this study, the average increase of BVG group was-6020.58 ± 7957.22 and average of elderly gymnastics

group was 0.11 ± 2.54. In this study, it was concluded that elevated serum BDNF levels among the treatment group were significantly different with the control group ($p = 0.027$). This indicates that BVG not only improves functional cognitive functioning as evaluated by MoCA-Ina but also plays an important role in improving neuroplasticity. As is known, BDNF plays an important role in short and long-term memory regulation, through the mechanism of long-formation reinforcement, consolidation, re-consolidation, care and elimination involving various molecular signaling pathways, including PI3K, PLC-gamma, and ERK1/2, in addition to potassium sodium ion pump regulation, and NMDA and AMPA receptor modulation [24]. Thus, the increase in BDNF levels due to BVG, as found in this study, is expected to have a positive impact on improving cognitive function, both in healthy elderly and people with dementia. This is also supported by experimental animal studies conducted by Laksmidewi, et al., (2016), where BDNF increased after the 7th, 14th, 28th day of training when exercise is regular and not excessive. BDNF levels reportedly rose much higher after 14 days of regular training compared to the seventh-day post-exercise [23]. BDNF increment is evidence that BDNF is a key protein in the regulation of the successful balance of neuronal growth and also shows the association of physical exercises with brain plasticity.

Interleukin-6 (IL-6) is a proinflammatory cytokine secreted mainly by astrocytes in the CNS. Although IL-6 has a beneficial effect because it is neurotropic, its excessive expression is generally neurotoxic [43]. Changes in cognitive function after acute systemic inflammation are thought to be the result of cellular and molecular interactions, especially in the hippocampus. Acetylcholine inhibits the release of IL-6 pro-inflammatory cytokines to control inflammation in the brain [44], [45]. BVG in this study proved to lower IL-6 levels in the elderly as assessed after four weeks of gymnastics. The median pre-test and post-test IL-6 levels were each 2212.00 and 3197.50 pg/mL. There were 12 people with higher IL-6 levels after than before gymnastics, and 26 people showed IL-6 levels to be lower than before exercise. In this study, BVG training for 4 weeks regularly was proven to lower IL-6 levels when compared with elderly gymnastics group.

Furthermore, in this study, S100β was found decreased among the treatment group (BVG) but did not differ significantly with the control group (elderly gymnastics) with $p = 0.404$. S100β is a protein attached to calcium, secreted by glial cells, Schwann cells, and astrocytes under the influence of metabolic stress conditions. The synaptic transmission of S100β affects the excitability of nerve cells and cerebral blood flow, thereby increasing neurobehavioral and cognitive symptoms [44]. The release of S100β by astrocytes is associated with oxidative stress mechanisms, but also has a beneficial effect on the maintenance of nerve cells, neurogenesis, and cognitive function described through brain repair processes especially in the hippocampus region [46], [47], [48]. Increased secretion of S100β by activated astrocytes suggests a neurodegenerative process, found in Alzheimer's dementia, stroke, schizophrenia, and traumatic brain injury [49], [50].

In this study, the BVG administration for 4 weeks did not demonstrate a significant difference between treatment and control group with regards to S100β levels decrement. Referring to the dualism nature of S100β secretion related to oxidative stress and neurogenesis (along with cognitive function), the physical exercise should be conducted longer and more regularly as well as evaluated both on pre- and post-test. The insignificant result of S100β levels among the treatment and control group was assumed due to the post-test evaluation only.

In conclusion, BVG performed regularly in the elderly at intervals of 2 times a week with a duration of 20-30 minutes per session for 4 weeks has been shown to improve cognitive function and brain plasticity, as well as decrease the neuroinflammation process is shown by increasing MoCA-Ina score and BDNF levels, IL-6 levels, respectively, were statistically significant compared to elderly exercise. Meanwhile, BVG did not show any significant effect on S100β compared to elderly gymnastics.

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Clinical Science

Retrospective Study of Clinical and Epidemiological Parameters of Patients Undergoing Percutaneous Coronary Intervention with Their Follow-Up

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Abbreviations: ACC - American College of Cardiology; ACS - acute coronary syndrome; AWMI - anterior wall myocardial infarction; BMS - bare-metal stents; CSA – chronic stable angina; CTO - Chronic total occlusion; DES – drug-eluting stents; DVD - Double vessel disease; IWMI – inferior wall myocardial infarction; JNC 8 - Eighth Joint National Committee; LAD - left anterior descending artery; LCX – left circumflex artery; LVEF - left ventricular ejection fraction; MJPJAY - Mahatma Jyotiba Phule Jan Arogya Yojana; NIC - National Interventional Cardiology; NSTE-ACS - non-ST elevation acute coronary syndrome; PAMI - Primary angioplasty in myocardial infarction; PCI - undergoing percutaneous coronary intervention; RCA – right coronary artery; STEMI - ST-elevation myocardial infarction; SVD - Single vessel disease; TVD – Triple vessel disease; US NCDR - United States National Cardiovascular Data Registry

Abstract

AIM: To study clinical and epidemiological parameters of patients undergoing percutaneous coronary intervention (PCI) and to follow them up for understanding the outcomes of the procedure.

MATERIAL AND METHODS: This is a retrospective data analysis of 862 patients who underwent PCI from January 2016 to November 2017

RESULTS: Out of 862 patients, 611 (70.88%) were male & 251 (29.12%) were female, with the mean age being 55. 243 (28.19%) were diabetic, 470 (54.52%) were hypertensive, 158 (18.32%) patients were tobacco chewer, 215 (24.92%) were smokers and 111 (12.87%) were alcoholic. 636 (73.78%) patients had STEMI, 153 (17.74%) had NSTE-ACS, 61 (7.07%) had CSA.578 (67.05%) were SVD, 262 (30.39%) were DVD and 19 (2.20%) were TVD. Out of SVD, 350 (60.55%) patients had LAD involvement and among DVD patients, LAD and RCA were most commonly involved in 107 (40.83%) patients. On follow-up of mean 604.42 days (minimum 236 days, maximum 909 days), 2 (0.23%) episodes of subacute stent thrombosis occurred and 11 (1.27%) patients had ISR but no mortality was reported.

CONCLUSION: The study shows affection of young population predominately and genders inequality, suggesting primarily male disease. PCI is often sought in ACS and CSA is predominately treated medically. Thrombolysis remains the first treatment received by STEMI patients. SVD is the most common angiographic diagnosis with LAD predominately affected vessel. This real world-data on clopidogrel with aspirin as dual antiplatelet therapy and second-generation stent shows negligible event of stent thrombosis and ISR.

LIMITATION: Due to non-invasive follow-up, the exact amount of stent restenosis cannot be calculated.

IMPACT ON DAILY PRACTICE: This real world-data on clopidogrel with aspirin as dual antiplatelet therapy and second-generation stent shows negligible event of stent thrombosis and ISR. This can help reduce the cost burden on society and help better distribution of health budget.

Introduction

Percutaneous coronary intervention (PCI) is the cornerstone in the treatment of acute coronary syndrome (ACS). PCI is an evolving science and outcomes of which depend on characteristics of the individual patient, operator experience, availability of newer and more sophisticated hardware, improvement in our understanding of the science of atherosclerosis, etc. Since the first balloon angioplasty done by Dr Andreas Gruntzig in 1987, the art of PCI has changed a lot. Balloon angioplasty evolved into angioplasty with stenting and from bare-metal stents (BMS) to eluting drug stents (DES). DES is constantly changing from the type of drug being used to the amount of metal, the thickness of struts, platform design, etc. The stent classification system developed by Dr Sundeep Mishra helps in understanding the importance of newer stent with newer drugs [1]. Also, at the same time when stents are undergoing evolution, newer antiplatelet drugs with improved

efficacy and potency are being launched. American college of cardiology has put newer antiplatelets ticagrelor and prasugrel as first-line antiplatelet for patients undergoing PCI [2]. But the real-world data is quite different from trials data which are done in a controlled environment. Hence, came the concept of the registry to maintain real-world data which is usually free from pharmaceutical industry bias. Reviewing the registry helps us understand the real-world scenario and analysing it makes one more competent in dealing with patients in one's locality.

Objectives: - To understand the age-wise & gender-wise distribution of patients; - To know the percentage prevalence of risk factors viz. diabetes mellitus, hypertension, smoking, tobacco chewing and alcohol intake among patients requiring PCI; - To know the prevalence of ST-elevation myocardial infarction (STEMI), non-ST elevation acute coronary syndrome (NSTE-ACS) & chronic stable angina (CSA) among patients who underwent PCI in real-world scenario; - To study the distribution of left ventricular ejection fraction (LVEF) among patients undergoing PCI; - To learn about the incidence of involvement of type of coronary artery and dimensions of coronary stents required for PCI; - To follow-up the cohort to calculate incidence of stent thrombosis, in-stent restenosis, other complications or deaths.

Table 1: Summary of variables

Variable	Observation (n = 862)
Male	611 (70.88%)
Female	251 (29.12%)
Age	
Teenage (< 20)	1 (0.11%)
Very Young (20 to ≤ 35)	30 (3.48%)
Young (35 to ≤ 55)	405 (46.98%)
Peri-retirement (56 to 64)	222 (25.75%)
Elderly (≥ 65)	204 (23.66%)
Diabetes	243 (28.19%)
Hypertension	470 (54.52%)
Tobacco chewer	158 (18.32%)
Smoker	215 (24.92%)
Alcoholic	111 (12.87%)
STEMI	636 (73.78%)
NSTE-ACS	153 (17.74%)
CSA	61 (7.07%)
Thrombolysis	302 (47.48%)
PAMI	3
AWMI	384 (60.37%)
IWMI	262 (41.19%)
CTO	47 (5.45%)
SVD	578 (67.05%)
DVD	262 (30.39%)
TVD	19 (2.20%)
LVEF	
≤ 30	158 (18.32%)
30-45	239 (27.72%)
46-60	233 (27.03%)
≥ 60	232 (26.91%)
Follow-up means	604.42 days
Local complication	9 (1.04%)
Stent thrombosis	2 (0.23%)
ISR	11 (1.27%)

Methods

This is retrospective data analysis of 862 patients who underwent PCI from January 2016 to November 2017 at the department of cardiology in our institute under Mahatma Jyotiba Phule Jan Arogya Yojana (MJPJAY). This cohort was followed up in July 2018 with a mean duration of 604.4 ± 161.4 days.

Exclusion criteria: Patients who were in cardiogenic shock or required vasopressors or support ventilation before or at the time of PCI were excluded from the study.

Age-wise distribution is done as follows: A) teenage – defined as age between 13 years to 19 years; B) very young (3) – defined as age from 20 years to 35 years; C) young (4) – defined as age from 36 years to 55 years; D) peri-retirement – defined as age from 56 years to 64 years; and E) elderly [5], [6] – defined as age more than or equal to 65 years. Risk factors considered in the study were diabetes mellitus, hypertension, smoking, tobacco chewing or alcohol intake. Diabetes mellitus was defined in the study as per WHO guidelines, 2006 [7]. Hypertension was defined in the study as those patients who required pharmacologic therapy for hypertension as per the Eighth Joint National Committee (JNC 8) guidelines [8]. The individuals were classified as "chewing tobacco" if the patient or his/her relatives answered "yes" to the question of chewing tobacco. Similarly, individuals were categorised as "smokers" if the patient or his/her relatives answered, "yes" to the question of "smoking tobacco" [9].

In the same way, alcohol intake was based on response either "yes" or "no" of the question to patients or their relatives when asked about alcohol drinking habit [10]. Acute coronary syndrome either STEMI [11] or NSTE-ACS [12] and chronic stable angina [13] were defined by the American College of Cardiology guidelines (ACC). Thrombolysis, whether done in our institute or patient, was thrombolysis at other centre and referred for further management was defined thrombolysis irrespective of thrombolytic agent used. Primary angioplasty in myocardial infarction (PAMI) was defined as when the patient presented within 6 hours of the onset of symptoms of ACS and was taken for PCI directly. STEMI was subclassified into anterior wall myocardial infarction (AWMI) or inferior wall myocardial infarction (IWMI) depending upon whether ST elevation is recorded in anterior chest lead or inferior leads respectively. Chronic total occlusion (CTO) of the coronary artery was defined as at least 3 months of total occlusion of coronary artery or infarct-related artery total occlusion when the primary event was recorded 3 months earlier plus the use of guide-wire for angioplasty with a tipping load of more than 4 gm. Single vessel disease (SVD) was the involvement of only one coronary artery with a significant lesion that requires intervention, while double vessel disease (DVD) was when two coronary arteries required intervention. Similarly, triple vessel disease (TVD) was defined when all the three left anterior descending (LAD), left circumflex (LCX) and right coronary artery (RCA) required stenting during PCI.

Stent details

The two stents that belong to second-generation DES used during this period for PCI at the department were as follows: 1) ProNOVA (Vascular concepts Ltd, India) and 2) Endeavor (Medtronic, United States of America)

Antiplatelet details

A loading dose of 300 mg of aspirin with 600 mg of clopidogrel was used in all patients analysed in the study and dual antiplatelet therapy with aspirin 75 mg and clopidogrel 75 mg once daily post PCI were prescribed. Stent thrombosis was defined as per timings and definition of academic research consortium [14]. Restenosis was defined in this study as more than 50% diameter stenosis in the patient who presents with either ACS or refractory symptoms despite optimal anti-anginal drugs. Local complications considered were pseudoaneurysm, hematoma, arterio-venous fistula. Deaths any reported during this period were classified into cardiac or non-cardiac based on verbal autopsy.

Data regarding all clinical and epidemiological variables were obtained from MJPJAY database of the hospital record. Follow-up of all patients that underwent PCI under MJPJAY scheme at our hospital excluding those mentioned in exclusion criteria was done in July 2018 at one point in time in a cross-sectional manner and any event in the past from the date of PCI was recorded as per protocol. Individual characteristic was expressed using the percentage of the total event.

Results

Out of 862 patients, 611 (70.88%) were male, and 251 (29.12%) were female. Patients' age was uniformly distributed from 18 years of age to 85 years of age as shown in Table 2, with both median age and mean age being 55. Two hundred forty-three (28.19%) were diabetic and 470 (54.52%) were hypertensive. 158 (18.32%) patients were tobacco chewer, 215 (24.92%) were smokers and 111 (12.87%) were alcoholic. Six hundred thirty-six (73.78%) patients had STEMI, 153 (17.74%) had NSTE-ACS, 61 (7.07%) had CSA.

Table 2: Age-wise distribution

Age	Number	Percentage
Teenage (< 20)	1	0.11
Very Young (20 to ≤ 35)	30	3.48
Young (35 to ≤ 55)	405	46.98
Peri-retirement (56 to 64)	222	25.75
Elderly (≥ 65)	204	23.66

Out of 636 patients with STEMI, 302 patients were thrombolysis (47.48%) before being referred to our centre and only 3 patients received PAMI. Also, out of STEMI patients, AWMI has diagnosed in 384 (60.37%) patients and IWMI in 262 (41.19%) patients.

Table 3: Distribution according to LVEF

LVEF	Number	Percentage
=<30	158	18.32
30-45	239	27.72
46-60	233	27.03
>=60	232	26.91

Table 3 shows the distribution of patients according to LVEF. Forty-seven (5.45%) patients were CTO, out which 5 (10.63%) attempts failed. As per this PCI registry, 578 (67.05%) were SVD, 262 (30.39%) were DVD and 19 (2.20%) were TVD. Out of SVD, 350 (60.55%) patients had LAD involvement and among DVD patients, LAD and RCA were most commonly involved in 107 (40.83%) patients. Five hundred seventy-seven times LAD PCI was done with average stent diameter of 2.97 mm and length of 25.68 mm, 186 times LCX PCI was done with average stent diameter of 2.86 mm and length of 22.84 mm and 327 times RCA PCI was done with average stent diameter of 3.20 mm and length of 25.86 mm.

On follow-up of mean 604.42 days (minimum 236 days, maximum 909 days), 9 (1.04%) had a local complication which included one event of pseudoaneurysm and one event of radial artery thrombosis both of which were treated conservatively. There were 2 (0.23%) episodes of subacute stent thrombosis and 11 (1.27%) patients had ISR.

Discussion

The PCI registry data described here comes from patients who underwent PCI under the government health scheme for poor at academic/university government hospital. Epidemiology of ischemic heart disease in India is of concern. According to the present PCI registry almost of half of the population belonged to young age strata in contrast to United States National Cardiovascular Data Registry (US NCDR) database were mean age of PCI patients was 64.6 ± 12.1 and Japanese PCI registry where the mean age was 68.2 ± 9.8 [15]. The PURE study [16] describing epidemiological factors in the causation of cardiovascular diseases across different countries helps us understand these contrary findings in different countries. Diet, nutrition, the prevalence of diabetes & hypertension, access to healthcare facilities, funding for treatment of disease, etc. are the major determinants in making of this epidemiological picture. Also, as per the report of National Interventional Cardiology (NIC), 2011 for data on Indian coronary heart disease, 13.6% of patients were below 40 years of age [17]. Almost one

quarter i.e. 23.66% of patients undergoing PCI were elderly. This data shows a significant increase in the elderly population undergoing PCI for coronary artery disease but still, the young population is the majority affected. 70.88% of males are affected which is almost similar across the world statistics. More than half were hypertensive & more than one quarter were diabetic. Smoking, tobacco chewing and alcohol intake were involved as a risk factor in decreasing order. Three-fourth of patients undergoing PCI had STEMI which is way beyond described in US NCDR (14.44%), Japanese registry (23.01%) and NIC data (29.5%). Almost half of STEMI still undergo thrombolysis, and PAMI is occasional treatment received by the patient. Elective PCI for CSA is the least proportion of the population (~ 7%) while 22.3% population had CSA in NIC PIC registry, 31.69% in US NCDR and 50.59% in Japanese registry. Five percent CTO intervention is equitable to 2.2% CTO in NIC registry, 3.2% US NCDR and 6.4% in Japenese registry. Patients undergoing PCI had LVEF as normal, mild, moderate and severe left ventricular dysfunction approximately one quarter in each level. LAD is the most common coronary artery that requires stenting and LAD with RCA is the most common double vessel disease encountered. Incidence of stent thrombosis and ISR is negligible to make out any inferences.

In conclusion, the epidemiological study of PCI data is of huge concern as it shows affection of young population predominately and gender inequality, suggesting primarily male disease. PCI is often sought in ACS and CSA is predominately treated medically. Thrombolysis remains the first treatment received by STEMI patients and PAMI is occasional. Single vessel disease is the most common form of coronary artery involvement with left anterior descending artery being the most commonly involved vessel. No mortality reported in approximately 2 years of follow-up with the negligible incidence of stent thrombosis or in-stent thrombosis shows the skill & knowledge of the operators of the centre and also presents real-world data for the second-generation stents and anti-platelet therapy used.

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Risk Factors Associated With Hip Fractures among Adult People in Babylon City, Iraq

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Keywords: Risk factor; Hip fracture; Elderly adult; Case-Control study; Osteoporosis

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Abstract

BACKGROUND: The fractures of hip joint considered as a serious problem in public health in the medical and socioeconomic issues, the incidence of the fracture neck femur is significantly increased with the increment of general population life span.

AIM: The goal of this study is to highlight and focus on the most important risk factor for the hip fractures in our Babylon society, and to improve our understanding of the medical and social aspects of these predisposing factors.

PATIENTS AND METHODS: A case-control study of older adults (above 60 years old). The study was done on tow samples. First, one consisting of 75 cases those having fracture neck femur considered as cases, and second sample as a control group, consisting of 150 people as a healthy control group having no fracture. A pre-tested questionnaire was prepared to collect data from both samples; the questionnaire included demographic data and information about potential risk factors of hip fracture.

RESULTS: Most of the people in the study samples in both groups were, married women, housekeepers, illiterate and from urban dwellers. There was highly significant association between case-control groups regarding, Continuous using of medication such as cortisone which was found to be a potential risk factor of hip fracture (Unadjusted OR = 3.636), low income was positively associated risk factor of hip fracture in this study (OR = 2.377), low milk intake, low sun exposure, tobacco smoking were positively associated with this health problem (OR = 1.794), while physical exercise was protective factor (OR = 0.489).

CONCLUSION: The highest risk factors associated with increased occurrence of hip fracture were using cortisone, Osteoporosis, tobacco smoking, consuming soft drinks, and less exposure to sunlight.

Introduction

The fractures of hip joint considered as a serious problem in public health in the issues of morbidity, treatment cost, long inpatient stay, social impact and mortality. With the significant improvement in medical services and increasing the general population life span, the incidence of the fracture neck femur is significantly increased as well, due to osteoporosis. It accounts for over 40% of the estimated burden of osteoporosis worldwide [1].

Among adults, 65 years of age and older, the hip fracture cause more than 340,000 hospitalisations per year. The older adults especially the women who have fragile bone due to osteoporosis have a high incidence of hip fracture as they tend to have frequent falls, as they usually have many comorbidities beside the general frailty due to age and week quadriceps muscles, the effects of medications for the transient ischemic arracks, anaemia, and heart diseases. Mortality rates post-hip fracture range between 12% and 32% per year [1], [2].

It is about 90% of all hip fracture occur in people older than 50 years. The incidence is doubling for each subsequent decade after the age of 50; it is 2-3 times higher in women than in men. And with the expansion of the old age group population, the number of hip fractures can also be expected to

increase, even if the age-related incidence of hip fracture remains unchanged [3].

In the United States, it is about one third only of the hip fractures occurring in men. While two thirds in females, as the males do not experience a precipitous decrease in endogenous sex hormones analogous to menopause. And usually, they have a shorter life span. The rates of females affection were stable in the US, but from (2000 to 2009) showed a declined [4].

Although it is only 30% of all hip fracture, occur in men, but the loss of independence after a hip fracture and the morbidity, mortality, is greater in men than women [4], [5].

Hip fractures still considered one of the most serious health care problems, in spite of much researchers concern. And in spite of some evidence of real declining in the prevalence rate of hip fractures, it is still considered as a persistent cause of excessive morbidity, unsatisfied life quality and early mortality among older adults. And because of the management protocols are not universally applied worldwide, it is expected to see an increased, rather than decreased annual incidence of hip fracture over the next few decades [6]. As it is known, there are intracapsular and extracapsular fractures; the AVN is the most dangerous complication of the intracapsular fractures [2].

Many risk factors need to be monitored and to take the appropriate action to treat, so we can prevent the osteoporosis and then decrease the prevalence rate of hip fractures.

Besides the age and osteoporosis, other possible risk factors may be the following: (cigarette smoking, lack of physical activity, medications that cause osteoporosis, disturbed mentality, consumption of excessive alcohol and caffeine, low body weight with tall stature, vision problems, generalised weakness, disability, or unsteady gait that increased risk for falls.) [7].

Importance of the study

Many risk factors are associated with hip fractures; maybe the age is the most important one. In the last few decades, some geographic variations noticed in the incidence among the elderly in different regions of the world. In recent systematic review concluded that the rate of hip fractures might be lower in Asian, but it is increasing with the time. This variation in incidence may be related to some etiological and environmental factors. In Taiwan, for example, the hip fractures are considered a serious medical and geriatric economic issue. Except for Japan, there are few studies about hip fractures in Asia. For that reason, there is a real need for epidemiological studies in Asia to assess the potential changes in the expected risk factors of hip fractures, to help the health administration authorities to put the proper plans and policies for the future care of those populations. It is important to know about the long term medical outcomes and how to prevent the second occurrence of hip fractures among older adults in Asia countries [8], [9], [10]. In the developed world countries, with the improvement in medical care and increased life expectancy, the hip fractures become a serious public medical problem, as the elderlies are liable for frequent falls due to generalised muscle weakness and many types of walking disabilities.

Although most of the older adults are osteoporotic but that is alone does not result in hip fracture without sustaining a trauma, so if we plan to decrease the rate of incidence of hip fractures we need to study the two aspects of the problem, the medical and social aspects of the problem for any policy of the hip fracture prevention to be successful [9], [10], [11], [12].

The goal of this study is to highlight and focus on the most important risk factor for the hip fractures in our Babylon society, and to improve our understanding of the medical and social aspects of these predisposing factor, so we can help the medical health authority to put the proper plan to prevent and treat older adults those vulnerable to sustain hip fractures.

Patient and Method

This is a case-control study. The sample was selected purposively (non-probability) of age group 60 years and above (Elderly), 75 cases of patients with hip fracture were taken and compared with 150 cases of a healthy control group without hip fracture. The study was conducted in (Babylon province/Iraq) from 21 July 2018 to 18 February 2019.

Figure 1: frequency distribution of old adult patients with hip fracture

Method of data collection: A questionnaire was used to collect data through the interview the participant in this study.

The questionnaire included a socio demographical characteristics of the patient like (age, gender, level of education, place of residence, occupation, smoking, exercises, family monthly income), any previous disease such as (DM,

Hypertension, Arthritis, asthma and other chronic disease, as well as any history of drug intake. And information about the fracture; the type of accident that leads to hip fracture, family history of hip fracture, did he do serum vitamin D test and its value (sufficient or nonsufficient).

Data analysis

The data were analysed using the statistical package of socio science program 24 (SPSS). Data were presented in tables, and graphs value less than 0.05 considers statistical significant in this study. The chi-square test was used to measure the associations between different variables. Odds ratio (OR) was collected to measure the risk of exposure.

Results

From Table 1, we can see that most of the population was older 70 – 79 age group 37.3%, and most of them were female 68%, most of them were currently married 52%, majority of them live at urban regions 64.3%, most of them the females are housewives 38.7%, economic status of most of them was not enough 68%, those with enough and more only 5.3%.

Table 1: Frequency distribution of participants with hip fractures (cases) by demographical data

Item	Frequency	Percentage
Age		
50 – 59	15	20%
60 – 69	18	24%
70 – 79	28	37.3%
80 – and higher	14	18.7%
Total	75	100%
Gender		
Male	24	32%
Female	51	68%
Total	75	100%
Marital status		
Currently Married	39	52%
Widowed	31	41,3%
Divorced	5	6.7%
Single	0	0
Total	75	100%
Address		
Urban	48	64.3%
Rural	27	36.7%
Total	75	100%
Educational level		
Cannot read and write	12	16%
Read and write	25	33.3%
Middle school	15	20%
Secondary school	11	14.7%
College and higher	9	12%
Total	75	100%
Occupation		
Housewife	29	38.7%
Have no work	17	22.7%
Retired	11	14.6%
Employee	13	17.3%
Military field	5	6.7%
Total	75	100%
Economic status		
Not enough	51	68%
Enough	20	26.7%
Enough and more	4	5.3%
Total	75	100%

From Table 2 we may notice that most of the population doesn't practice exercise 84%, most of them were not smokers 61.3%, and the reason of fractures in most of them was fall out 64%.

Table 2: Distribution of patients with hip fracture according to possible risk factors and health-related variables

Item	Frequency	Percentage
Do you practice exercise?		
Yes	12	16%
No	63	84%
Total	75	100.%
Smoking		
Smoker	29	38.7%
Not smoker	46	61.3%
Total	75	100%
Type of smoking		
Cigarette	22	75.9%
Bubble	7	24.1%
Others	0	0%
Total	29	100%
Reason of fracture		
Fall out	48	64%
Another reason	27	36%
Total	75	100%
Another current disease		
DM	20	26.7%
Hypertension	27	36%
Rheumatism	16	21.3
Osteoporosis	9	12
Others	3	4
Total	75	100%
Period stayed in the hospital		
Did not admitted	50	66.7%
Short period (1 - 5) days	16	21.3%
Middle period (6 –14) days	6	8%
Long-period 15 days &more	3	4%
Total	75	100%
Complication		
Presence	44	58.7%
Absent	31	41.3%
Total	75	100%
Presence of similar cases in the family		
Present	32	42.6%
Absent	43	57.3
Total	75	100%
Is any person take care of the patient?		
Yes	59	78.7%
No	16	21.3%
Total	75	100%
Have you ever attended a seminar on prevention of pelvic bones fracture or falls?		
Yes	6	8%
No	69	92%
Total	75	100%
Have you ever had a previous downfall?		
Yes	10	13.3%
No	65	86.7%
Total	75	100%
Take medication		
Yes	59	78.7%
No	16	21.3%
Total	75	100%
Type of medication		
Cortisone	20	33.8
Analgesic	8	13.6
DM drugs	16	21.3
Hypertension drugs	12	20.3
Others	3	5.1
Total	59	100%
Ingest milk and milk products daily		
Yes	27	36%
No	48	64%
Total	75	100%
Amount per day		
Little	14	51.8%
Medium	9	33.3%
Abundant	4	14.8%
Total	27	100%
Drink soft drinks more than twice a week		
Yes	48	64%
No	27	36%
Total	75	100%
Exposed to direct sunlight		
Yes	28	37.3%
No	47	62.7%
Total	75	100%
Period of exposed		
Continuously	5	17.8%
Weekly	13	46.4%
Sometimes	10	35%
Total	28	100%
Have you ever tested the vitamin D level?		
Yes	22	29.3%
No	53	70.7%
Total	75	100%
If yes, What are the results were?		
Low level	15	68.1%
Normal	7	31.8%
Total	22	100%

Hypertension was another current disease in 36% of them, 58.7% of them have got certain complications, family history was negative for having such fracture in 57.3%, and 78.7% have a person take care of the patient, 92% have not attended a seminar

about prevention of pelvic bone fractures or how to avoid falls, 78.7% currently taking medications, in 33.8% it was cortisone, 36% ingesting milk and milk products daily and 51.8% of them ingesting only little amounts per day, 37.3% have exposed to direct sunlight, 70.7% have never tested vitamin D level.

Table 3: Distribution of the study group according to demographic characteristics

Item	Cases	Controls
Age(year)	No. (%)	No. (%)
50 – 59	15 (20)	28 (18.7)
60 – 69	18 (24)	56 (37.3)
70 – 79	28 (37.3)	45 (30)
80 – and higher	14 (18.7)	21 (14)
Total	75 (100%)	150 (100%)
Gender		
Male	24 (32)	61 (40.7)
Female	51 (68)	89 (59.3)
Total	75 (100%)	150 (100%)
Marital status		
Currently Married	39 (52)	87 (58)
Widowed	31 (41.3)	59 (39.3)
Divorced	5 (6.7)	4 (2.7)
Single	0 (0)	0 (0)
Total	75 (100%)	150 (100%)
Place of residence		
Urban	48 (64)	98 (65.3)
Rural	27 (36)	52 (34.7)
Total	75 (100)	150 (100%)
Educational level		
Cannot read and write	12 (16)	18 (12)
Read and write	25 (33.3)	67 (44.7)
Middle school	15 (20)	35 (23.3)
Secondary school	11 (14.7)	20 (13.3)
College and higher	9 (12)	10 (6.7)
Total	75 (100%)	150 (100%)
Occupation		
Housewife	29 (38.7)	74 (49.3)
Have no work	17 (22.7)	37 (24.7)
Retired	11 (14.6)	12 (8)
Employee	13 (17.3)	16 (10.7)
Military field	5 (6.7)	11 (7.3)
Total	75 (100%)	150 (100%)
Economic status		
Not enough	51 (68)	43 (28.6)
Enough	20 (26.7)	82 (54.7)
Enough and more	4 (5.3)	25 (16.7)
Total	75 (100%)	150 (100%)

From Table 3 for those of (case group), indicated that most of the population were elderly(70 – 79 years) age group 37.3%, and most of them were female 68%, most of them were currently married 52%, majority of them live at urban regions 64%, most of them are housewives 38.7%, regarding the economic status most of them with(not enough) 68%.

While in the other Table of (control group) indicated that most of the population were ranging from(60 – 69 years) age group 37.3%, and also most of them were female 59.3%, most of them are currently married 58%, majority of them live at urban regions 65.3%, higher percentage of them are educated, at least they are reading and writing 44.7%, most of them were housewives 49.3%, economic status was enough in most of them 54.7% and those (not enough) only 28.6%.

From Table 4 if we look for the (case group), we may see, that most of the patients don't practice exercise 84%, most of them were not smokers 61.3%, and the smoker group of them (38.7%), those were using cigarette 75.9%, the reason of fractures was fall out in 64%. Hypertension was in 36% of them as another current disease, 58.7% present with complications, 57.3% have negative family history of hip fracture, 78.7% have a person takes care of the patient, 92% have not attended a seminar on prevention of pelvic bone fracture or fall prevention, 78.7% taking medication, in 33.8% it was cortisone, 36% ingest milk and milk products daily, and 51.8% were ingesting a little amounts per day, 37.3% have exposed to direct sunlight, 70.7% have not tested vitamin D level.

Table 4: Odds ratio of fracture hip among the (study group)

Item	cases	controls
Do you practice exercise?		
Yes	12 (16)	67 (44.7)
No	63 (84)	83 (55.3)
Total	75 (100%)	150 (100%)
Smoking		
Smoker	29 (38.7)	39 (26)
Not smoker	46 (61.3)	111 (74)
Total	75 100%	150 (100%)
Type of smoking		
Cigarette	22 (75.9)	24 (61.5)
Bubble	7 (24.1)	15 (38.5)
Others	0 (0)	0 (0)
Total	29 (100%)	39 (100%)
Reason of fracture		
Fall out	48 (64)	
Another reason	27 (36)	
Total	75 (100%)	
Another current disease		
DM	20 (26.7)	48 (32)
Hypertension	27 (36)	39 (26)
Rheumatism	16 (21.3)	11 (7.3)
Osteoporosis	9 (12)	5 (3.3)
Others	3 (4)	47 (31.3)
Total	75 (100%)	150 (100%)
Period stayed in the hospital		
Did not admitted	50 (66.7)	
Short period (1-5) days	16 (21.3)	
Middle period (6-14) days	6 (8)	
Long-period 15 days more	3 (4)	
Total	75 100%	
Complication		
Presence	44 (58.7)	
Absent	31 (41.3)	
Total	75 (100%)	
Presence of similar cases in the family		
Present	32 (42.7)	37 (24.7)
Absent	43 (57.3)	113 (75.3)
Total	75 (100%)	150 (100%)
Is any person take care of the patient?		
Yes	59 (78.7)	128 (85.3)
No	16 (21.3)	22 (14.7)
Total	75 (100%)	150 (100%)
Have you ever attended a seminar on prevention of pelvic bone fracture or falls?		
Yes	6 (8)	15 (10)
No	69 (92)	135 (90)
Total	75 (100%)	150 (100%)
Have you ever had a previous downfall?		
Yes	10 (13.3)	11 (7.3)
No	65 (86.7)	139 (92.7)
Total	75 (100%)	150 (100%)
Take medication		
Yes	59 (78.7)	90 (60)
No	16 (21.3)	60 (40)
Total	75 (100%)	150 (100%)
Type of medication		
Cortisone	20 (33.8)	7 (7.8)
Analgesic	8 (13.6)	28 (31.1)
DM drugs	16 (21.3)	24 (26.7)
Hypertension drugs	12 (20.3)	21 (23.3)
Others	3 (5.1)	10 (11.1)
Total	59 (100%)	90 100%
Drinking milk and milk products daily		
Yes	27 (36)	88 (58.7)
No	48 (64)	62 (41.3)
Total	75 (100%)	150 (100%)
Amount per day		
Little	14 (51.8)	25 (28.4)
Medium	9 (33.3)	39 (44.3)
Abundant	4 (14.8)	24 (27.2)
Total	27 (100%)	88 (100%)
Drink soft drinks more than twice a week		
Yes	48 (64)	53 (35.3)
No	27 (36)	97 (64.7)
Total	75 (100%)	150 (100%)
Exposed to direct sunlight		
Yes	28 (37.3)	116 (77.3)
No	47 (62.7)	34 (22.7)
Total	75 100%	150 (100%)
Period of exposed		
Continuously	5 (17.9)	39 (33.6)
Weekly	10 (35.7)	54 (46.6)
Sometimes	13 (46.4)	23 (19.8)
Total	28 100%	116 (100%)
Have you ever tested the vitamin D level?		
Yes	22.(29.3)	37 (24.7)
No	53 (70.7)	113 (75.3)
Total	75 (100%)	150 (100%)
If yes, What are the results were?		
Low	15 (68.1)	9 (24.3)
Normal	7 (31.8)	28 (75.7)
Total	22 (100%)	37 (100%)

While in the other column of Table 4 for the (control group) indicated that most of the population doesn't practice exercise 55.3%, most of them were not smokers 74%, but who are smoking are only (26%), 61.5% of them were using a cigarette. Another current disease was hypertension among 26% of them, 75.3% have negative family history for such fracture, 85.3% have a person take care of the patient, 90% have not attended a seminar on prevention of pelvic bone fracture or fall prevention, 60% are currently taking medication, only in 7.8% was the cortisone, 58.7% are daily ingested milk and milk products.

Table 5: Odds Ratios of hip fractures of exposed cases to different independent variables

Medical conditions as a risk factor of hip fracture	Cases		Controls		Odds ratio
	F	%	F	%	
Hypertension	27	36	39	26	1.384
DM	20	26.7	48	32	0.834
Rheumatism	16	21.3	11	7.3	2.917
Osteoporosis	9	12	5	3.3	3.636
Smoking	29	38.7	39	26	1.488
Don't practice exercise	63	84	83	55.3	1.245
Family income (Not enough)	51	68	43	28.6	2.377
Don't drink milk and products daily	48	64	62	41.3	1.549
Present similar cases in the family	32	42.7	37	24.7	1.728
Type of medication (Cortisone)	20	33.8	7	7.8	4.333
Analgesic	8	13.6	28	31.1	0.437
DM drugs	16	21.3	24	26.7	0.797
Hypertension drugs	12	20.3	21	23.3	0.858
Soft drink more than twice a week	48	64	53	35.3	1.813
Vitamin D (deficiency)	15	68.1	9	24.3	2.802

We may assess the medical conditions as a risk factor of hip fractures among study participants, as seen in Table 5 which shows the medical problems of the study sample which are considered as a risk factor among (case group), the highest percentage of the case sample had family income (not enough) (68%) and the highest percentage of osteoporosis (12), while in control group only (28.6) of them was the level of family income (not enough), and the lowest percentage of having osteoporosis (3.3%) and type of medication (cortisone) was the lowest percentage (7.8%) comparing with 33.8% in the case group.

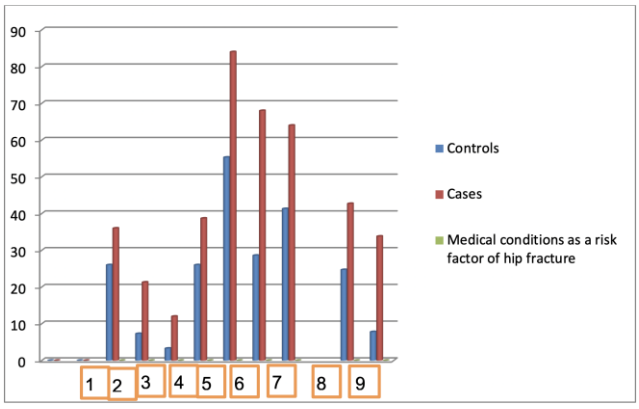

Figure 2: Odds Ratio of risk factors; 1-HRT; 2-Rheumatism; 3-osteoporosis; 4-smoking; 5-lack exercise; 6-income not enough; 7-no milk intake; 8-family history +ve; 9-cortisone intake

The Odds Ratio of different medical problems among study participants in both case and control groups as associated risk factors, are shown in Figure 2, as following: hypertension (OR = 1.384), DM (OR = 0.834) ,Rheumatism (OR = 2.917), Osteoporosis (OR = 3.636), Smoking (OR = 1.488), Don't practice exercise (OR = 1.245), Family income (Not enough) (OR = 2.377), Don't drinking milk and products daily (OR = 1.549), Present similar case in the family (OR = 1.728), Type of medication (cortison) (OR = 4.333), Anxiolytic (OR = 0.437), Hypertension drugs (OR = 0.858), DM (OR = 0.797), Soft drink more than twice a week (OR = 1.813), vitamin D deficiency (OR = 2.802).

Discussion

There is very little information available about the epidemiology of hip fractures in Iraq, and the most significant risk factors of this disabling fracture. Preventing the occurrence of hip fracture is a public health priority in our society, given the ongoing transition to the super-ageing society. The risk for hip fractures can be reduced by preventing falls. It is therefore important to identify those individuals most at risk of falling to maximise the effectiveness of any proposed public health and family nursing interventions [13].

Regarding the socio-demographic characteristics of the study sample, most of them in both case and control groups were illiterate, married, keeping homemakers, from an urban area.

The results in this study go with the findings of the study which was done by Coutinho et al. Many medical conditions that are considered as a risk factor of hip fracture among elderly adults, most of them have an increased associated risk factor with hip fracture like Osteoporosis, Hypertension and using antihypertensive drugs, ambulation problems like osteoarthritis of knee joints, history of hip fracture, and history of falls respectively [14], [15].

In our study, the results are almost similar to what had been mentioned by Suzuki et al., in Japan, who found that these medical disorders were highly associated with increased risk factors of hip fractures in elderly. And also goes with the findings of other articles like that done by Ribeiro et al., in 2014 in South Brazil, and that of Welfare in 2010, done for Australian Institute of Health [16], [17], [18].

In our study, we studied the pattern of lifestyle of the participants, (Milk and dairy products and its amount per day, sun exposure and how often per week, smoking, and physical exercises). Those who have a little physical exercise (less than four times per week) have increased risk of getting hip fractures. Also, we found the role of currently heavy smoking is a risk factor of hip fracture, and in some studies, they

considered the smoking is a greater risk of hip fracture, whether the patient is ex- or current smoker in comparison to those who do not smoke [14].

In this study, we found that there is a positive association between low income and hip fracture. This finding goes with findings of other researches abroad [19], [20]. Despite the problem of hip fractures considered a great public problem [19], [21], [23], actually a few studies only that have analysed the association between hip fractures and socioeconomic status, so it remains unclear.

Some of the articles found an increased incidence [20], [22], [23], [24], [25], [26], [27] while others found a decreased incidence of Hip fractures with low socioeconomic status [28], [29]. Some articles found an association with only some socioeconomic indicators [30], [33]. In another status, they found an increased risk of hip fractures of some socioeconomic indicators and decreased or no association of other socioeconomic markers [34], [38]. However, we may conclude that although it is an important factor, not all the studies found a direct association between the hip fractures and socioeconomic status [39], [40].

We recommend an educational program should be done to raise the level of awareness among older adults and their families about the preventive measures, to avoid the risk factors of hip fractures, avoidance of miss use of medication, encouraging physical exercise among elders but protecting them from fall.

In summary, osteoporosis, smoking, lack of physical exercise and lack of sunlight exposure, less milk and milk products intake, heavy consumption of soft drinks, were the main risk factors for hip fracture among elderly persons.

Ethical Approval

All the variable information consents of the patients and control groups were taken after explaining the purpose of the study to the patients, Those who refused to participate in the study were excluded. And all are approved according to the ethical standards of our institutional research committee.

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Active Smoking is Associated with Lower Dialysis Adequacy in Prevalent Dialysis Patients

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Abstract

BACKGROUND: Dialysis adequacy measured by single pool Kt/V (spKt/V) lower than 1.2 or urea reduction rate (URR) lower than 65% is associated with a significant increase in patient mortality rate. Patients' adherence to the medical treatment is crucial to achieve recommended targets for spKt/V. Smoking is a recognized factor of non-adherence.

AIM: In this study we sought to assess the association of active smoking and dialysis adequacy.

METHODS: A total of 134 prevalent dialysis patients from one dialysis center were included in an observational cross-sectional study. Clinical, laboratory and dialysis data were obtained from medical charts in previous 6 months. The number of missed, on purpose interrupted or prematurely terminated dialysis sessions was obtained. Dialysis adequacy was calculated as spKt/V and URR. Patients were questioned about current active smoking status. T-test and Chi-Square test were used for comparative analysis of dialysis adequacy with regard to smoking status.

RESULTS: The majority of patients declared a non-smoking status (100 (75%)) and 34 (25%) were active smokers. Male gender, younger age and shorter dialysis vintage were significantly more often present in the active smokers ((9 (26%) vs 25 (73%), p = 0.028; 57.26 ± 12.59 vs 50.15 ± 14.10, p = 0.012; 118.59 ± 76.25 vs 88.82 ± 57.63, p = 0.030)), respectively. spKt/V and URR were significantly lower and Kt/V target was less frequently achieved in smokers ((1.46 ± 0.19 vs. 1.30 ± 0.021, p = 0.019; 67.14 ± 5.86 vs. 63.64 ± 8.30, p = 0.002; 14 (14%) vs. 11 (32%), p = 0.023), respectively. Shorter dialysis sessions, larger ultra filtrations and higher percentage of missed/interrupted dialysis session on patients' demand were observed in smokers (4.15 ± 0.30 vs. 4.05 ± 0.17, p = 0.019; 3.10 ± 0.78 vs. 3.54 ± 0.92, p = 0.017; 25 (0.3%) vs. 48 (1.8%), p = 0.031), respectively.

CONCLUSION: Active smokers, especially younger men, achieve lower than the recommended levels for dialysis adequacy. Non-adherence to treatment prescription in smokers is a problem to be solved. Novel studies are recommended in patients on dialysis, to further elucidate the association of dialysis adequacy with the active smoking status.

Introduction

Dialysis adequacy measured by single pool Kt/V (spKt/V) lower than 1.2 or urea reduction rate (URR) lower than 65% is associated with a significant increase in patient mortality rate [1], [2]. Duration of dialysis session [2], [3], blood flow [4], [5], adequate vascular access [6] and dialyzer membrane surface [3] influence dialysis adequacy. Patients adherence to the medical treatment is crucial to achieve recommended targets for spKt/V [7], [8]. Smoking is a recognized factor of non-adherence, and it is associated with chronic kidney disease [9], [10]. Patients on chronic dialysis are burdened not only by the disease itself, but also by the treatment regime, dialysis prescription and many diet restraints. Non-compliance is well known among dialysis patients, affecting their dialysis adequacy [11], [12]. Recent studies recognize smoking as a hazard for morbidity and mortality in dialysis patients [13], [14]. In this study we sought to assess the association of active smoking and dialysis adequacy.

Material and Methods

A total of 134 prevalent dialysis patients from one dialysis center were included in an observational cross-sectional study. Dialysis duration was prescribed for 4-5 hours, three times per week. Low flux dialyzers with membrane surfaces from 1.3 to 1.8 m^2 were used. Dialysis vintage of less than 9 months, twice a week dialysis prescription and significant residual renal function were exclusion criteria. Clinical, laboratory and dialysis data were obtained from medical charts for the previous 6 months. Number of missed or on purpose interrupted/prematurely terminated dialysis sessions was noted. Dialysis adequacy was calculated as spKt/V and URR. Patients were questioned about their current active smoking status. T-test and Chi-Square test were used for comparative analysis of dialysis adequacy with regard to smoking status.

Results

Sociodemographic, clinical and laboratory data are shown in Table 1. The mean age of study participants was over 55 years, 57% were men and the dialysis vintage was 111.034 months. Diabetes was present in 18% of the patients and 25% were active smokers. Anemia was managed to mean level of hemoglobin 116.36 ± 8.45 g/L. The mean albumin level was 38 g/L, CRP was 7.06 g/L and BMI 23.74 Kg/m^2.

Table 1: Sociodemographic, clinical and laboratory data of the study population

N = 134	Mean ± SD
Age (years)	55.45 ± 13.33
Dialysis vintage (months)	111.034 ± 72.95
Men (%)	76 (57%)
Diabetes (%)	24 (18%)
Active smokers (%)	34 (25%)
Hemoglobin (g/L)	116.36 ± 8.45
Albumin (g/L)	38.45 ± 2.54
C-reactive protein (mg/L)	7.06 ± 8.71
Body Mass Index (kg/m^2)	23.74 ± 4.6

Dialysis variables are shown in Table 2. The mean dialysis adequacy measurements spKt/V and URR were in recommended target ranges: 1.38 ± 0.20 and 66.27 ± 6.7%, respectively. The mean dialysis session time was 4.08 hours, while the mean ultrafiltration volume per dialysis session was 3.22 litres.

Table 2: Dialysis variables

N = 134	Mean ± SD (%)
Catheter as vascular access	5 (4%)
Kt/V	1.38 ± 0.20
URR (%)	66.27 ± 6.7
Dialysis session time (hours)	4.08 ± 0.21
Ultrafiltration (L)	3.22 ± 0.84

The comparative analysis between patients with regard to smoking status is presented in Table 3.

Table 3: Comparative analysis between patients regarding smoking status

variable	Non Smokers N = 100	Active smokers N = 34	p
Men	9 (26%)	25 (73%)	0.028
Diabetes	15(63%)	9 (37%)	0.194
Catheter	5 (5%)	0 (0%)	0.329
Age (years)	57.26 ± 12.59	50.15 ± 14.10	0.012
Vintage (months)	118.59 ± 76.25	88.82 ± 57.63	0.030
Albumin (g/L)	38.66 ± 2.54	39.40 ± 2.54	0.156
Hb (g/L)	116.63 ± 8.89	115.57 ± 7.03	0.487
CRP (mg/L)	6.87 ± 7.54	7.62 ± 11.56	0.669*
BMI (Kg/m^2)	23.74 ± 4.7	23.77 ± 4.33	0.966
Kt/V	1.46 ± 0.19	1.30 ± 0.021	0.019
Kt/V < 1.2	14 (14%)	11 (32%)	0.023
Time (hours)	4.15 ± 0.30	4.05 ± 0.17	0.019
URR (%)	67.14 ± 5.86	63.64 ± 8.30	0.002
UF (L)	3.10 ± 0.78	3.54 ± 0.92	0.017

* non-parametric test was applied.

Out of 58 women, 9 (15%) were active smokers and out of 76 men, 25 (33%) were active smokers. Men had 1.26 higher odds to be smokers (OR 1.26 95% CI: 1.229-2.516), p = 0.028 (Figure 1).

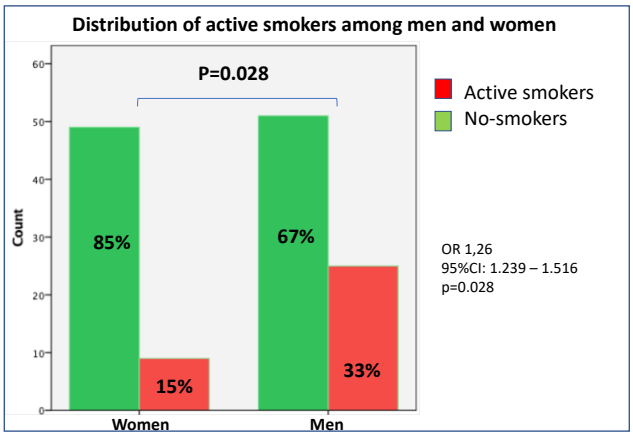

Figure 1: Distribution of gender and smoking habits

Non-smokers were significantly longer time on dialysis (118.59 ± 76.25 *vs.* 88.82 ± 57.63, p = 0.030, respectively). The mean age of patients who smoked was 50.15 and of non-smokers 57.26 years and this difference was significant, as shown in Figure 2.

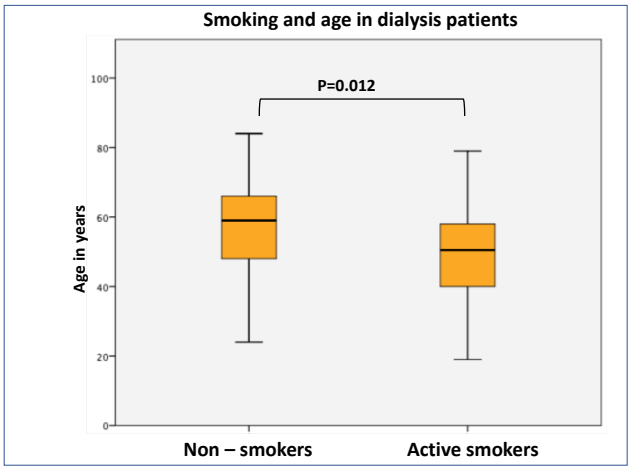

Figure 2: Distribution of age according to smoking status

The two groups did not differ in the laboratory values for albumin, hemoglobin and CRP as well as for BMI. In terms of dialysis variables, in non-smokers out of 7700 prescribed dialysis sessions, [25] 0.3% were missed/interrupted and for the 2618 dialysis sessions in active smokers that percentage was significantly higher: (48)1.8%, (p = 0.031). Also, the mean time per dialysis session in non-smokers lasted longer and the ultrafiltrations were lower than in smokers (4.15 ± 0.30 *vs.* 4.05 ± 0.17, p = 0.019; 3.10 ± 0.78 *vs.* 3.54 ± 0.92, p = 0.017), respectively. Non-smoking patients achieved better adequacy when calculated spKt/V and also URR (1.46 ± 0.19 *vs.* 1.30 ± 0.021, p = 0.019; 67.14 ± 5.86 *vs.* 63.64 ± 8.30, p = 0.002), respectively. Considering targets for spKt/V > 1.2, lower percentage of patients with spKt/V below 1.2 were present in non-smokers (14 (14%) *vs* 11 (32%), p = 0.023), respectively.

Discussion

In spite of therapeutic and medical technology improvements, patients receiving renal replacement treatment still have low survival rates. In the latest European Renal Association-European Dialysis and Transplant Association Registry Annual report for patients commencing RRT during 2006-10, the 5-year unadjusted patient survival probabilities on all RRT modalities combined was 50.0% (95% confidence interval 49.9-50.1) [15]. There is still a real need for recognizing the modifiable factors influencing the high mortality. In dialysis patients both traditional and non-traditional factors affect the all-cause and cardiovascular mortality [16]. The interplay among those factors aggravates the burden of comorbidities and survival. In this study we sought to associate the dialysis adequacy and compliance with smoking, as both are well recognized survival factors in dialysis patients [2], [12]. There are not many published studies on this matter, but in general, smoking is, non-debatably, a risk hazard for human health [17], and it is also connected with acceleration of chronic kidney disease [10], [18], [19]. It is known that chronic exposure to nicotine increases or upregulates the number of high-affinity nicotine binding sites and the receptors undergo long lasting changes and desenzitization which explains the receptor modulation and addiction to nicotine [20]. Perry *et al*., found markedly higher nicotine levels in hemodialysis patients compared to control subjects, when measured before and after hemodialysis, and also after several hours [21]. To our knowledge, there are no studies on nicotine dialyzability [22]. We assume that if dialysis does not provide adequate clearance of nicotine, non-compliance and shortening dialysis sessions would aggravate the problem of addiction. A recent meta-analysis of 26 studies and 6536 dialysis patients showed that active smoking is associated with a significant increase in all-cause mortality [14]. The percentage of active smoking was 15%, but the CHOICE study implied that the number was mostly underestimated [23]. In our study that percentage was much higher (25%) which is in line with the Longeneckers study [20]. Compliance to life style is in general less achieved in younger generations and it especially affects smoking [24]. Considering the association of age and smoking, we found younger persons to be more likely smokers, and this finding is in agreement with other dialysis groups [14] as well. A limitation of our study could be that we did not clarify if non-compliance was due to smoking, or maybe to younger age and male sex, which was more commonly present in the smoking group. Also, we cannot underestimate the lower dialysis vintage of the smoking group as a potential factor for non-compliance because these patients could presumably be less well educated and experienced, and thus comply less well to the rules that are beneficial to them. We studied compliance to dialysis prescription through time of dialysis session, ultrafiltration and number of skipped/interrupted dialysis sessions. Smokers performed worse on all of these markers resulting in lower spKt/V values. Patients who smoked had significantly lower Kt/V, URR and achieved lower than the recommended values for Kt/V. With this study we associated non-compliance with lower dialysis adequacy. Considering the prognostic value of KtV for survival [1], [2], we found education of young patients for smoking cessation of great importance. Our study was performed in patients treated by low-flux hemodialysis. We consider this as a limitation. Further studies should address dialysis adequacy in patients treated by high-flux hemodialysis, since it is today current treatment modality.

In conclusion, active smokers, especially younger men, achieved lower than the recommended levels for dialysis adequacy, when treated with low-flux dialysis. Non-adherence to dialysis treatment in smokers was an additional problem to be solved. Novel studies are recommended in patients treated with high-flux dialysis to further elaborate the association between active smoking status and dialysis adequacy.

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Clinical Science

Osteopontin for Early Detection of Microvascular and Macrovascular Type 1 Diabetic Complication

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Keywords: Osteopontin; cIMT; Urinary albumin/creatinine ratio; Type 1 diabetes

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Abstract

AIM: To evaluate the relationship between osteopontin and diabetes complication in type 1 diabetic patient.

PATIENTS AND METHODS: Seventy types 1 diabetic and 60 healthy volunteers were studied. Full history, examination, laboratory tests of glycosylated haemoglobin (HbA1c), serum lipids {cholesterol, triglyceride (Tg), high-density lipoprotein-cholesterol (HDL-c), low-density lipoprotein – cholesterol (LDL-c)}, oxidised low-density lipoprotein (OxLDL), Osteopontin and urinary microalbuminuria (albumin/creatinine ratio) were done. Image study in the form of a carotid intimal medial thickness (cIMT) and aortic intimal medial thickness (aIMT), renal doppler for resistivity index was also done for all participant included in the study.

RESULTS: Urinary albumin/creatinine ratio, lipid profile, osteopontin, cIMT and aIMT were higher in people with diabetes. Osteopontin was higher in people with diabetes with positive microalbuminuria and cIMT. Systolic blood pressure, microalbuminuria and cIMT had a positive correlation with osteopontin in people with diabetes. Stepwise multiple regression analysis showed that osteopontin had a significant correlation with cIMT. Receiver operating characteristic (ROC) curve showed that the cut off value of Osteopontin for detection of cIMT was > 60 with a specificity of 100% and sensitivity 80.5%, while that of albumin/creatinine ratio was > 64 with a specificity of 66.7 and sensitivity of 92.3.

CONCLUSION: Osteopontin is higher in type 1 diabetics and is useful for early detection of diabetic microvascular and macrovascular complication.

Introduction

Osteopontin (OPN) is an adhesive molecule, rich in phosphorylated sialic acid and is a way between cells and minerals [1]. It increases in autoimmune diseases and chronic inflammation and is important for cytokine production in macrophages, dendritic cells, and T-cells [2]. It has a role in the occurrence of adipose tissue inflammation and insulin resistance [3]. It increases in aortic atherosclerotic plaques and other cardiovascular diseases [4]. OPN is useful for early detection of coronary calcification [5], diabetic retinopathy [6] and nephropathy [7] in type 2 diabetes (T2DM).

cIMT and aIMT is an easy, useful, non-invasive method for early diagnosis of subclinical atherosclerosis in type 1 diabetic patients [8], [9]. From our knowledge, a very little publication was done for estimation of the relationship of OPN with a diabetic complication in adolescent type 1 diabetes. We aimed to assess the serum level of OPN in adolescent type 1 diabetes and to detect the association between OPN and diabetic complication (micro and macrovascular).

Patients and Methods

Seventy adolescent type 1 diabetics with duration of diabetes more than 5 years and 60 healthy volunteers from the endocrine clinic, Medical Center of Excellence, National Research Centre were enrolled in this cross-sectional study after taking approval from the ethical committee of the National Research Centre and a written consent from Patients

or their parents and healthy volunteers. Patients presented with any cardiac or vascular disease, hypertension, familial hyperlipidemia, receiving other medication rather than insulin were excluded from the study.

A detailed history was taken; the general and local examination was done for all diabetics and controls. Anthropometric assessment including weight, height, midarm circumference, waist circumference and hip circumference were done. Calculation of body mass index (kg/height (m^2), waist/hip ratio and waist/height ratio were done. Blood pressure was measured 3 times after 5-time rest in the sitting position by mercury sphygmomanometer. Venous blood samples were obtained from each subject in a sterile EDTA vacutainer tube for measuring glycated haemoglobin (HbA1c). It was measured using The NycoCard READER II (Alere Technologies AS, Kjelsåsveien 161, P.O. Box 6863 Rodeløkka, NO-0504 Oslo, Norway). Glycated haemoglobin (HbA1c) was done every 3 months, and the mean value was calculated per year.

A 2^{nd}-morning urine sample was taken for diagnosis of microalbuminuria by measuring albumin/creatinine ratio. Microalbuminuria was diagnosed if the albumin/creatinine ratio was 30-299 µg/g creatinine (measured by an immuno-nephelometric method) in 2 out of 3 samples (6 months period) done every 2 months. Fasting venous blood (12 hr) for determination of lipid profile was done [10]. LDL-c (by Friedewald equation) and Tg (in a Techno Con AutoAnalyzer II, Tarrytown, NY, USA) were calculated. Serum OPN and OxLDL determination by ELISA kit (PELO-BIOTECH GmbH, Germany) were also done.

Carotid intimal medial thickness cIMT

Carotid Doppler was done by ultrasound General Electric: Vivid 7 Pro, GE Vingmed ultrasound AS-Nl90, Horton-Norway, 7.5 – 10 MHz linear-array transducer. Doppler was done in the supine position with an extended neck and turned head 45° to contralateral side [11]. The average of 3 measurements each side was taken for calculation of cIMT [12], [13].

Aortic intimal medial thickness aIMT

Abdominal aorta till aortic bifurcation was assessed by using 7.5 MHz pediatric phased array transducer. Aortic intimal medial thickness was measured by 10 MHz linear array transducer [14], [15]. The average of 3 measurements was taken to calculate the aIMT.

Renal Doppler

Renal colour duplex ultrasound scans using 3-6 MHz convex array transducer (Toshiba, Xario ultrasound machine). Patients were scanned in the supine position. The transducer was placed in a longitudinal position just to the Lt. of the midline, recording colour flow and Doppler spectrum from the abdominal aorta where peak systolic velocity of the abdominal aorta was recorded. Then, the transducer was placed in transverse position just distal to the origin of superior mesenteric artery, to achieve transverse view of the aorta at the origins of both renal arteries where peak systolic velocity of both renal arteries was recorded, and renal artery stenosis was ruled out in all patients by tracing and examining different segments of both renal arteries from origin to renal hilum. Then, resistivity indices were recorded in the segmental, interlobar and arcuate arteries, on both sides [16], [17].

Statistical Analysis

Statistical analysis was conducted using Statistical Package for Social Science (SPSS) program version 20 (Chicago, Illinois, USA) t-tests for independent variables was done. Pearson's correlation, followed by stepwise multiple regression analysis, was done. Receiver operating characteristic curve (ROC curve) was also done to detect sensitivity and specificity of OPN about cIMT and albumin/creatinine ratio.

Results

Diabetic patients had higher urinary albumin/ creatinine ratio, lipid profile (total cholesterol, Tg, LDL-c), OxLDL, osteopontin, cIMT and aIMT (Table 1).

Table 1: Comparison between demographics, laboratory data, anthropometric and image study of diabetic patients and controls

Variables	Patients N = 70		Controls N = 60		P-value
	Mean	SD	Mean	SD	
Age of diabetics (yrs)	17.99	2.59	17.50	2.67	0.6
Systolic blood pressure (mmHg)	118.45	13.33	123.75	10.61	0.30
Diastolic blood pressure (mmHg)	76.55	10.06	80.00	10.69	0.40
Midarm circumfrence (mm)	75.14	379.53	25.79	4.41	0.30
Body mass index (kg/m^2)	24.44	3.89	21.86	6.47	0.30
Waist/hip ratio	0.88	0.08	0.88	0.07	0.90
Waist/height ratio	0.51	0.07	0.48	0.10	0.40
Albumin/creatinine ratio (µg/g creatinine)	71.94	73.49	11.27	4.28	0.0001
Total cholesterol (mg/dl)	194.86	63.65	159.94	22.20	0.0001
Triglyceride (mg/dl)	106.59	53.12	88.21	30.37	0.03
HDL-c (mg/dl)	49.31	16.35	48.78	10.01	0.40
LDL-c (mg/dl_)	116.49	39.10	100.74	28.60	0.03
OXLDL (mg/dl)	4.33	1.42	2.66	1.37	0.0001
Osteopontin (mg/ml)	75.12	20.90	18.71	3.17	0.0001
Carotid intimal medial thickness (mm)	0.52	0.06	0.41	0.03	0.0001
Aortic intimal medial thickness (mm)	0.72	0.11	0.46	0.04	0.0001
Resistivity index	0.67	0.04	0.65	0.05	0.30

LDL: Low-density lipoprotein; HDL: high-density lipoprotein; OxLDL: Oxidized low-density lipoprotein.

Diabetic patients with higher urinary

albumin/creatinine ratio and cIMT had a higher level of serum osteopontin (Table 2).

Table 2: Comparison between osteopontin about microalbuminuria and to a carotid intimal medial thickness in type 1 diabetic patients

Osteopontin (mg/ml)	Negative microalbuminuria		Positive microalbuminuria		P-value
	Mean	SD	Mean	SD	0.01
	63.20	16.44	83.38	23.48	
Osteopontin (mg/ml)	Negative cIMT		Positive cIMT		
	Mean	SD	Mean	SD	0.0001
	53.25	7.23	77.20	20.55	

cIMT: carotid intimal medial thickness.

Osteopontin had a relationship with albumin/creatinine ratio, systolic blood pressure and cIMT (Table 3).

Table 3: Correlation between Osteopontin with demographics, laboratory data, anthropometric data and image study of diabetic patients

Variables	Osteopontin	
	r	P-value
Demographic data:		
Age of diabetic patients (yrs)	0.10	0.44
Duration of diabetes (yrs)	0.09	0.52
Onset of disease (yrs)	0.22	0.10
Insulin dose (u/kg)	0.08	0.55
Blood pressure:		
Systolic blood pressure (mmHg)	0.28	0.03
Diastolic blood pressure (mmHg)	0.01	0.92
Anthropometric data:		
Midarm circumference (mm)	0.07	0.62
Body mass index (kg/m2)	0.03	0.85
Waist/ hip ratio	0.10	0.45
Waist/height ratio	0.12	0.40
Laboratory data:		
HbA1c (%)	0.19	0.16
Urinary albumin/ creatinine ratio (µg/g creatinine)	0.48	0.0001
Total cholesterol (mg/dl)	0.04	0.76
Triglyceride (mg/dl)	0.17	0.25
HDL-c (mg/dl)	0.04	0.80
LDL-c (mg/dl)	0.06	0.70
OxLDL (mg/dl)	0.01	0.92
Image study:		
carotid intimal medial thickness(mm)	0.64	0.0001
Aortic intimal medial thickness (mm)	0.1	0.6
Resistivity index	0.15	0.26

HbA1c: glycosylated haemoglobin; LDL: Low-density lipoprotein; HDL: high-density lipoprotein; OxLDL: oxidised low-density lipoprotein.

Stepwise multiple regression analysis of osteopontin as a dependent factor with demographic, anthropometric, laboratory data and image studies as an independent variable of diabetic patients revealed that osteopontin had a relationship with cIMT (Table 4).

Table 4: Stepwise multiple regression analysis of osteopontin about demographics, anthropometric data, laboratory data and image study in type 1 diabetic patients

	Unstandardized coefficent		Standardized coefficent		
	B	SE	Beta	t	P-value
(Constant)	-76.75	42.13		-1.82	0.09
cIMT (mm)	290.23	76.43	0.67	3.80	0.0001

Dependent variables are osteopontin; cIMT: carotid intimal medial thickness.

ROC curve of osteopontin demonstrate that the area under the curve (AUC) of cIMT was 0.9 with cut off value > 60 and high specificity and sensitivity (100, 80.5% respectively), on the other hand, AUC of urinary albumin/creatinine ratio was 0.8 with cut off value > 64, specificity and sensitivity (66.7 and 92.3 respectively) (Table 5).

Table 5: ROC curve of osteopontin about carotid intimal medial thickness and albumin/ creatinine ratio in type 1 diabetic patients

Variables	Cut off	AUC	SE	95%CI	Sensitivity	Specificity	+LR	-LR
cIMT	> 60	0.9	0.05	0.8 -1.0	81.5	100	0.2	100
Albumin/creatinine ratio (µg/g creatinine)	> 64	0.8	0.1	0.7 – 0.9	92.3	66.7	2.8	0.1

Discussion

In the current study, diabetic patients had higher albumin/creatinine ratio, lipid profile (cholesterol, Tg, LDL-c), OxLDL and aIMT were higher than cIMT which is comparable with [15], [18] who revealed that dyslipidemia is a risk factor of cardiovascular disease. Several previous studies revealed that cIMT and aIMT are increased in type 1 diabetic patients indicating the early occurrence of subclinical atherosclerosis [19], [20].

In our study, all diabetic patients had higher OPN, and it is particularly higher in those with a diabetic complication in the form of diabetic nephropathy (positive microalbuminuria) or atherosclerosis (in the form of increased cIMT) which is comparable with the previous study [19].

Gordin et al., [21] demonstrated that adult type 1 diabetic patient with diabetic nephropathy had higher OPN and in follow up study patients who had higher baseline OPN level develop diabetic nephropathy (microalbuminuria or macroalbuminuria), cardiovascular or retinal disease later on.

OPN had a relationship with systolic blood pressure, albumin/creatinine ratio and cIMT in our diabetic patients and no relation was found with glycemic control and stepwise multiple regression analysis revealed that the most important factor related to OPN is the cIMT.

On the contrary, Abo El-Asrar et al., [19], reported that uncontrolled diabetes, microalbuminuria is associated with high OPN level. Also, a previous study revealed that high serum level of OPN is associated with long duration of diabetes, increase in waist/ hip ratio, high systolic blood pressure, microalbuminuria and increase high sensitive CRP (hs CRP) level and no relation was found with glycemic control (HbA1c) [21].

ROC curve of OPN in our study was done to estimate the cut off level at which we can early predicate patients with complication (cIMT and microalbuminuria). The area under the curve (AUC) of cIMT and microalbuminuria were 0.9 and 0.8 respectively with cut off value > 60 and high specificity

and sensitivity (100, 80.5% respectively) for cIMT and cut off value > 64, specificity and sensitivity (66.7 and 92.3 respectively) for urinary albumin/creatinine ratio. Our study is comparable with Abo EL Asrar et al., [19] study which found that 90 is a cut-off value of OPN for detection of microvascular complications with a sensitivity and specificity of 81.7, 95.8% respectively and AUC is 0.8.

Yan et al., [22] revealed that OPN is associated with the development and degree of nephropathy and coronary affection in diabetic patients and can be used as a predictor of diabetic vasculopathy while Berezin and Kremzer [5] found that OPN can be used in coronary heart disease type 2 diabetic patients as an early marker of coronary artery calcification.

In conclusion, type 1 diabetic patients had subclinical atherosclerosis that can be diagnosed easily by non-invasive and an easy method cIMT and aIMT. OPN is increased in adolescent type 1 diabetic patients and can be used as an early marker for the diagnosis of diabetic nephropathy and subclinical atherosclerosis.

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Case Report

An Unusual Case of Anteriorly Displaced Posterior Cruciate Ligament Tibial Attachment Avulsion Injury

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Abstract

BACKGROUND: Avulsion injury to the tibial attachment of posterior cruciate ligament (PCL) is frequently occur. The purpose of this report is to highlight an unusual variation of PCL avulsion injury.

CASE PRESENTATION: A 24-year-old female was suffered a motorcycle accident 1 day before admission. The patient complained of severe right knee pain. Plain radiograph and CT-scan revealed an anteriorly displaced tibial attachment PCL avulsion fracture. Open surgical fixation was done to the patient. A satisfactory outcome was observed until the final 1-year follow-up.

CONCLUSIONS: The bony fragment of the tibial attachment of posterior cruciate ligament avulsion injury can be displaced to the anterior compartment of the knee. Concomitant injury to other knee structures should be suspected when finding this case. Special consideration is also needed during the management of this unusual case.

Introduction

A posterior cruciate ligament (PCL) is one of the most important structures of knee stabiliser. It prevents tibia to posteriorly translated especially during knee flexion and rotation. PCL is a strong ligament. Therefore avulsion injury to the tibial attachment frequently occurred [1]. It may lead to chronic knee pain, instability, further injury to other knee structures and induced early joint arthrosis if left untreated [1], [2]. The treatment itself varied from conservative treatment, surgical open reduction-internal fixation to arthroscopically assisted fixation [2], [3], [4].

Rademakers *et al.,* [5] adapted the Meyers and McKeever classification [6] of anterior cruciate ligament (ACL) for into PCL avulsion injury. It was described that PCL Avulsion could be classified into three grades: Grade I: non-displaced; Grade II: partially displaced; and Grade III: complete displaced [5]. We reported a case of grade III PCL avulsion injury which the bony fragment was displaced to the anterior compartment of the knee in a young female patient. To our knowledge, there has been no previous literature reporting this variation of the PCL avulsion injury. Informed consent was obtained from the patient before this publication.

Case Presentation

A 24-year-old female was suffered a motorcycle accident 1 day before admission. The patient complained of severe right knee pain. She was brought to emergency department at a district

hospital. Plain radiograph and CT-Scan of the knee was also performed at the district hospital. She then requested to be referred to Prof. Dr R. Soeharso Orthopaedic Hospital for further management.

Physical examination revealed a severely swollen knee, with the sign of skin brushing and excoriation at the anterior part of the knee (Figure 1A). There was tenderness both on the anterior and posterior part of the knee. Neurovascular status was normal. Knee joint range of motion was limited to 90 degrees. A positive result was obtained on the posterior drawer test. Anterior drawer, valgus-varus test were negative. The patient refused any other complaints on another part of the body. Plain radiograph of the knee revealed a large bony fragment at the anterior compartment of the knee (Figure 1B).

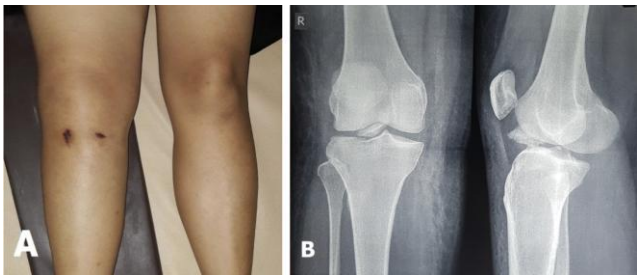

Figure 1: Clinical picture of the knee at 1 day after trauma. The right knee was swollen with skin brushing and excoriation on anterior part A); Preoperative plain radiograph of the knee. The displaced fragment located at anterior compartment of the knee, masquerading of an ACL fragment B)

An avulsion injury to the anterior cruciate ligament (ACL) was suspected based on the plain radiograph results. However, the result of CT was different. It was shown that the large anterior bony fragment was originated from a posterior part of the tibia which indicates a PCL avulsion injury (Figure 2A and 2B). We ask the patient to perform a magnetic resonance imaging (MRI) study of the knee for further evaluation of other soft tissue structures. However, it was rejected by the patient and family due to financial issue.

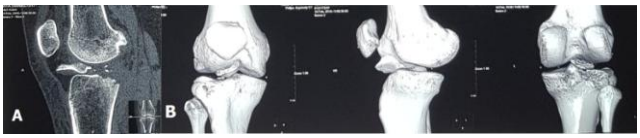

Figure 2: Sagittal CT image of the knee showed the fragment was originated from the posterior side of the tibial plateau A); Three-dimension CT showed the exact site of PCL avulsion fracture B)

Operative treatment was planned for the patient. Under general anaesthesia, the surgical procedure was performed to the patient in a prone position. Preoperative antibiotic with 2 g of Cefazolin was administered before skin incision. Burks and Schaffer approach [3] was utilised to expose the fracture site. Intraoperatively, reposition of the fracture fragment from the anterior compartment was done with a mosquito clamp, then fixation of the fragment was performed with a 4.5 mm malleolar screw (Figure 3A). Rupture of the posterior root of lateral meniscus also observed and repaired with an absorbable suture. After irrigation with normal saline, the wound was then closed with a suction drain. Cefazolin injection was continued until 48 hours postoperatively, followed by oral cefadroxil for 5 days.

Postoperative rehabilitation includes the application of posterior knee splint which retained until 2 weeks, isometric quadriceps exercise and ankle pump exercise started at day 1 after surgery. The range of movement exercise of the knee started after 2 weeks and increased gradually to obtained full knee range of movement at 6 weeks after the surgery. Non-weight bearing ambulation was applied for the first 4 weeks, followed by partial weight-bearing as tolerated. Full weight-bearing ambulation was allowed after 8 weeks. The patient was followed monthly for progression of a range of motion and evaluation of knee stability. During follow-up, full knee range of movement was obtained at 6 weeks postoperatively and maintained until the final 1 year of follow-up (Figure 3B, and 3C). Posterior drawer test revealed some posterior laxity (grade 1). However, the patient has no subjective complaint about her knee. Plain radiograph also showed a complete bony union (Figure 3D).

Figure 3: Immediate postoperative radiograph A); Clinical outcome at 1-year follow-up (B, and C); Radiograph at 1 year follow-up D)

Discussion

This case report described an unusual variation of PCL avulsion injury. The PCL avulsion injury classification described by Rademakers *et al.,*

[5] did not mention whether the fragment is anteriorly displaced or remain in the posterior compartment. To the best of our knowledge, this was the first report describing this pattern of injury. We hope this report may give some different insight into the spectrum of PCL avulsion injury.

The main issue to discuss is about the mechanism of injury. There were three mechanisms for PCL injury, include 1. Hyperflexion injury which resulted in a mid substance rupture; 2. Dashboard injury which resulted in a substance rupture at the level of tibial plateau or avulsion injury; and 3. Hyperextension injury which resulted in a substance rupture at the proximal part of PCL [7]. In our recent case, the anteriorly displaced fragment was quite large (approximately 3 x 2 x 1 cm). A large anterior opening with frank posterior tibial translation and some distraction force may be needed to allow the large fragment migrated from posterior to the anterior compartment of the knee. This situation may be only possible when the knee joint is in flexion position. Also, there was an evidence of skin brushing and excoriation on the anterior part of the knee which indicates a previous posterior direct hit on the proximal part of the tibia. Therefore, the most possible mechanism of this case it might be a “Dashboard injury”.

Another unusual variation of PCL avulsion injury has been reported by Ogawa *et al.,* [8]. They found a case of PCL avulsion injury associated with a lateral tibial condyle avulsion fracture. The authors believed that the mechanism of injury was not associated with the three previously described mechanism. An extension-distraction injury with more predominantly distraction believed to be the mechanism. That was due to no injury observed on the posterior capsule of the knee. Similarly to Ogawa’s case, we also believed that some distraction mechanism also occurred in our recent case.

The investigation is another important issue. A missed diagnosis to an ACL avulsion injury might happen if the plain radiograph is the only available imaging study. As the bony fragment is completely located in the anterior compartment of the knee and masquerading an ACL avulsion injury (Figure 1B). A preoperative CT and/or MRI is mandatory to prevent missed and incomplete management. The intraoperative combined procedure with arthroscopy diagnostic and treatment also could be recommended.

Consideration of concomitant injury to other knee structures are also crucial. Liu *et al.,* [9] reported a series of 21 cases of PCL avulsion injury associated with posterior root meniscal repair with a satisfactory outcome. Conversely, Ogawa *et al.,* [8] did not find any other injured part of the knee including ACL, both meniscus and cartilage. In our recent case, we found a posterior root of lateral meniscus rupture which also has been repaired. The patient has no complained of persistent pain, snapping or locking of the knee until the final follow-up. The presence of concomitant injury to other structure of the knee should be suspected when there was a complete displaced fragment, especially if the bony fragment is large (> 20 mm).

In conclusion, the bony fragment of the tibial attachment of posterior cruciate ligament avulsion injury can be displaced to the anterior compartment of the knee. Concomitant injury to other knee structures should be suspected when finding this case. Special consideration is also needed during the management of this unusual case.

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Case Report

Simultaneous Presentation of Benign Paroxysmal Positional Vertigo and Meniere's Disease – Case Report

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Keywords: Benign paroxysmal positional vertigo; Meniere's disease; Dix-Hallpike manoeuvre; Epley manoeuvre

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Abstract

BACKGROUND: Benign paroxysmal positional vertigo (BPPV) is one of the most common clinical entities, which develops spontaneously in most of the cases, but it can be secondary as a result of different conditions such as head injuries, viral neurolabyrinthitis, Meniere's disease and vertebrobasilar ischemia. The aim of presenting this case is to point out to the need of taking a precise clinical history and performing Dix-Hallpike manoeuvre in all patients who complain about vertigo regardless of the previously diagnosed primary disease of the inner ear.

CASE PRESENTATION: A 63-year-old female patient presented with the classical triad of symptoms for Meniere's disease (fluctuating sensorineural hearing loss in the right ear, tinnitus and fullness in the same ear and rotary vertigo), two years later complained of brief episodes of vertigo linked to changes in head position relative to gravity. Dix-Hallpike manoeuvre showed a classical response in the head-hanging right position. Benign paroxysmal positional vertigo (BPPV) in the same ear was diagnosed in this patient. After treatment with Epley's canal repositioning manoeuvre for a few days, the symptoms of positional vertigo resolved.

CONCLUSION: The authors recommend complete audiological and otoneurological evaluation in all patients with vertigo for timely recognition/diagnosis of any inner ear associated pathology.

Introduction

Vertigo is a symptom of many diseases that involve the inner ear, which is not surprising having in mind its complex constitution and function. Benign paroxysmal positional vertigo (BPPV) is one of the most common clinical entities that can be easily treated, but unfortunately in many cases, it is not recognised by the doctors [1], [2], [3]. The annual incidence of BPPV ranges from 0.06 to 0.6%, and its prevalence increases proportionally with the age of patients [4], [5], [6]. It is rarely found in children and population younger than 35 years, where it is often associated with head injuries [7]. It is considered that pathophysiological mechanisms that cause positional vertigo are detached otoconia floating inside the semicircular canals (canalithiasis) or otoconia adhering to the cupula, most often in the posterior semicircular canal (cupulolithiasis) [8], [9], [10]. Although its pathogenesis is still not yet elucidated, in general, it is assumed that it develops spontaneously in 85% of cases as a result of degeneration of utricular neuroepithelium [11]. In 25% of cases, BPPV can be associated with other different conditions in the inner ear that might cause otoconia detachment or secondary BPPV [12], [13], [14].

Meniere's disease is an inner ear disease that is characterised by episodes of vertigo, fluctuating and progressive hearing loss, tinnitus, sense of fullness in the affected ear. The aetiology and pathophysiology of Meniere's disease are still unknown although 156 years have passed since it was recognised for the first time by Prosper Meniere, after whom this disease was named. Schuknecth's theory is about the rupture of membranous labyrinth and spilling of potassium-rich endolymph through the rupture, causing a potassium intoxication of the vestibular nerve. His theory is based on pathohistological findings of numerous temporal bone specimens from patients with Meniere's disease [15]. Recent studies based on the documented age when the symptoms of Meniere's disease appear, an age that is similar to that when BPPV prevalence is the highest, consider detached saccular otoconia to be the fundamental cause of

Meniere's disease [16], [17].

The frequency of BPPV in MD in N Macedonia is unclear. BPPV is largely underdiagnosed, while Menière's disease, which is about 10 times less frequent than BPPV, appears to be overdiagnosed.

The latest studies have indicated the possible coexistence of these two conditions in the inner ear and suggest their interaction [18]. BPPV, along with MD, was mostly observed in the ear affected by hydrops in females and patients with more advanced disease. Canalolithiasis of the horizontal semicircular canal was more common in patients with BPPV associated with MD than in idiopathic BPPV. BPPV in MD was more prone to recurrence and required more canal repositioning manoeuvres [19].

According to Taura et al., about one-third of patients with Meniere's disease developed benign paroxysmal positional vertigo (BPPV)-like attacks [20]. Thus, vertigo attacks in patients with Meniere's disease must be carefully treated because the therapy for such vertigo attacks is different from the therapy for BPPV.

Case Report

In March 2014, a 62-year-old woman came to the University Clinic of Ear, Nose and Throat, accompanied by her husband. The patient was pale, scared and with difficulty walking. She complained about vertigo, which she explained as if subjects moved in a circle from right to left; she felt ringing in the right ear similar to "ocean whisper", fullness in the right ear and the need of permanent swallowing and making Valsalva manoeuvre. Her six-month-clinical history revealed episodes of vertigo in several hours. In the beginning of the disease, auditory symptoms in the period between vertigo episodes had resolved, but later they became permanent.

The patient underwent a neurological evaluation, tonal audiometry, tympanometry and standard test protocol for visual and vestibular stimulation [21]. Neurological evaluation revealed normal mental status, speech and normal function of cranial nerves. The patient was unstable while standing, and the Romberg test showed lateralisation towards the side of the affected ear. The first audiometric examination registered a conductive hearing loss of 10 db at 250 Hz in the right ear and normal hearing in the left ear. The check-up made at 6 months showed sensorineural hearing loss of 10 db at 250 and 500 Hz; 2 months later sensorineural hearing loss was found for low and high frequencies except at 2000 Hz, and the check-up after 24 months since the onset of symptoms showed a moderate sensorineural hearing loss for all frequencies (flat audiometric curve) Figure 1.

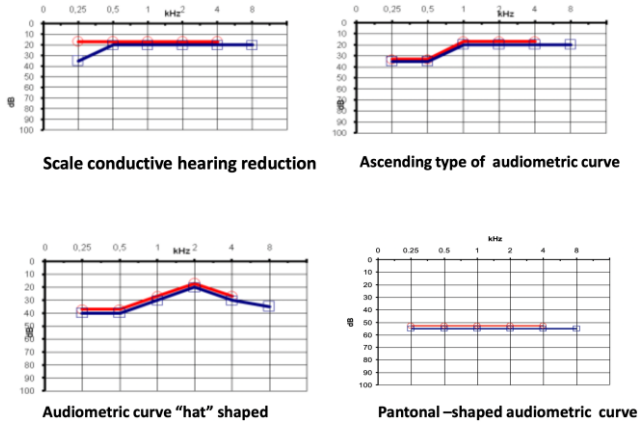

Figure 1: Pure tone results

Type A curve, Figure 1, according to Jeger's classification, was seen in both ears on tympanometry, confirming normal admittance and tympanometric peak pressure, [22].

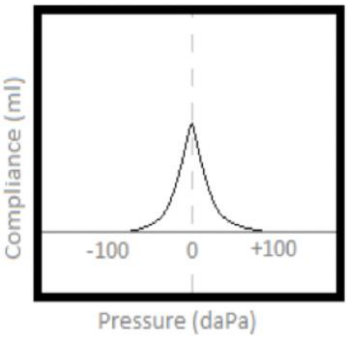

Figure 2: Tympanogram type A

Unilateral, rhythmic, conjugated horizontally rotary nystagmus was registered in a reverse direction from the affected ear. A caloric test with hot and cold water was realised. The test showed an asymmetric horizontal canal function of 25% and confirmed a depressed response on the disease side. The diagnosis of definitive Meniere's disease was made based on the guidelines of the American Academy of Otolaryngology-Head and Neck Surgery Foundation (AAO-HNS) [23].

After 24 months since the beginning of the symptoms, the patient came to our Clinic complaining about brief attacks of vertigo, which had appeared two weeks after the first vertigo attack that differed from the previous attacks. She described vertigos as sudden sensation as if "the room was spinning", very often accompanied by nausea and short-term instability. She had this kind of vertigo most often in the morning when she got up from bed or when turning over to the right in bed. She did not have vertigo while standing or turning the head.

Based on patient's anamnesis Dix Hallpike

manoeuvre was realised and it showed a classical response when in head-hanging-right position. A short time of about 5 seconds after repositioning the head, geotropic, torsional rotary nystagmus of about 30 sec. Was registered. The patient complained of vertigo and nausea. When the test was repeated, the response was significantly weaker, and after several repetitions, it completely disappeared. Complete symptom remission was achieved after 7-day-treatment with Eplay's manoeuvre. At the check-up visit after several months, the patient said she had no vertigo when changing the position of the head.

Discussion

The association between BPPV and Meniere's disease has been described in the literature, but there are still some unresolved questions related to the possible pathophysiological mechanisms and the exact incidence of this combination. The incidence of BPPV among patients with Meniere's disease has been presented in a wide range from 0.3 to 30% [24], [25].

In a study including 718 patients with diagnosed BPPV, 9% of patients had existing inner ear disease, of which one third had Meniere's disease [26]. Lately, the incidence increase of patients with concomitant Meniere's disease and BPPV might be due to the higher awareness of the doctors about the possible coexistence of these two different conditions in the affected ear.

In our patient, the symptoms of Meniere's disease preceded the symptoms of BPPV in the affected ear, which is in conjunction with the results of others suggesting that Meniere's disease predispose patients to BPPV [27], [28].

The matter of argument is whether there is a possible relation between these conditions or their association is accidental. According to many authors, simultaneous existence/appearance of Meniere's disease and BPPV in the same ear indicates an association between these two disorders [26], [29].

In our patient, BPPV symptoms appeared two weeks after the last vertigo attack and receded after the 7-day-treatment with Epley's manoeuvre. Lee *et al*. reported that in the largest percentage of patients, the BPPV symptoms appear one week after the vertigo attack. According to these authors, the simultaneous onset of symptoms is unusual and is found in a small percentage [26]. Regarding the rate of symptoms resolution after repositioning procedures, literature data show a higher rate in patients with idiopathic BPPV than in those with BPPV associated with Meniere's disease or other disorders in the inner ear. These authors also state that it might be due to different pathophysiological mechanisms associated with different diseases of the inner ear [30], [31], [32]. Contrary to these reports, BPPV in MD can affect either ear and be not associated with poorer outcomes than idiopathic BPPV [33].

The symptoms of BPPV in our patient appeared in the late phase of Meniere's disease, which is similar to other studies that indicate a higher secondary BPPV rate in patients during the late stages of Meniere's disease [24], [34]. Concerning the pathophysiological mechanism, we think that the common hydropical attacks are the possible cause for the damage to the maculae of the utricle and saccule, which results in detached otoconia in the endolymph and are the cause of simultaneous development of these two different entities in the affected ear. Our assumptions are similar to those of other authors [24], [25].

According to other authors, the sleep position is in a tight relation and cause for the coexistence of these two different conditions in the same ear. Patients with Meniere's disease usually sleep on the head-lying side with reduced hearing so that the ear with a good hearing is free. These authors say that otoconia dislodged from the utricle can fall in the lateral or posterior semicircular canal in the lower portion of the inner ear during sleep [29], [30]. Our patient did not tell us that she usually/often sleeps on her right ear.

In conclusion, authors think that due to the possible association of two different clinical entities in the same ear, the initial diagnosis of Meniere's disease should not be considered definitive, emphasising the need of taking Dix Hallpike test in all patients with vertigo.

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Dental Science

Microleakage of Aesthetic Restorations Following Functional Simulation and Immersion in Saudi-Traditional Mouth Rinses

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Keywords: Composite resin; Glass ionomer cement; Microleakage; Traditional mouthwashes

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Abstract

AIM: The study is aimed to assess the effect of 3 Saudi-traditional types of mouth rinses (Karadah, Myrrh, salted water) on the microleakage of composite and glass-ionomer restorations subjected to thermal cycling and cyclic loading.

MATERIAL AND METHODS: Class V cavities in both buccal and lingual surfaces of eighty extracted premolars were restored with both nano-filled composite and glass-ionomer restoratives. Half the number of restored teeth (group 1, n = 40) were subjected to further thermal cycling and cyclic loading to mimic the in-service functional stresses. The rest of the teeth were left as control with no functional simulation (group 2, n = 40). Teeth of each group were then stored wet for one month in 4 subgroups (n = 10) according to the storage media (distilled water, salted water, Myrrh and Karadah extracts). Following wet ageing, all teeth were immersed in methylene blue solution for 24 hrs, followed by sectioning in Bucco-lingual direction. The microleakage was inspected using stereomicroscope and rated from 0-4 according to its penetration depth. The collected non-parametrical data was then analysed statistically using Kruskal-Wallis One-way ANOVA at $\alpha = 0.05$.

RESULTS: There was no statistically significant difference observed in microleakage between specimens treated with any of the mouthwashes for both glass ionomer and composite restorations in the presence and absence of thermal cycling and cyclic loading ($p = 0.889$).

CONCLUSION: Given the results of the present study, the Saudi-traditional types of mouth rinses are not contributory to microleakage in aesthetic-based composite and glass-ionomer restorations.

Introduction

Tooth aesthetic usually governs the healthy appearance of an individual. Accordingly, many carious and non-carious tooth defects of the aesthetic areas should be restored with materials that mimic the regular tooth appearance [1], [2]. Both resin composites and glass-ionomer-based restoratives show a respectable degree of success doing that job [1], [2], [3]. However, incidences of microleakage had been reported at restorations-tooth interfaces in response to materials' setting contraction and deteriorated bonding to tooth tissues [4], [5], [6]. This drove the attention of dental manufacturers to modify their bonding products and to develop self-adhesive and minimal-shrink restoratives [5], [6], [7]. Although these new developments seem promising, their bonding performance could be affected by both thermal and mechanical stresses that are generally developed on function [7], [8]. Also, individuals in different communities may depend on traditional, cultural-known medicines and topicals to cure certain oral diseases [9], [10] and the influence of these agents on restorations is not yet known.

Microleakage has been detected in different types of aesthetic restorations. It is generally due to questionable material-tooth bonding, and sometimes, mismatch of material-tooth properties. Microleakage either from small or microscopic openings between the margins of the composite restoration and tooth was considered a significant cause of restoration failure [11], [12]. Thus, it can result in bacteria penetrating the tooth-restoration space and dentinal tubules, where secondary decay may occur, and bacterial toxins will irritate the pulp. The oral environment (including occlusal forces and temperature variation) and several differences between the physical properties of teeth and

restorative materials (including polymerisation shrinkage, the coefficient of thermal expansion, and modulus of elasticity) can additionally contribute to microleakage [13], [14]. Therefore, microleakage can create clinical problems, including hypersensitivity, recurrent caries, staining of restoration margins, pulp irritation and failure of the restorative material. Thus, prevention of microleakage is of paramount consideration in the development of adhesive systems, for application in tooth restorative [15]. Although recently-developed materials and techniques seem promising with respect to the incidence of microleakage, the privilege of their excellent bonding and accordingly, the rate of microleakage could be affected in function and in contact with different fluids.

The use of chemical plaque control methods has been on the rise in the past couple of decades. However, the adverse effects of chemical products, as well as the widespread antimicrobial resistance, have diverted the attention of clinicians and patients towards more traditional methods specifically in specific communities. Nonetheless, how these alternative products interact with the oral environment and dental restorations needs investigation.

Hence, the present study was conducted to assess the effect of 3 Saudi-traditional types of mouth rinses (Karadah, Myrrh, salted water) on the microleakage of composite and glass-ionomer restorations subjected to thermal cycling and cyclic loading. The tested null hypothesis was that the Saudi-traditional based mouth rinses have no additional adverse effect on the rate of microleakage in both composite and glass-ionomer restorations.

Material and Methods

An in vitro comparative study was conducted to test the stated null hypothesis. Eighty freshly extracted, caries-free premolars were collected out of the orthodontic outpatient clinic, College of Dentistry, King Khalid University. Ethical clearance was obtained from the Institutional Review Board, King Khalid University (2013-2014 / 28).

Study Protocol

After cleaning both soft and hard deposits off, all teeth received standardised cervical cavities on both buccal and lingual surfaces using # 811 FG 033 diamond abrasives. All buccal cavities were restored with nano-filled composite (Feltik Z350, 3M ESPE, St.Paul, MN) restorative and Single Bond Universal (3M ESPE, St.Paul, MN) 7th generation resin adhesive. Following poly-acrylic acid conditioning, all lingual cavities were restored with glass-ionomer restorative (Ketac Fil Plus Aplicap, 3M ESPE, St.Paul, MN). Half the number of restored teeth (Group 1, n = 40) were subjected to cyclic fatigue loading under a standardised weight of 5kg for 10,000 cycles followed by thermal cycling at 4, 37 and 60°C for 3500 cycles with 60s dwelling time, while the rest remained with no functional conditioning (Group 2, n = 40). Teeth of each group were then subjected to one month of wet ageing at 37°C in 4 different solutions (water, salted water, Myrrh and Karadah, n = 10 for each) (Table 1).

Table 1: Specimen test groups

	Restorative Material	Groups (n = 40) (Conditioning)	Subgroups (n = 10) (Storage media)
80 Extracted premolars (buccal and lingual)	Nano-Filled composite (n = 80)	Functional Simulation (n = 40)	Water
			Salted water
			Myrrh
			Karadah
		No Simulation (n = 40)	Water
			Salted water
			Myrrh
			Karadah
	Glass-ionomer (n = 80)	Functional Simulation (n = 40)	Water
			Salted water
			Myrrh
			Karadah
		No Simulation (n = 40)	Water
			Salted water
			Myrrh
			Karadah

Teeth of all subgroups were incubated in methylene blue solution at 37°C for 24 hours before their longitudinal sectioning in buccolingual direction. The sectioned specimens were then inspected under low angle illumination using stereomicroscope (0.8 x) to detect the degree of stain ingress (microleakage) at the restoration-tooth interface. The detected leakage was ranked for each restoration from 1-4 depending on the depth of stain penetration. The scoring criteria used was as follows: Score 0 = No leakage; Score 1 = Stain ingress limited to enamel thickness; Score 2 = Stain ingress along the side walls of restored cavities; and Score 3 = Stain ingress to the pulpal wall of the restored cavities.

Statistical Analysis

The data was entered in a Microsoft Excel worksheet and analysed using IBM SPSS v. 21 (IBM Statistics, Chicago, USA). Since the data did not follow a normal distribution (assessed using the Shapiro-Wilk test), a non-parametric test was used for analysis. The data were analysed using Kruskal Wallis One-way ANOVA with $\alpha = 0.05$.

Results

Table 2 shows the percent microleakage scores in the different aesthetic restorations investigated in the present study. Restorations immersed in salt-water showed higher microleakage

compared to all the subgroups. Also, the aged restorations showed higher scores of microleakage as compared to their non-aged counterparts.

Table 2: Percent microleakage scores in different aesthetic restorations

Mouth rinses	Ageing	Aesthetic Restorations							
		Composite				Glass-ionomer			
		Score 0	Score 1	Score 2	Score 3	Score 0	Score 1	Score 2	Score 3
Water	Non-aged	50	40	10	0	70	30	0	0
	Aged	40	50	10	0	50	50	0	0
Salted water	Non-aged	40	30	20	10	50	20	30	0
	Aged	50	10	20	20	40	20	40	0
Myrrh	Non-aged	50	50	0	0	70	20	10	0
	Aged	40	60	0	0	70	20	10	0
Karadah	Non-aged	50	30	20	0	50	40	10	0
	Aged	60	20	10	10	50	20	30	0

The microleakage scores in different subgroups are presented in Table 3. Kruskal-Wallis One-way ANOVA comparing microleakage scores in different subgroups did not identify statistically significant differences in the scores between groups ($p = 0.889$). Thus, ageing showed no statistically significant effect on microleakage in all composite and GI subgroups subjected to various mouth rinses. Since Kruskal-Wallis One-way ANOVA observed no statistical significance, further multiple comparisons using post-hoc analysis were not indicated.

Table 3: Microleakage scores in different test groups

Study Group	Subgroups		Median (IQR)	p-value
Composite	No Ageing	Water	0.5 (1)	0.889
		Salted Water	1 (2)	
		Myrrh	0.5 (1)	
		Karadah	0.5 (1)	
	Ageing	Water	1 (1)	
		Salted Water	0.5 (2)	
		Myrrh	1 (1)	
		Karadah	0 (1)	
Glass Ionomer	No Ageing	Water	0 (1)	
		Salted Water	0.5 (2)	
		Myrrh	0 (1)	
		Karadah	0.5 (1)	
	Ageing	Water	0.5 (1)	
		Salted Water	1 (2)	
		Myrrh	0 (1)	
		Karadah	0.5 (2)	

Kruskal-Wallis One-way ANOVA, $p < 0.05$ is significant.

Discussion

The present study evaluated the effect of 3 Saudi-traditional types of mouth rinses (Karadahah, Myrrh, salted water) on the microleakage of composite and glass-ionomer restorations subjected to thermal cycling and cyclic loading. According to the results of the current study, the null hypothesis tested was accepted since none of the tested mouthwashes increased the microleakage of both composites and glass ionomer in the absence and presence of functional stimulation. An extensive literature search revealed no studies evaluating the effect of herbal mouth rinses on microleakage of aesthetic restorations.

Microleakage tests provide invaluable data on the sealing ability of adhesive resins. Dye penetration technique is the most commonly used technique for microleakage evaluation [15], [16]. Hence, it was used in the present study. Microleakage of aesthetic restorations is attributed to mechanical stresses as a result of polymerisation shrinkage. There are several factors involved in polymerisation stress, for instance, C-factor, cavity size, the technique of placement, the light-curing technique employed, and the mechanical properties of the composite resin [15]. In the present study, efforts were made to maintain all these variables at the same level for both groups. Cavity preparation was standardised and restored, using the same composite resin and one light-curing unit and glass ionomer cement. Also, to simulate oral conditions, all the samples underwent a uniform thermocycling procedure.

In the present study, statistically significant microleakage was not observed with any of the mouthwashes as compared to control. This is in contrast to a study conducted by Ajami et al., [15] where they evaluated the effects of commercially available mouth rinses on microleakage of composite resin restorations bonded with two adhesive systems [15]. This contrast could be attributed to the fact that they used commercially available mouthwashes (Listerine, Oral-B and Rembrandt plus) which have a higher alcoholic content as compared to the traditional mouthwashes used in our study. Furthermore, they also studied the samples after subjecting them to bleaching with 10% carbamide peroxide, as one of the objectives of their research, which could have been the reason for the higher microleakage observed in their study.

The use of antimicrobial mouth rinses is an approach to limit the accumulation of dental plaque, with a primary objective to control the development and progression of periodontal diseases and dental caries [17], [18]. However, the frequent use of mouth rinses may have adverse effects on oral and dental tissues [19], [29]. Despite the increased use of mouth rinses, research comparing resin composite microleakage associated with the use of mouth rinses is limited. Villalta et al., [21] have shown that low pH and alcohol concentration of solutions might affect the surface integrity of composite resins and cause staining.

Myrrh is a natural gum or resin extracted from some small, thorny tree species of the genus *Commiphora*. In Saudi Arabia, myrrh was as an antiseptic in mouthwashes, gargles, and toothpaste. Myrrh has also been recommended as an analgesic for toothaches and can be used in liniment for bruises, aches, and sprains. Myrrh is a common ingredient of tooth powders. Myrrh and borax in the tincture can be used as a mouthwash [22].

It is estimated by the World Health Organization that about 4 billion people in the world are using the Karad plant (Arabic: Karad, Latin:

Acacia nilotica L) for its therapeutic properties. The tender twig of this plant is used as a toothbrush in south-east Africa, Pakistan and India. The extract of *Acacia* can be used in dental products like mouthwash to prevent gingivitis. A. nilotica is a medicinal plant acknowledged to be rich in phenolics, consisting of condensed tannin and phlobatannin, gallic acid, protocatechuic acid, pyrocatechol, (+)-catechin, (-) epi-gallocatechin-7-gallate and (-) epigallocatechin-5, 7-digallate [23]. The simulation of all clinical factors that influence the effects of mouthwashes on aesthetic restorative materials is not possible in vitro. Saliva, salivary pellicle, foods, and beverages may affect the physical properties of resin restorative materials. Further, in vivo studies are necessary to determine the effects of different types of mouth rinses. Also, in the present study, we did not observe any significant microleakage with the traditional mouthwashes as compared to the control (water). Therefore, it may be hypothesised that the traditional mouthwashes are safer as compared to the commercially available ones. Further research comparing traditional and commercially available mouthwashes is also warranted.

In conclusion, within the limitations of the present study, the Saudi-traditional types of mouth rinses are not contributory to microleakage in aesthetic-based composite and glass-ionomer restorations. However, the results of the present study should be used with caution since in-vitro results need to be corroborated with clinical studies, as the oral environment cannot be entirely simulated in laboratory settings.

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Dental Science

Preparation and Histological Study of Multi-Walled Carbon Nanotubes Bone Graft in Management of Class II Furcation Defects in Dogs

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Keywords: Multi-walled carbon nanotubes; Bone graft; Furcation defects; Bone regeneration; Histological analysis

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Abstract

BACKGROUND: The main target of periodontal disease and alveolar bone defeat treatment is the regeneration of the lost structures.

AIM: This work deals with the evaluation of the effect of functionalised multi-walled carbon nanotubes (MWCNTs), as grafting material in the management of furcation defects created in dogs.

MATERIAL AND METHODS: Potential cytotoxicity of the grafting material was assessed. Scanning electron microscope (SEM) and energy dispersive x-ray (EDX) analysis after incubation of the grafting material in simulated body fluid (SBF) at pH 7.4 and 37°C for one week was done. In six healthy mongrel dogs' full-thickness mucoperiosteal flaps were raised on the buccal aspects to create two walls intrabony defects at the furcation areas. The mandibular premolar area received the grafting material. Histological evaluation was carried out at 1, 2- and 3-months' period.

RESULTS: Cytotoxicity results proved the safety of grafting material application. The prepared material exhibited good Ca-apatite crystal patterns at the surface revealed by SEM and high calcium content showed by EDX results. Good bone formation ability was also apparent histologically.

CONCLUSION: The prepared grafting material (MWCNTs) can serve as a delivery vehicle for osteogenic cells and osteogenic growth factor proteins in the bone development process.

Introduction

The ultimate goal of periodontal disease treatment and alveolar bone reconstruction is the regeneration of defeated structures as a result of infection, trauma or congenital abnormalities [1]. The renovation of the lost structures to reach optimum aesthetics and function is a major patients' demand [2]. The innate potential of bone regeneration or remodelling allows the impulsive repair of small bone defects. However, in multiple situations, the existing defect may be severe or the local environment is not favourable for adequate self-repair of the damaged or missing tissues [3], [4]. Bone grafts or bone graft substitutes are thus required to aid the healing of such decisive size or non-healing bone defects [5]. Various grafting materials contain most of the essential elements required for stimulating bone regeneration, and therefore have been superior in current clinical practice for restoring damaged, diseased or resorbed bone tissue. These materials have been used effectively in the management of intrabony defects [6]. Filling the regenerative defect sites with a biocompatible substance offers a scaffold for host inhabitant cells to assist in the regenerative process via osseoinduction or osseoconduction pathways [7].

Since the autografting procedure has several disadvantages, not only because of the limited amount of the available bone that can be harvested, but also because the surgery is time-consuming and causes invasion of the intra or extraoral donor structures to increase the risk of donor site morbidity. The use of other bone substitute materials such as allografts and xenografts although numerously

documented as being osteoinductive/osteoconductive is still controversial due to the graft material resorption and the possible immunogenic reactions that might occur [8]. Therefore, the search for an ideal synthetic graft material continues [9]. In hard tissue management, a diversity of materials has been developed to imitate the explicit highly organised nanoscale structure of the bone, which consists of collagenous fibres and mineralised apatite nanocrystals [10]. Nanocomposite materials and natural polymers are getting a great deal of attention with the vision that these composite systems can offer a variety of benefits in preserving the structural and biological functions of the damaged tissues like or superior to the naturally present system [11], [12].

Carbon nanotubes (CNTs) have been under research in the precedent decade for a variety of applications due to their exceptional and multipurpose properties [13]. In the meadow of regenerative medicine, they have demonstrated immense promise to improve the quality of tissue engineering scaffolds [14]. CNTs a multi-functional nano-material have been introduced due to the outstanding electronic, mechanical, optical and chemical properties. Recently the use of CNTs or CNTs based composites (i.e. associated with different biological molecules or polymers) classified as single-walled carbon nanotubes (SWCNT) and multi-walled carbon nanotubes (MWCNT) in bone repair/regeneration has been documented as an innovative biomaterial in the reconstruction or regeneration of tissues [15], [16], [17].

Functionalisation of MWCNTs using different materials such as beta-cyclodextrin and natural plant extracts led to lowering of the cytotoxic behaviour of the resulting composites and allow using them as carriers according to the different previous reported works [18], [19],20], [21], [22], [23].

The aim of this work deals with evaluating the effect of functionalised MWCNTs, as grafting material in the management of furcation defects created in dogs.

Material and Methods

In the present study, functionalised MWCNTs material was prepared and used as grafting material in created furcation defects in dogs. Histological evaluation of this material on the surrounding tissues, the signs of its biodegradation, and the evidence of new bone formation was investigated.

This study was carried out according to the ethical guidelines of the World Medical Association; Declaration of Helsinki as revised in 2000 for studies involving animal subjects and the study protocol was approved by the Medical Ethical Committee at the National Research Centre (NRC) with a code no. 16467. Sample size calculation revealed that (n = 6) was enough to detect the main effects of the grafted material with a satisfactory level of power set at 80% and a 95% confidence.

Functionalisation of the MWCNTs grafting material

Pristine MWCNT (100 mg) was dispersed in mixed concentrated sulphuric and nitric acids (3:1, v/v) at a ratio of 50 ml acid mixture. The resulted mixture was then heated at 110°C overnight with continuous stirring to produce oxidised carbon nanotubes (MWCNTs-COOH). The sample was washed with ultrapure water until getting a neutral filtrate (pH 7). The collected solid was dried under vacuum at 70°C for 12 h and kept for further functionalisation and analysis [24].

Measurement of Potential Cytotoxicity

Potential Cytotoxicity of the studied material was examined using the method of Skehan et al., [25] in the Clinical Pharmacy Department at the National Cancer Institute, Egypt, to obtain the IC50 value which is the half-maximal inhibitory concentration to measure the potency of a substance in inhibiting a specific biological or biochemical function. Normal cell line (baby hamster kidney cells, BHK-21, Sigma-Aldrich, Germany) was plated in 96-multiwell plate (104 cells/well) for 24 h before application of the tested material to allow attachment of the cells to the wall of the plate. Different concentrations of the attested material (0, 1, 2.5, 5, and 10 µg/ml) were added to the cell monolayer triplicate wells prepared for each dose. Monolayer cells were incubated with the material for 24 h at 37°C and in an atmosphere of 5% CO_2. After 48 h, cells were fixed, washed, and stained with sulforhodamine B stain. Excess stain was washed with acetic acid, and the attached stain was removed with Tris-EDTA buffer. Colour intensity was measured in an enzyme-linked immunosorbent assay reader (ELISA reader, BioTek, USA). The relation between surviving fraction and material concentration is plotted to get the survival curve of each cell line after specified compound concentration.

In vitro bone bioactivity testing

The attested material was soaked in simulated body fluid (SBF) for 7 days then removed, rinsed using de-ionized water, and dried to complete the investigations. The material surface after soaking was examined via scanning electron microscope (SEM) and Electron dispersive x-ray (EDX) to confirm the formation of apatite. The bioactivity test was carried out three times for the sample to ensure the bioactivity behaviour [26].

Characterisation

Scanning electron micrographs (SEM) were recorded using JXA-840A Electron Probe Micro analyser JEOL-SEM. For SEM, the substrate was mounted on metal stubs and coated with gold-palladium with the thickness of deposit about 75Å at vacuum 7 × 10-2 millibar and 2.4-kilovolt cathodic voltage before being examined using Polaron SEM Coating Instrument.

Experimental Design

Six healthy young adult male mongrel dogs with age range 19-24 months and weighing from 13 to 16 kg, were selected for the study. Before the experiment, all dogs were examined by a vet, and those having good systemic and periodontal health were included in the study. The mandibular right third premolar (RP3) was considered as the control group and after the creation of the bony defect, it was left to undergo normal healing, while the mandibular right fourth premolar (RP4) on the same side received the CNT material (study group). Initially scaling and tooth brushing were performed for the selected teeth to control gingival inflammation for one week. During this period, plaque control was maintained by daily topical application of 0.12% chlorhexidine solution.

Surgical procedures

All surgical procedures were performed under systemic anaesthesia with an intravenous injection of sodium thiopental solution following sedation. The systemic anaesthesia was complemented with infiltration anaesthesia to ensure local homeostasis. Following the intrasulcular incisions around the selected teeth, full-thickness mucoperiosteal flap was raised on the buccal aspect to create two-wall intrabony defects" box- type" (4 mm inferior, 3 mm horizontal from the buccal surface of the roots in a buccolingual direction to the lingual bone wall, using a line tangential to the buccal root surface as reference and 5 mm height from the alveolar crestal bone to the base of the defect) at the furcation area using a water-cooled diamond fissure bur [12]. Each root surface was scaled and planed completely to remove remaining cementum and periodontal ligament. An experimental notch was placed at the most apical part of the exposed root using a round diamond bur with abundant irrigation. These notches were placed on the buccal aspects of the roots and extended interproximally and into the furcation areas as deep as the involvement of the furcation defect permitted, as a guideline for histological analysis. Following placement of the notches, the surgical sites were thoroughly irrigated with sterile saline. The study material was inserted in the created defect and the created defect at the control site was left to heal normally. The flaps were repositioned and sutured with non-absorbable sutures. From the evening before surgery and for 4 days after surgery intramuscular injections with Vibramycin (0.1 g/15kg weight) were administrated. The animals were fed only water-softened dog food to reduce the possibility of local trauma to the site of operation. After 7 days, the sutures were removed. Plaque control was maintained by daily topical application of 0.12% chlorhexidine solution until the time of sacrifice, which was performed by a thiopental overdose at 1, 2, and 3 months after surgery.

Tissue Processing

After euthanasia, which was performed by a thiopental sodium overdose 1, 2 and 3 months after the regenerative surgeries, the mandibles were dissected, and the tissues containing the experimental specimens were obtained.

The specimens were fixed in 10% buffered natural formaldehyde for 24 hours. The samples were kept in separate coded dishes containing 10% ethylene diamine tetraacetic acid (EDTA) for 8 weeks. As decalcification is completed, the specimens were washed in running water to remove all traces of EDTA. The specimens were gradually dehydrated in ascending concentration of ethyl alcohol (50%, 70%, 90%) and 2-3 changes of absolute alcohol to ensure replacement of water by alcohol. The specimens were defatted in two changes of xylene under vacuum. The specimens were infiltrated with paraffin into a constant temperature furnace, at about 60°C, until the xylene in the tissues was replaced completely by paraffin. The specimens were cut parallel to the long axis of the tooth so that serial sections could be performed and representing most of the induced defective area. Semiserial 7-mm-wide histological sections were made in a mesiodistal direction and the sections were stained with hematoxylin and eosin (H&E) for histological examination for signs of biodegradation as well as bony graft integration of the graft material and evidence of new bone formation.

Histological Analysis

Nine non-serial histological sections of each block, corresponding to the first three, central and last three sections, which contained notches in the roots were examined with a light microscope under conventional and polarised light to evaluate the areas of new bone formation in the induced furcation defects. Histological analysis was performed using the Leica Qwin 500 Image Analyzer Computer System (Leica Microsystems, Swizerland) with optical magnification of 100 and 200 x on the H & E slides.

The presence of the following reparative criteria was subjectively assessed: new woven bone formation, osteocytes and variability in their size, in addition to attempts of new mature bone (osteon) formation.

Measuring the area percent of newly formed bone

The area percent of newly formed bone was estimated using Leica Qwin 500 Image Analyzer Computer System, (Leica Microsystems, Switzerland). The cursor was used to outline the areas of newly formed bone trabeculae, which were then masked by a binary colour that could be measured by the computer. The image analyser is calibrated automatically to convert the measurement units (pixels) produced by the image analyser program into actual micrometre units. The area percent of newly-formed bone was estimated in 8 different fields in each group using magnification (x 100). Mean values and standard deviation (SD) were calculated for each group.

Statistical analysis

The data obtained from computer image analysis were tabulated and statistically analysed. Analysis of variance (ANOVA) test was used for statistical analysis of the difference between groups. Tukey's post hoc test was performed to reveal any significant difference. The unpaired t-test was used for pair-wise comparisons. P-value ≤ 0.05 was considered statistically significant.

Results

Potential cytotoxicity results

In vitro cytotoxic behaviour of the grafting material (MWCNTs) showed that the toxicity was increased with increasing the material concentration. The results revealed that the IC50 of MWCNTs was almost 73.1 µg/ml. These results were within the safety level of the application according to previous studies [27], [28].

Scanning electron microscope (SEM) results

SEM-micrographs showed the morphology of the prepared grafting material after incubation in SBF. It can be observed that calcium-apatite was formed with excellent crystal-like structures, as shown in Figure 1.

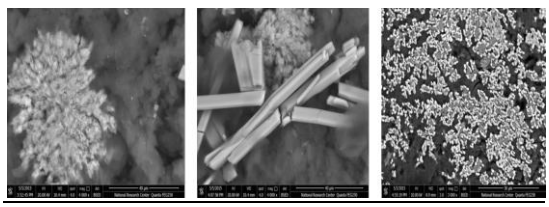

Figure 1: Scanning electron microscope (SEM) micrographs of the prepared FMWCNT

Energy dispersive x-ray (EDX) results

EDX results presented in Table 1 showed the high level of calcium content (Ca) of the grafted material.

Table 1: Energy dispersive x-ray (EDX) results showing the high calcium content after incubation of the grafted material

Element	Weight %	Atomic %
Carbon	11.14	27.38
Oxygen	2.63	4.86
Sulfur	22.92	21.11
Calcium	63.31	46.65

Clinical results

Clinical healing proceeded uneventfully. The animals tolerated the surgical procedures well, and postoperative signs were consistent with these following a localised periodontal flap surgery. At the time of sacrifice, all dogs showed a healthy periodontal condition without gingival recession or inflammation. There was no difference between the different experimental surgical sites based on clinical observation.

Histological results

Control group

One month after surgery: collagen-rich connective tissue (CT) matrix suggesting granulation tissue formation with little fibroblast and inflammatory cells was seen. At the lesion`s edge, there were areas of condensed non-mineralized tissue within the undifferentiated CT, revealing the beginning of the new formation of bone tissues (Figure 2).

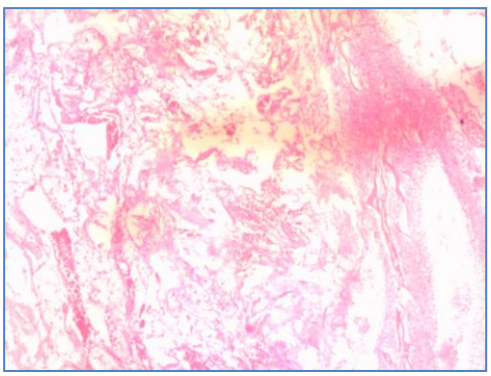

Figure 2: Photomicrograph of the control group (1 month) showing condensed collagen fibres with little fibroblast, inflammatory cells in collagen-rich CT matrix suggesting granulation tissue & ectopic bone formation within the granulation tissue (H & E)

Two months after surgery: the results showed mild bone remodelling which could be detected at the expense of granulation tissue in the form of ectopic in-growth of osteoid bone formation that was highly vascularised, irregular and with a limited number of entrapped bones forming cells (Figure 3).

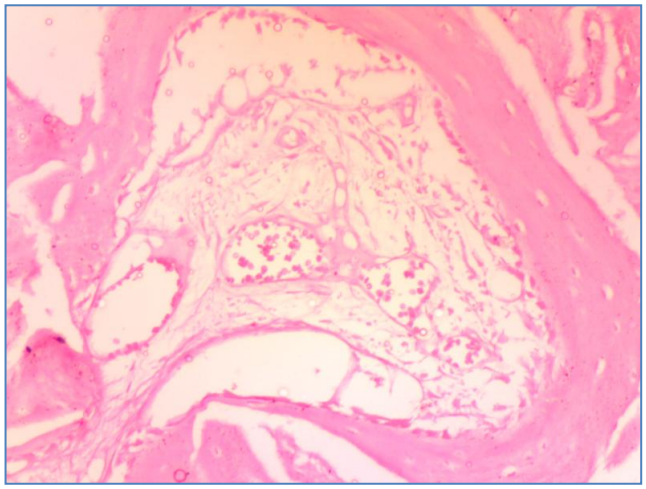

Figure 3: Photomicrograph of the control group (2 months) showing newly formed osteoid bone trabeculae replacing the granulation tissue with little number & small entrapped rounded osteocytes. Dilated blood vessels are seen within the granulation tissue (H & E)

Three months after surgery: newly formed tissue consisting of interconnected islands of newly formed bone trabeculae containing flattened osteoblasts, consistent with maturation into bone-lining cells was revealed. A complex layer of connective tissue comprised of blood vessels and collagen fibres oriented haphazardly to the bone surface covering the external aspect of the newly formed alveolar bone. Attempts of incomplete Haversian systems were also observed. No evidence of inflammatory reaction or fibrosis was detected (Figure 4).

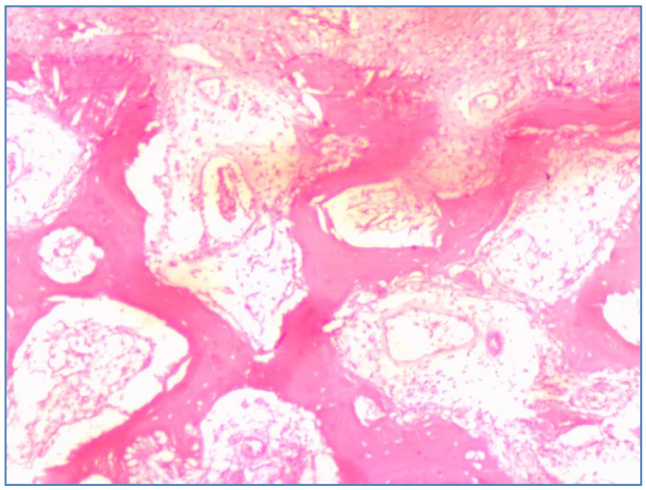

Figure 4: Photomicrograph of the control group (3 months) showing interconnected islands of thin trabeculae of new bone formation with flattened osteoblasts. Attempts of an incomplete Haversian system were also observed (H & E)

Study group (CNT group)

One month after surgery: the grafted material remained in the surgical area. The unresorbed material was predominantly surrounded by granulation tissue which demonstrated significant inflammatory cell infiltration. Bone festooning (an indicator of resorption) and multinucleated giant cells having the histological features of osteoclasts were seen residing in their lacunae on the brink of the grafted material. Residual bone spicules bordered the bone defect and extended into the graft area as seen in Figure 5.

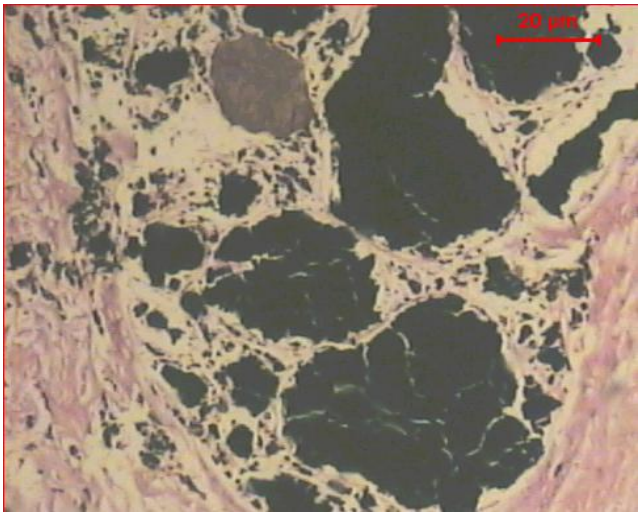

Figure 5: Photomicrograph of CNT group (1 month) showing big and small areas of the implanted material which appear black enclosed in fibrous capsules. Areas of collagenous fibres are also seen (H & E)

Two months after surgery: the outsized amount of the grafted material can still be seen in the surgical area. Little amount of newly formed bone was seen in the area. Some granulation tissue reaction can be seen next to the grafted material. A dense accumulation of fibrous tissue was observed walling off the material from the surrounding tissues. Heavy infiltration of inflammatory cells predominates in the surgical field (Figure 6).

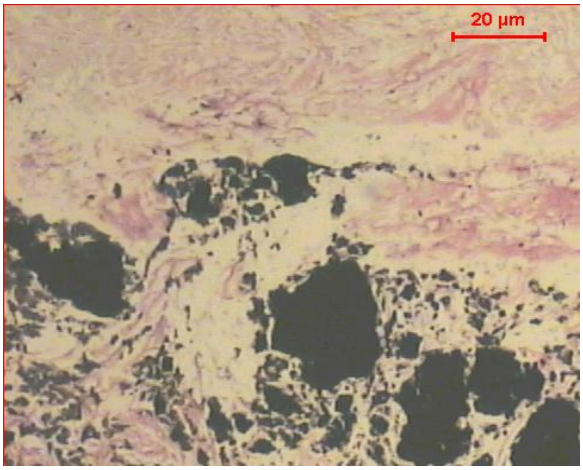

Figure 6: Photomicrograph of CNT group (2 months) showing signs of resorption of the implanted material. Evidence of newly formed bone and blood vessels are also apparent (H & E)

Three months after surgery: thinned bone trabeculae are seen radiating from the CNT material. Some of the grafted material can be seen in the surgical area. Mild inflammatory reaction was demonstrated. These slim trabeculae enclosed

irregular sized and shaped marrow spaces and widened osteocytic lacunae. A considerably thick collagen bundle is seen walling off the material from the surrounding bone tissues, as reported in Figure 7.

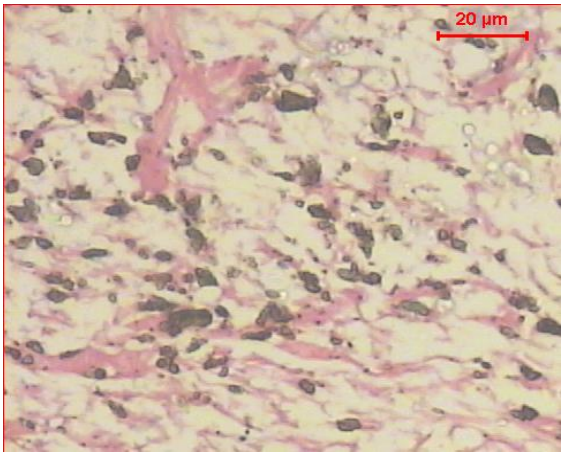

Figure 7: Photomicrograph of CNT group (3 months) showing lamellae of newly formed bone which are still associated with small areas of the grafting material (H & E)

Area percent of the newly formed bone

The greatest mean area percent of newly formed bone was recorded in the control group, whereas the least mean value was recorded in the study group. ANOVA test revealed a statistically significant difference ($p < 0.0001$).

Table 2: Mean area percent of newly formed bone and the significance of the difference between the groups

Group	Mean ± SD	Unpaired t-test	
		P-value of the different control group	P-value of difference study group
Control group	67.16 ± 2.27	----------	< 0.0001*
Study group	40.6 ± 5.73	< 0.0001*	----------
P value (ANOVA)	< 0.0001*		

*statistically significant at $p < 0.05$.

Comparing the control group with the study group using the unpaired t-test revealed a statistically significant difference ($p < 0.0001$) (Table 2).

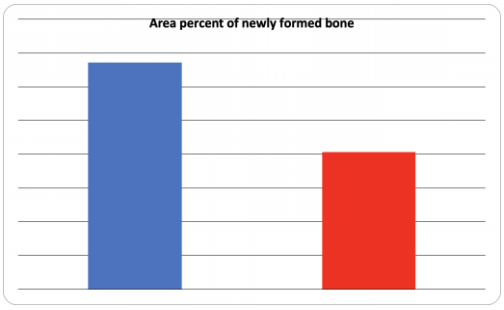

Figure 8: Column chart showing mean area percent of newly formed bone in different groups

Discussion

Bone tissue engineering has evolved as a substitution approach that depends on the use of biodegradable polymers which are of interest in medicine because of their commercial availability, biocompatibility, degradation into non-toxic products and the ability to control the material's characters as mechanical properties, porosity and surface charges [1].

Carbon nanotubes considered as one of the most important materials in nanotechnology and nanoscience nowadays. CNTs for bone regeneration are being developed, which use negatively charged functional groups with calcium bonded to them. This can provide a scaffold to which hydroxyapatite, the majority of the inorganic component of bone, can join. CNTs act as an exceptional substrate for cell growth and differentiation [29], [30], [31].

Little attention has been focused on the histological behaviour following grafting of CNTs in such a bony defect orally. Hence, it is of great interest to validate the healing and the potential for bone formation in a two walled bony defect created in the bifurcation of the mandibular premolar area in mongrel dogs using CNTs.

For any material to be used as a graft, it should have a strong mechanical and physical integrity to be able to function properly; it should also be biocompatible [12]. To test the material biocompatibility and tissue response in vivo, animal studies are considered to be the best assessment method. Dogs which were implanted with CNTs subcutaneously had no mortality, evident inflammation, behavioural changes or noticeable signs of physical self-mutilation during the post-operative examinations and at the time they were scarified. This is by the findings of Martins-Junior et al., [23] and Rajesh et al., [32].

The study design included a control group where the defects created are going to heal normally and a study group where CNTs were placed into the defects. The defects in both groups were left to heal for a similar time interval after which the animals were sacrificed, and the healing of both defects was assessed histologically.

In the control group evidence of woven bone formation was seen at the edge of the defect by the end of the first month, which became vascularised and denser at the end of the second month. Subsequently, by the end of the third month, the newly formed bone trabeculae were found to be lined by flattened osteoblasts denoting the formation of lamellar bone. These findings are consistent with those of Streitzel et al., [22] and conform to the normal healing pattern expected to be seen in such a bony defect.

It was obvious that the material resulted in a strong tissue response at the end of the second month. This was evident by the infiltration of the defect by multinucleated giant cells and fibrous encapsulation of the material walling it off from the surrounding bone. At the end of the third month, tiny traces of CNTs were still present in the defect with scarce evidence of new bone formation. The rate and amount of bone formation were slow when compared to the natural healing in the control group. This is contradictory with other results which demonstrate the higher potentiality of CNTs for bone regeneration [32].

This could be explained by the fact that the most frequent applications for these carbon materials are their inclusion as reinforcement in polymer matrices due to its weak mechanical properties. This was evident in the results of Mwenifumbo et al., [33] and White et al., [34] who used CNTs to reinforce hydroxyapatite which was then implanted in a bone defect in the rabbit's femur. Their four months' results demonstrated the growth of a web-like soft callus from the host bone towards the reinforced CNTs with a hydroxyapatite implant, indicating a strong host bone interaction.

In conclusion, the results obtained from the proposed study demonstrated the potential of CNTs for bone regeneration applications. It concluded that more time interval might be needed to achieve a better bone regenerative effect. Also, reinforcement of CNTs as a carrier in polymer matrices could attain enhanced bone modelling in PDL bony defect and might have a significant impact on the ability to restore functional activity in injured patients. Further investigation is needed to validate the potential role of CNTs in periodontal ligament bony defects.

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Dental Science

Oral Health-Related Quality of Life in Single Implant Mandibular Overdenture Retained by CM LOC versus Ball Attachment: A Randomized Controlled Trial

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Abstract

AIM: This randomised clinical study aimed to detect whether CMLOC attachment could improve Oral Health-Related Quality of Life (OHRQOL) when compared to ball attachment.

METHODS: Eighty edentulous patients were recruited to receive a single symphyseal implant for mandibular overdenture, after three months, randomisation was done to divide them into two groups; Dalbo ball (control group) and Cendres and Metaux locator (CM-LOC) (intervention) attachments respectively, oral health impact profile for edentulous patients (OHIP-EDENT)questionnaire was recorded before implant placement, two weeks after pick up, at 3, 6, 9, and 12 months.

RESULTS: Results revealed a lack of statistical significance between the two groups except for psychological discomfort at 2 weeks after pick-up (p-value = 0.029)

CONCLUSION: Single implant overdenture is a simple, reliable treatment modality for treating edentulous mandible and both CM LOC and Ball attachments are good alternatives for such treatment modality.

Introduction

The dental implant had offered a fixed prosthetic treatment modality for edentulism and added retention to complete dentures [1].

The introduction to single mandibular implant overdenture (SMIOD) went back to Cordiolli et al., in 1997, it was only preceded with a single report in 1991 where Naert et al., had used an overdenture attached to one implant until an additional implant installation [2].

SMIOD had offered minimal surgical procedure with less expected postoperative complications, and fewer expenses compared to two or more MIOD. It also offered a prosthetic implant solution in case of insufficient bone at the canine region and saved the patient a longer duration more complicated grafting procedure [3].

Ball attachment is commonly employed in single implants because its elastic retainer allows for the slight rotation of the overdenture transmitting the load to surrounding bone tissue and balancing the axial load resulting in less implant-bone tissue damage [4]. On the other hand, it had expressed high maintenance frequency, and inability to use with divergent implants as it is impossible to establish an axis of rotation [5].

A novel locator attachment system with a matrix made from polyether ketone (PEKK), was manufactured by Cendres and Métaux which claimed to offer high chemical and mechanical resistance against wear and high tensile, fatigue and flexural strengths [6].

CMLOC attachment (the commercial name) had expressed high initial retention values for both vertical (22.5N) and tilted (27.4 N) implants [7].

Patient-relevant outcomes are becoming increasingly popular in dentistry in assessing dental service and prosthesis [8]. The term "quality of life" was preferred by Locker and Allen as it was defined as being broader than merely "health" or "disease" [9]. Oral health impact profile (OHIP) was justified for being a sensitive OHRQOL tool to record clinically significant differences between different prosthodontic treatments [10].

OHIP-EDENT is a modified form of (OHIP) addressing edentulous subject [9]. It stands for 19 questions denoting seven domains: functional limitation, physical pain, psychological discomfort, physical disability, psychological disability, social disability, and handicap [11].

The OHIP-EDENT could detect changes in the OHRQoL among denture wearers before and after they receive new prostheses [10].

This study was therefore conducted to assess the improvement of the CM-LOC over ball attachments regarding ORQOL in patients receiving SMIOD.

Methods

The study was Randomized controlled, 1: 1 allocation ratio, Parallel grouped with superiority frame.

Eighty eligible patients of age range 50-69 years, were recruited in the out-patient prosthodontics clinic, at faculty of Dentistry-Cairo university.

Patients with absolute contraindication for implant placement were excluded (i.e., recent myocardial infarction, valvular prosthesis surgery, immunosuppression, active treatment of malignancy, drug abuse and psychiatric illness) [12]. Patients with a recent glycosylated haemoglobin analysis test (HbA1c) (i.e., less than a month test) results that showed greater than 8% were also excluded (According to American Association of Diabetes) [13].

Based on Bilhan et al., 2011 [14], the absolute difference of total score of QOL 6.4 ± 8.3. A total sample size of 58 patients (29 in each group) was sufficient. This number had to be increased to 66 to correct for non-parametric usage and again to 78 patients to compensate for losses during follow up. The sample size was calculated by the G power program.

The selected patients were informed about all procedures and asked to sign an informed consent before study joining. Dentures assessment and reconstruction (when necessary) were guaranteed followed by adaptation period for denture settling. OHIP-EDENT was recorded for complete denture wearers before implant installation

Dentures were duplicated resulting in a clear acrylic resin stent with Radiopaque auto polymerising resin (Jet XR™ Opaque Powder, Lang Dental Mfg Co Inc, USA) at the lower central incisors space to act as a reference for the implant site after cone beam radiograph. A CBCT scan was used for surgical planning. This radiographic template was modified during surgery to act as a surgical stent. A dose of 2g amoxicillin-clavulanic (Augmentin, Egypt) was given 1 hour before surgery [15]. Zero-point two percent Chlorohexidine mouth wash (Hexitol, Egypt) preoperative oral rinse was also instructed.

Crestal incision was cut in the inter-canine region using surgical blade number 15 (Swann-Morton-England). If the bone width was less than 5 mm, a short releasing incision was done for better accessibility to bone plateauing. A full-thickness flap was reflected using a mucoperiosteal elevator (Martin-Germany). Drilling was started with a cortical drill (if no plateauing was done), followed by a pilot, intermediate and final drill of a size 3.4 mm in diameter and 10 mm length. All implants installed in this study were Zimmer Dental (Implants ZDI, Tapered screw vent Indiana America) of diameter 3.7 mm, and length 10 mm. Soft tissue depth over the implant was measured by a periodontal probe to select the height of the healing cap before its placement. The flap was repositioned and properly adapted with either interrupted or continuous mattress suture Figure 1.

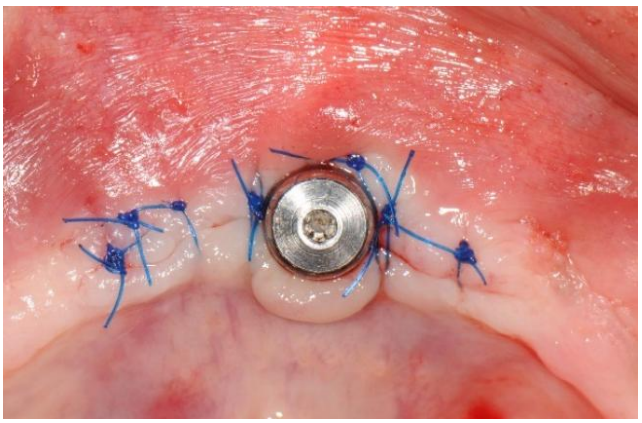

Figure 1: A single midline implant with a healing cap

Denture adjustments were made to suit the new condition with adequate relief opposite the healing cap, a small amount of soft liner (Coe soft, GC America, USA) was used anteriorly in the fitting surface of the mandibular denture. Post-operative instructions and oral hygiene measures were emphasised for all patients.

The healing phase lasted for three months after which patients were assigned to one of the two attachments groups (ball or CM-LOC) using non-

transparent sealed envelopes for randomisation.

Blinding was impossible due to the difference in the attachment shape.

After selecting the proper attachment height (with a plastic periodontal probe) the attachment was torqued, and a housing was seated over it, denture was relieved until lacking housing interference, dryness, bonding and light-curing then pick up with self-curing bis-acrylate resin material (Luxa-pick up, DMG, Hamburg, Germany) was done Figure 2.

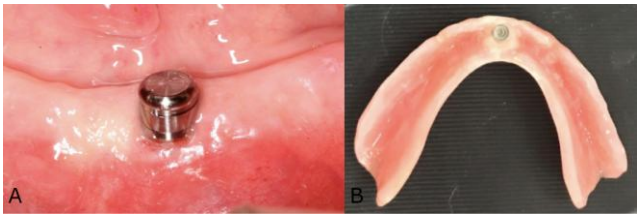

Figure 2: A) CM-LOC attachment; B) Nylon cap and housing (of Dalbo ball)

A translated form from a validated questionnaire was used to address the Egyptian population; all patients had to answer the same questionnaire two weeks after pickup, 3, 6, 9, 12 months respectively. Each answer was given a score from 0 to 4 where the lesser the score the better the quality of life, data was collected, and privacy was ensured Figure 3.

A-functional limitation.1.Have you had difficulty chewing any foods because of problems with your denture?

2 .Have you had food catching in your dentures?

3.Have you felt that your dentures haven't been fitting properly?

B-physical pain.4.Have you had painful aching in your mouth?

5.Have you found it uncomfortable to eat any food because of problems with your dentures?

6.Have you had sore spots in your mouth?

7.Have you had uncomfortable dentures?

C-Psychological disorder.8.Have you been worried by dental problems?

9.Have you been self-conscious because of your dentures?

D- Physical disability10-Have you had to avoid eating some food because of problems with your dentures?

11Have you been un able to eat with your dentures because of problems with them?

12. Have you had to interrupt meals because of problems with your dentures?

E- Psychological disability

13.Have you been upset because of problems with your dentures?

14. Have you been a bit embarrassed because of problems with your dentures?

F- Social disability

15.Have you avoided going out because of problems with your dentures?

16.Have you been less tolerant of your spouse or family because of problems with your dentures?

17Have you been a bit irritable with other people because of problems with your teeth, mouth or denture?

G Handicap

18. Have you been unable to enjoy other people company as much because of problems with your dentures?

19.have you felt that life in general was less satisfying because of problems with your dentures?

0=never	1=hardly ever
2=occasionally	3=fairly often
4=very often	

Figure 3: OHIP-EDENT questionnaire

Results

Eighty patients were grouped equally into either ball, or CM-LOC 6 failures occurred after implant installation (lack of osseointegration), and three dropouts (two travelled and one jailed)before attachment placement, after pickup one patient died in the ball group and five dropouts (2 from ball and 3 from CM-LOC group)through a whole year either due to hospitalisation, travelling, starting a distant job and post-divorce depression. The summary of the flowchart is in Figure 4.

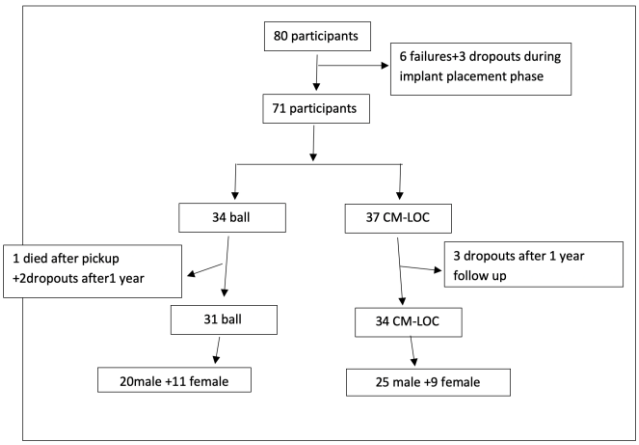

Figure 4: Participants flow chart

Scores of OHIP EDENT were tabulated in an excel sheet. Data were statistically described in terms of mean ± standard deviation (± SD) Comparison of numerical variables between the study groups was done using Mann Whitney U test for independent samples. Within group, comparison of numerical variables was done using Wilcoxon signed rank test for paired (matched) samples.

To detect the effect of gender variability in each group, exact test was used instead of Chi-square (χ^2) test as the expected frequency was less than 5. p values < 0.05 was considered statistically significant. In this study, p value was > 0.05 denoting lack of statistical significance between groups regarding the gender.

Table 1: Mean score of OHIP-EDENT of both groups at different time intervals

Group		Total-Baseline	Total-2w	Total-3m	Total-6m	Total-9m	Total-12m
Ball	Mean	23.50	9.05	8.04	8.11	10.96	10.11
	Std. Deviation	17.065	9.437	8.324	8.750	14.339	14.436
CM LOC	Mean	18.71	11.04	7.76	6.86	7.44	5.45
	Std. Deviation	12.770	12.252	8.155	9.058	9.791	7.037
	p value	0.258	0.859	0.783	0.748	0.367	0.327

All statistical calculations were done using computer program IBM SPSS ((Statistical Package for the Social Science; IBM Corp, Armonk, NY, USA) release 22 for Microsoft Windows. The effect of time

on each group was shown in Table 1; the base line denoted scores before implant placement i.e. during complete denture (CD) period, 2 w = 2 weeks after pick-up, (m) referred to months, i.e., 3 m meant three months follow up results.

From the above table, it was observed the lack of statistical significance in both groups, despite the statistically significant improvement of both groups when compared to complete denture ($p < 0.05$).

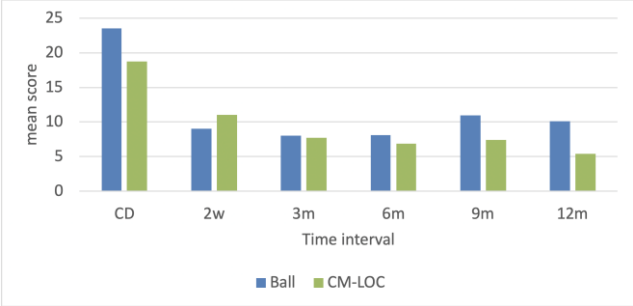

Figure 5: Mean score of OHIP-EDENT between the ball and CM-LOC expressing statistical significance compared to baseline and lack of significance through 1 year follow up

Clinically, patients with CM-LOC attachment had expressed relatively better results than those with ball group except two weeks after pick- up where CM-LOC patients found difficulty in insertion and removal of the attachment which had significantly affected the psychological discomfort domain ($p = 0.029$) when compared to ball group Figure 6.

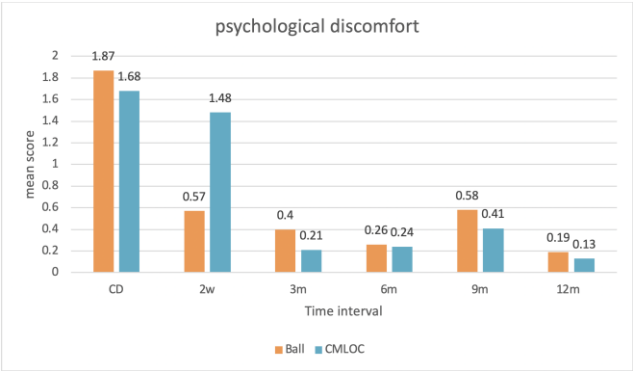

Figure 6: Psychological discomfort domain between two groups at a different time interval

Discussion

When comparing SMIOD with a complete denture, a statistically significant difference was observed at all domains regardless of the attachment type, the attachment insertion highly improved patient mastication, decreased pain due to decrease in denture motion (better fixation) which had reflected positively on patient comfort and social interaction.

This result confirmed with Cheng et al., 2012, where both locator and magnet attachments were interchanged in a cross-section study to find improvement from complete denture regardless of the attachment fastened [16]. It also confirmed with Ismail et al., 2015, in two years of study where ball and magnets had shown similar improvement from the complete denture baseline records [17].

Ball had shown relatively better results 2 weeks after pickup (zero mean score) due to high initial retention values compared to CMLOC, that added confidence and psychological stability to the patient in addition to the ease of insertion and removal caused by its spherical geometry unlike the cylindrical CM-LOC this conformed with Harder et al., 2011, in his three years study which had shown significant improvement in chewing ability and quality of life with ball attachment [18]. The quality of life improvement of SMIOD was also proven by Passia et al., 2014, systematic review [19].

The total score of the whole questionnaire had exhibited similar results in both groups except at nine and 12 months, where it favoured the CM-LOC group. This was related to the change of PEKK matrices at six months causing retention promotion compared to the nylon cap which necessitates changing at nearly 12 m follow up.

In conclusion, Single implant overdenture is a simple, reliable treatment modality for treating edentulous mandible and both CM LOC and Ball attachments are good alternatives for such treatment modality. CM-LOC seemed to offer promising results, but care should be given to the frequency of matrix changing and maintenance.

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Dental Science

Evaluation of Flexibility, Microstructure and Elemental Analysis of Some Contemporary Nickel-Titanium Rotary Instruments

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Keywords: NiTi instruments; Endodontic files; Flexibility; Microstructure; Elemental analysis; Heat treatment

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Abstract

BACKGROUND: Contemporary nickel-titanium (NiTi) rotary endodontic instruments had a revolutionary impact on the success of root canal treatment.

AIM: To evaluate the flexibility, microstructure and elemental analysis of four different recent NiTi rotary instruments, namely; Wave One Gold, TF adaptive, HyFlex EDM and Gr_Reciproc Blue compared to conventional Protaper Universal (F2).

MATERIAL AND METHODS: The NiTi rotary files were subjected to cantilever bending test to evaluate their flexibility. The microstructural characteristics and elemental analysis were examined via scanning electron microscopy (SEM) and energy dispersive X-ray spectrometer (EDX).

RESULTS: The TF adaptive, HyFlex EDM and Wave One Gold endodontic files showed significantly lower cantilever bending values (i.e., higher flexibility) than Protaper F2 and Gr_Reciproc Blue ($p < 0.05$). The SEM micrographs showed that the bulk of all examined files showed multiple striations due to the cutting process, on the other hand, their external surfaces were different: The Protaper Universal F2 showed multiple voids, while the TF Adaptive surface exhibited more uniform structure. The Hyflex EDM had a crater-like surface, whereas Wave one Gold showed machining grooves with minimum defects, while Reciproc Blue displayed machining grooves with random scratch lines. There was a significant difference in bulk and surface elemental analysis of the various examined files, yet composed mainly of the same elements.

CONCLUSION: Chemical composition, heat treatment, manufacturing process and geometrical design of the NiTi rotary instrument have a great influence on their flexibility and microstructure.

Introduction

Rotary Nickel-Titanium (NiTi) instruments were introduced to the endodontic field because of their superelastic behaviour over traditional stainless-steel instruments [1]. This made them the materials of choice for the shaping of curved root canals [1]. The unique superelasticity and shape memory effect of NiTi alloys is gained from their nearly equiatomic ratio. NiTi alloys of the rotary instruments consist of approximately 56% by weight nickel and 44% by weight titanium, which possess in 1:1 atomic ratio of the main components [2].

NiTi can be found in three different forms named; martensite, austenite and R-phase, which determine the mechanical properties and characteristics of the alloy [3]. Moreover, the mechanical performance of NiTi alloy depends on the relative characteristics of their microstructure and their association with the metallurgical behaviours, which is beneficial to understand the NiTi instruments behaviour [2].

Manufacturing of rotary NiTi endodontic instruments with different geometrical designs and chemical compositions have been developed [4]. However, sudden fracture of NiTi rotary files during root canal therapy remains a tremendous problem in the clinical aspect [4]. Thus, recently, NiTi endodontic files were fabricated by various thermal and mechanical treatment technologies to optimise their microstructure and the flexibility compared to the traditional superelastic NiTi files [4].

The flexibility of NiTi instruments means that NiTi files can permit a significant deformation below their elastic limits and still able to retain their original form [5], [6]. The flexibility of NiTi instruments play a crucial role in successful endodontic treatment because especially in curved canals, it permits suitable canal enlargement while preserving the

instrument in a central position within the canal, leading minimum undesirable changes in the shape of curved canals [5], [6]. Flexibility is influenced by alloys chemical composition, heat treatment and geometric design such as file cross-sectional, and inner core area [7]. Low modulus of elasticity beside unique superelastic properties of NiTi endodontic instruments leads to superior flexibility [5], [6]. Flexibility evaluated usually in laboratory studies by cantilever bending test. The low bending result is indicative of the high material flexibility [7].

The metallurgical feature of NiTi instruments such as composition, microstructure and phase constitution has a great impact on their performance [8]. Metallurgical properties of NiTi instruments can be investigated by laboratory tests such as scanning electron microscopy (SEM) and energy dispersive X-ray spectrometer (EDX) [8].

Recent developments in the process of manufacturing of NiTi alloys are well documented, to produce contemporary rotary endodontic instruments with maximum flexibility [9]. New NiTi endodontic files with superior mechanical properties have been developed through special a series of thermo-mechanical processing treatment such as; producing NiTi alloys with the substantially stable martensite phase upon clinical conditions [9]. Alloys in its martensite form (M-Wire) become ductile, easily deformed and more flexible than austenite, thus reducing the risk of being broken under high stress [2].

Another manufacturing process is aiming to transform conventional NiTi alloy in the austenitic phase into a rhombohedral crystal structure (which called intermediate R-phase) between austenite and martensite. The rotary instrument made out of R-phase wire possesses good superelasticity, and lower rigidity than that of austenite, thus they exhibit more flexibility and can be twisted such as TF Adaptive files (TFA; SybronEndo, Orange, CA, USA) [3].

Hyflex EDM (HEDM; Coltene / Whaledent AG, Altstätten, Switzerland) was launched using electrical discharge machining (EDM) technique, which is a non-contact thermal erosion process via controlled electrical discharges machining process [10]. This process depends on electrical sparks which cause a local melting in addition to partial evaporation of small parts of metals leaving a typical crater-like surface finish. After that, the instrument is heat-treated at a temperature in a range of 300-600°C for 10 min-5 hours before or after the cleaning process [10]. The EDM process, being non-contact, is supposed to avoid early failure of material which may occur from conventional grinding techniques [4].

Wave One Gold endodontic file technology (Dentsply Maillefer, Ballaigues, Switzerland) is utilising a unique pre and post-manufacturing heat-treatment. The superelastic NiTi alloy is exposed to special heat treatment beneath constant strain in a range of 3-15 kg over a temperature range of 410°C-440°C. The finished instrument after machining is subjected to a further heat-treated in a range of 120°C-260°C. The manufacturer assumes that gold technology exhibited improved flexibility. They have two cutting edges; cross-section of the file was modified to a parallelogram [3].

Gr Reciproc Blue [RPC Blue; VDW, Munich, Germany] is released recently using innovative heat treatment that transforms the molecular structure of the M-Wire instrument providing a more flexible alloy; this treatment was also responsible for the blue colour of the file. They have two cutting edges; S-shaped cross-section and non-cutting tip [11].

Manufacturers have launched to market various thermally treated NiTi alloys (i.e., TF adaptive, HyFlex EDM) to optimise their microstructure and transformation, which in turn has a greater impact on their mechanical properties. The recent generation of NiTi instruments (i.e., Wave One Gold and Reciproc Blue subjected to a complex proprietary heating-cooling treatment providing a visible titanium oxide layer at the surface.

All this treatment and modification claimed by manufacturers to induce superior flexibility and high performance of the NiTi instruments. Thus, this study aims to evaluate the influence of NiTi alloy treatment of some recent NiTi rotary Instruments upon their bending behaviour compared to non-treated NiTi alloy (Protaper Universal, F2 [PTU; Dentsply Maillefer, Ballaigues, Switzerland]), in addition to the description of their microstructure and elemental analysis.

Material and Methods

Five different brands of rotary NiTi instruments (endodontic files) were tested in this study, Table 1: A) ProTaper Universal F2 (Dentsply Maillefer, Ballaigues, Switzerland); B) TF adaptive (SybronEndo, Orange, CA, USA); C) HyFlex EDM (Coltene / Whaledent AG, Altstätten, Switzerland); D) Wave One Gold (Dentsply Maillefer, Ballaigues, Switzerland); and E) Reciproc Blue (RPC Blue; VDW, Munich, Germany), (Figure 1).

Table 1: The endodontic rotary NiTi instruments tested

Brand Name / Taper	Manufacturer	ISO No. / Taper	Lot No.
Protaper Universal	Dentsply Maillefer, Ballaigues, Switzerland	25	1327775
TF Adaptive	SybronEndo Orange, CA, USA	25	051638929
Hyflex EDM	Coltene / Whaledent, Alstatten, Switzerland	25	H34710
WaveOne Gold	Dentsply Maillefer, Ballaigues, Switzerland	25	1332683
Reciproc Blue	VDW, Munich, Germany	25	041332

ProTaper Universal F2 file was established as the control group. All instruments tested with an ISO

size 25 mm length with 0.40 mm tip.

Figure 1: Rotary NiTi instruments: A) ProTaper Universal F2; B) TF adaptive; C) HyFlex EDM; D) Wave One Gold; E) Reciproc Blue

Flexibility (cantilever bending test)

These five different brands of rotary NiTi endodontic files were tested and divided into five experimental groups (n = 5). The rotary NiTi instruments' flexibility was evaluated by exposing the files to a cantilever bending resistance test using a universal testing machine (Model 3345; Instron Industrial Products, Norwood, MA, USA) with a load-cell of 5 kN. Each NiTi file was gripped horizontally in the lower fixed compartment of the universal testing machine by tightening screws. The tips of the specimens were subjected to a 45° displacement using mono-bevelled chisel attached to the upper movable compartment of the testing machine travelling at a cross-head speed of 0.5 mm/min. The chisel hit the file 5mm from its tip. Data were recorded using computer software (Instron® Bluehill Lite Software).

SEM and EDX analysis

Observations of the rotary NiTi instruments were conducted with a scanning electron microscope (JSM-5200, JEOL, Tokyo, Japan) equipped with EDX (Oxford Inca Energy 350, Oxford Instruments, Abingdon, UK) (n = 5). Specimens were prepared by cross-section cutting of the new files, then polishing it by silicon carbide sandpaper disc (400 to 2500 grit), under continuous running water. The chemical etching was done to reveal the metallographic features. The polished specimens were chemically etched in a solution of composed of 10 mL hydrofluoric acid, 45 mL nitric acid and 45 mL water and swabbed for 30 seconds according to ASTM E407-07 (Standard Practice for Micro-Etching Metals and Alloys) [12]. The analysis was performed along the cross-sectional area of the files; 5 mm from the tip of the file. Micrographs were taken at magnifications 400 X and 8000 X. The SEM micrographs and the EDX analysis were performed for both the bulk area and external surface of the different files to identify the microstructure and overall chemical composition respectively.

Statistical analysis

Statistical analysis of the data was conducted by using the One-way Analysis of Variance (ANOVA) and Tukey HSD test. The significance level was set to 5% ($P < 0.05$). Data obtained were analysed using IBM® SPSS® Statistics Version 20 for Windows (SPSS Inc., IBM Corporation; USA).

Results

Flexibility (cantilever bending)

The mean and standard deviation of the flexibility, measured by the load required to bend the NiTi instruments to 45°, are shown in Table 2.

Table 2: Mean and standard deviation of cantilever bending

Endodontic file	ProTaper Universal F2		TF adaptive		HyFlex EDM		Wave One Gold		Reciproc Blue		P-value
Cantilever bending (gram)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
	171.4[b]	19.5	86.5[a]	9.1	100.6[a]	9	66[a]	14.4	154.7[b]	10.5	≤ 0.001*

Mean with different small letters indicate significance difference * statistically; significant ($p < 0.05$).

The three endodontic files; TF adaptive, HyFlex EDM and Wave One Gold showed significantly lower cantilever bending values (i.e. higher flexibility) than Protaper F2 and Gr_Reciproc Blue ($p < 0.05$).

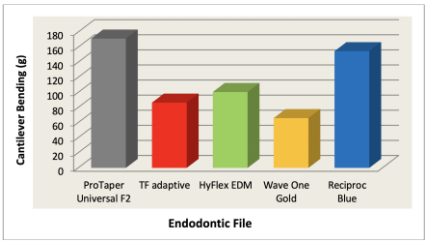

Figure 2: Bar chart showing mean cantilever bending values among the different endodontic files

SEM and EDX analysis

The SEM micrograph of Protaper Universal F2 displayed a convex triangular cross-section, Figure 3A. The bulk region showed multiple striations due to the cutting process, Figure 3B. This feature was not limited to the Protaper Universal F2 only, but all the tested endodontic files in this study. Where TF Adaptive, Hyflex EDM, Wave one Gold and Reciproc Blue showed nearly similar striations after cutting, Figures 4B, 5B, 6B and 7B respectively. Meanwhile, the external surface of Protaper Universal F2 showed multiple voids of varying sizes, Figure 3C.

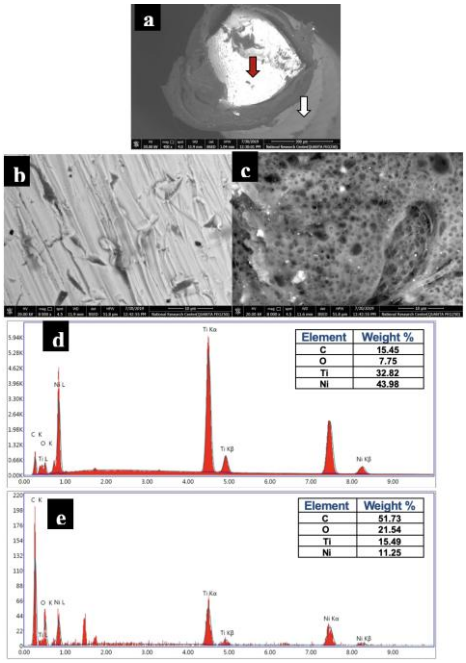

Figure 3: Representative SEM micrograph and EDX spectrum from a Protaper Universal F2 instrument; A) SEM micrograph of file cross-section after cutting, red arrow pointed to cut part of bulk and white arrow directed to external surface, magnification 400 X; B) SEM micrograph of cut part of bulk, magnification 8000 X; C) SEM micrograph of external surface, magnification 8000 X; D) EDX of cut part of bulk; E) EDX of external surface

Regarding TF Adaptive, its SEM micrograph revealed a triangular cross-section, Figure 4A. Contrary to the Protaper Universal F2, the external surface of TF Adaptive exhibited uniform structure with negligible voids, Figure 4C.

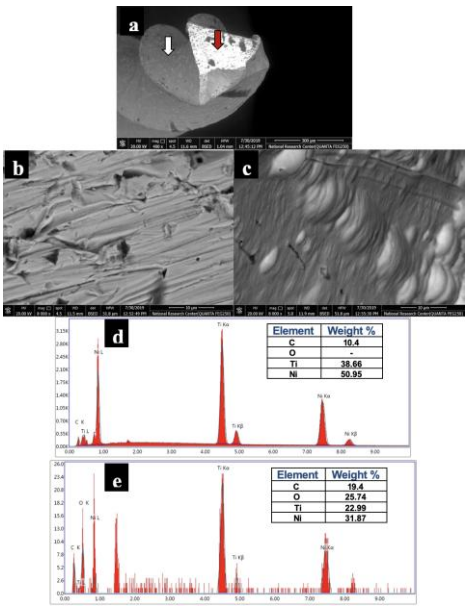

Figure 4: Representative SEM micrograph and EDX spectrum from a TF Adaptive instrument; A) SEM micrograph of file cross-section after cutting, red arrow pointed to cut part of bulk and white arrow directed to external surface, magnification 400 X; B) SEM micrograph of cut part of bulk, magnification 8000 X; C) SEM micrograph of external surface, magnification 8000 X; D) EDX of cut part of bulk; E) EDX of external surface

While, Hyflex EDM endodontic file had a rectangular cross-section, Figure 5A. Its external surface showed numerous corrugations with multiple pits and pores giving a crater-like surface, Figure 5C.

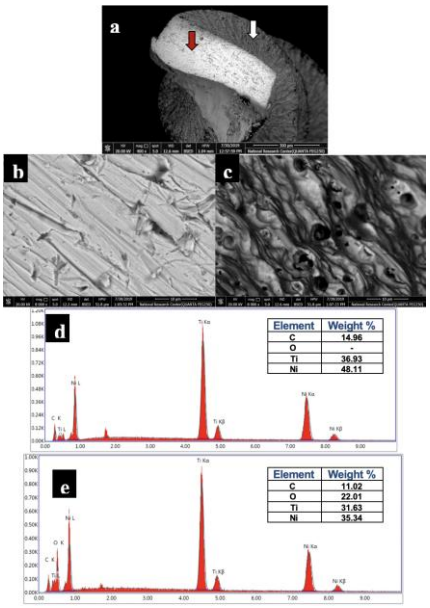

Figure 5: Representative SEM micrograph and EDX spectrum from a Hyflex EDM instrument; A) SEM micrograph of file cross-section after cutting, red arrow pointed to cut part of bulk and white arrow directed to external surface, magnification 400 X; B) SEM micrograph of cut part of bulk, magnification 8000 X; C) SEM micrograph of external surface, magnification 8000 X; D) EDX of cut part of bulk; E) EDX of external surface

The Wave one Gold showed parallelogram-shaped cross-section, Figure 6A. The SEM micrographs of its external surface-displayed machining grooves with minimum defects, Figure 6C.

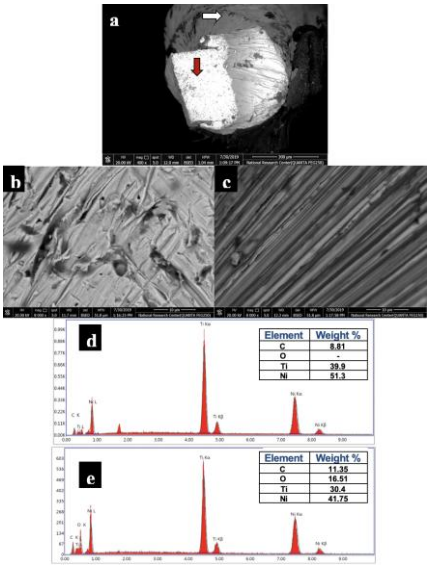

Figure 6: Representative SEM micrograph and EDX spectrum from a Wave one Gold instrument; A) SEM micrograph of file cross-section after cutting, red arrow pointed to cut part of bulk and white arrow directed to external surface, magnification 400 X; B) SEM micrograph of cut part of bulk, magnification 8000 X; C) SEM micrograph of external surface, magnification 8000 X; D) EDX of cut part of bulk; E) EDX of external surface

Concerning Reciproc Blue, its cross-section was dim S-shaped, Figure 7A. As for its external surface, machining grooves were shown with random scratch lines, Figure 7C.

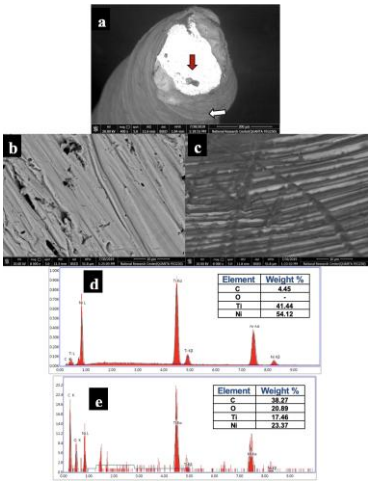

Figure 7: Representative SEM micrograph and EDX spectrum from a Reciproc Blue instrument; A) SEM micrograph of file cross-section after cutting, red arrow pointed to cut part of bulk and white arrow directed to external surface, magnification 400 X; B) SEM micrograph of cut part of bulk, magnification 8000 X; C) SEM micrograph of external surface, magnification 8000 X; D) EDX of cut part of bulk; E) EDX of external surface

EDX analysis showed that the bulk of all the examined five files were composed mainly of nickel and titanium, in addition to carbon in lower weight %, with a significant difference in these three elements among the different brands ($p < 0.05$), Table 3 and Figure 8.

Table 3: Mean and standard deviation of elemental analysis (EDX) of bulk material of the different endodontic files

Endodontic file	ProTaper Universal F2		TF adaptive		HyFlex EDM		Wave One Gold		Reciproc Blue		P-value
Elements (wt%)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Carbon	15.5^{e}	0.4	10.4^{c}	0.4	15^{d}	0.5	8.8^{b}	0.2	4.5^{a}	0.3	
Oxygen	7.8		-	-	-	-	-	-	-	-	≤ 0.001*
Titanium	32.8^{a}	0.6	38.7^{c}	0.4	36.9^{b}	0.7	39.9^{d}	0.3	41.4^{e}	0.5	
Nickel	44^{a}	0.3	51^{c}	0.9	48.1^{b}	0.5	51.3^{d}	0.3	54.1^{e}	0.6	

Mean with different small letters in the same row indicate significance difference * statistically; significant ($p < 0.05$).

Representative EDX spectra of bulk material of the different file brands are shown in Figures 3D, 4D, 5D, 6D and 7D. Oxygen was present in the bulk material of Protaper Universal F2 only, yet in very low percentage, Figure 3D. On the other hand, the oxygen was absent in the rest of the inspected files.

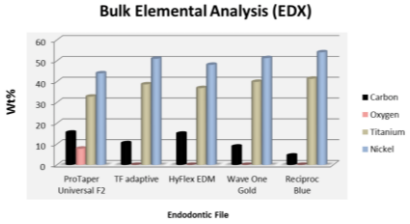

Figure 8: Bar chart showing elemental analysis (EDX) of bulk material of the different endodontic files

The surface EDX analyses of the different files are shown in Table 4 and Figure 9. There was a significant difference in external surface elemental analysis of various examined files, ($p < 0.05$) Representative EDX spectra of the surface of the different brands are shown in Figures 3E, 4E, 5E, 6E and 7E.

Table 4: Mean and standard deviation of elemental analysis (EDX) of the external surface of the different endodontic files

Endodontic file	ProTaper Universal F2		TF adaptive		HyFlex EDM		Wave One Gold		Reciproc Blue		P-value
Elements (wt%)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Carbon	51.7^{e}	0.6	19.4^{c}	0.2	11^{a}	0.6	11.4^{b}	0.3	38.3^{d}	0.5	
Oxygen	21.5^{c}	0.4	25.7^{e}	0.5	22^{d}	0.5	16.5^{a}	0.4	20.9^{b}	0.7	≤ 0.001*
Titanium	15.5^{a}	0.3	23^{c}	0.8	31.6^{e}	0.4	30.4^{d}	0.3	17.5^{b}	0.5	
Nickel	11.3^{a}	0.2	31.9^{c}	0.7	35.3^{d}	0.3	41.8^{e}	0.3	23.4^{b}	0.7	

Mean with different small letters in the same row indicate the statistically significant difference, *; significant ($p < 0.05$).

Contrary to the bulk, the EDX analysis of the external surfaces of most tested files revealed that the carbon and oxygen were present in higher weight% than their percentages in the bulk.

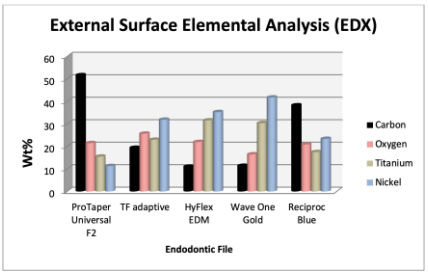

Figure 9: Bar chart showing elemental analysis (EDX) of external surfaces of the different endodontic files

Discussion

Using NiTi alloy for the production of endodontic instruments has brought pronounced advancement to endodontic treatment [13]. In this study, four rotary NiTi instruments were selected; TF adaptive, HyFlex EDM, Wave One Gold and Reciproc Blue. This selection was based on that they were subjected to different heat treatments and manufacturing process. Conventional Protaper Universal (F2) was used as a gold standard file [14].

Since the flexibility of NiTi files is considered an essential requirement in endodontic treatment, especially in curved canals [15], the flexibility of the examined file was compared in this research. It is usually assessed laboratory using bending test resembling the load exerted by the root canal curvature [7]. Lower values required for bending indicate higher flexibility of material [7].

In this study, Protaper F2 and Reciproc Blue endodontic files showed lower flexibility (higher cantilever bending values) compared to TF adaptive,

HyFlex EDM and Wave One Gold. This might be attributed to several causes as the flexibility is a multifactorial property influenced by several variables as geometric conformation, chemical composition and heat treatment [7].

The cross-sectional configuration of Protaper F2 and Reciproc Blue and their elemental composition may play a role in decreasing their flexibility. It was stated previously that the cross-section geometry of Protaper Universal F2 (convex triangle) decreased their flexibility [16]. While, a previous study compared the cross-sectional area of different files (Reciproc Blue, WaveOne Gold and Genius File). It was found that the Reciproc Blue had the largest cross-sectional area (S-shape) [17]. It was reported previously that flat sides of endodontic files reduce the cross-sectional diameter and area, rendering the endodontic file thinner and more flexible to explore curved canals [18]. Thus, the S-shaped cross-section of Reciproc Blue and the convex triangle of Protaper Universal F2 with their non-flat sides may provide bulky cross-section area which decreases their flexibility.

On the other hand, the flat sides of TF adaptive, HyFlex EDM and Wave One Gold might play a role in increasing their flexibility as their cross-sectional design were triangle, rectangular and parallelogram respectively. The later cross-section was attributed to the increased flexibility of endodontic files [19]. From a mathematical point of view, there are certain shapes which have a lower cross-sectional area as a triangle and slender rectangle compared to square and rectangle [20]. Similarly, triangle with linear sides compared to a triangle with convex sides [21].

Another cause which might lead to lower flexibility of Protaper F2 and Reciproc Blue endodontic files compared to TF adaptive, HyFlex EDM and Wave One Gold was the percentage of carbon content in the surface. In this research, Protaper F2 surface had the highest carbon content (51.7wt%), followed by Reciproc Blue (38.3 wt%), then TF adaptive (19.4 wt%), HyFlex EDM (11 wt%) and Wave One Gold (11.4 wt%). The increased carbon on the surface may lead to more carbide precipitations which may obstruct dislocation movement, increasing file rigidity of Protaper F2 and Reciproc Blue [22], [23].

It should also be noted that the manufacturing process and heat treatment play a tremendous role in determining material flexibility [24]. Files which are produced by just milling without heat treatment as Protaper F2 displayed lower flexibility [24]. While those exposed to heat treatment showed more flexibility as the crystal structure and phase transformation temperatures are affected [4]. The manufacturing process which transforms the conventional NiTi alloy with austenitic phase into R-phase with rhombohedral crystal structure increases the flexibility of NiTi alloy. Thus, it can be used for the production of twisted files such as TF Adaptive files which have high flexibility [3]. While Wave one Gold is exposed to heat treatment before and after file machining [9]. It was documented that heat treatment of file after machining overcome the machining procedure defects and modify the crystalline phase with a significant increase in flexibility [25]. As for Hyflex EDM, it is fabricated by electrical discharge machining (EDM) followed by heat treatment which exhibited considerable amounts of the R-phase and martensite which increases flexibility [26]. Although Gr Reciproc Blue is produced by pre and post milling heat treatments, yet they show lower flexibility. This might be attributed to the other factors which may hinder flexibility as bulky cross-sectional area and increased carbon in surface [18], [22].

Heat treatment of NiTi alloys does not affect the mechanical properties of the endodontic files only but also their optical properties. When the instruments are heat-treated and then cooled, this results in a colour of surface corresponding to the thickness of titanium oxide layer [15]. If the titanium oxide layer thickness is 60-80 nm, this results in blue colour, while if the thickness is 100-140 nm, this results in golden colour [15]. This is confirmed in this research by EDX which analysed only Ti, Ni, O and C in the surface of these coloured files. Therefore, the term gold used in describing some endodontic files does not denote the presence of gold element but denote the gold colour of file and the heat-treatment performed in the file which imitates that done for gold alloy by heating and then slowly cooling after the production phase [27], [28].

Regarding the SEM micrographs, the bulk of all the examined files showed the same morphology (multiple striations) after cutting due to the standardisation of the cutting procedure. However, the external microstructure differs among the various files, which may be due to the different manufacturing process used for each.

The external surface of Protaper Universal F2 showed multiple voids of varying sizes. These voids were observed in previous studies examining Protaper files and attributed this to Kirkendall effect which occurs due to unbalanced atomic diffusion between nickel and titanium atoms due to the difference in their diffusion rates when heated which leads to the formation of voids [29], [30]. The nickel atoms diffuse faster into titanium than the titanium atoms do in the reverse direction. Thus the mass transport is not balanced which leads to voids formation in the nickel side after alloying [30]. Others attributed this void to inclusions as oxides impurities which entered within alloy during the manufacturing process. And after machining the files, these voids may lead to further machining grooves and cracks [30]. The presence of oxygen within the alloy of Protaper file was confirmed by EDX analysis in this study which detected oxygen in bulk material and may be reflected as voids in surface [30]. On the other hand, obvious voids were not detected in the surfaces of the other examined

files nor oxygen was identified in their bulk materials. This might be attributed to the heat treatment processes performed in these files, as it was proven previously that heat treatments suppressed the formation of Kirkendall voids [31].

Contrary to the Protaper Universal F2, the surface of TF Adaptive exhibited uniform structure with negligible voids. This reason behind its great integrity with minimal defects may be due to the difference in its manufacturing process, as discussed previously. Its core structure is never sectioned but twisted which helps to preserve grain structure.

While the crater-like surface of Hyflex EDM may result from the electrical spark machining. As its surface showed numerous corrugations with a non-uniform structure where multiple pits and pores were observed as the electrical sparks cause local melting and partial evaporation of small parts of metals [32].

The external surface of Wave one Gold displayed even machining grooves without distortion with a uniform structure and minimum defects. This might be attributed to unique pre and post-manufacturing heat-treatment. It was stated previously that heat treatment of NiTi alloy led to a better crystalline arrangement which increases flexibility [33].

The Reciproc Blue surface showed machining grooves with random scratch lines; this might be attributed to its reported reduced micro-hardness compared to conventional M-Wire super-elastic NiTi [34]. Yet, it should be noted that hardness is a surface property and is not correlated to bulk properties as flexibility [35].

The EDX analysis results in this study are presented in weight percent for two mean reasons; first: in metallurgy, the weight percent is commonly used as it is used for determining how many grams would be added from each metal for alloy formation[36], second: for comparative reason as most previous researches used weight percent in describing their EDX results [37], [38], [39], [40].

The EDX analyses of the bulk material of the different files show that all of them consisted mainly of nickel and titanium [37]. Yet there was a significant difference in these elements among the different brands. This might be attributed to the discrepancies in the raw materials during manufacturing [38]. Carbon was also present in lower percentage as melting nickel and titanium was performed frequently using vacuum induction in graphite crucible [41], [42]. This procedure might lead to carbon contamination within the alloy [30], [42].

Contrary to the bulk, the EDX analysis of the surfaces of most tested files revealed that the carbon and oxygen were present in higher weight % than their percentages in the bulk. This may be due to the contact between the surface of NiTi endodontic file and the atmosphere which contains oxygen and carbon [43]. Thus, their concentration in the endodontic file increased from the surface and decreased towards the bulk [43]. Another justification of the increased carbon content in the surface was the decomposition of the oil used for lubrication during the machining process [44]. The oxygen on the NiTi surface is presented mainly as a titanium oxide layer which is responsible for tarnish and corrosion resistance and high biocompatibility of this material [45].

Within the limitations of the current study, it could be concluded that:

1. Chemical composition, heat treatment, manufacturing process and geometrical design of the NiTi rotary instrument have a great effect on their flexibility and microstructure.

2. Carbon and oxygen elements in the NiTi rotary instrument alloy affect their rigidity and structural integrity, respectively.

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Cost-Effectiveness of CM-LOC Attachment versus Ball Attachment Retaining Single Implant Mandibular Overdentures

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Keywords: Single implant; Overdenture; Cost-effectiveness

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Abstract

AIM: To investigate the cost-effectiveness of the novel CM-LOC attachment compared to the gold standard ball attachment in single implant mandibular overdenture.

MATERIAL AND METHODS: Fifty-two completely edentulous patients (50 to 69 years old) seeking to improve the retention of their complete mandibular dentures by installing a single implant in the midline of the mandible were recruited for this study. The patients were equally divided into two groups. The first group received a ball attachment abutment over the implant and the second group received CM-LOC attachment abutment. The initial cost and aftercare (maintenance) cost were calculated for each attachment and compared to each other.

RESULTS: The initial cost of the CM-LOC attachment was 2.2 times that of the traditional ball attachment. The after-care cost of the CM-LOC attachment was 2.39 times more than the ball attachment. The total cost of the CM-LOC attachment was 2.22 times that of the ball attachment.

CONCLUSION: The ball attachment system showed better cost-effectiveness compared to the CM-LOC attachment from the beginning of the study and throughout all the treatment period during the first year.

Introduction

Nowadays, edentulous patient's expectations and needs are very high, and by using complete denture as a prosthetic option large number of patients may not be satisfied. There are high potentials for problems to arise after the use of complete dentures.[1]The prosthodontic rehabilitation with implant-retained overdentures represents a superior treatment option to overcome these problems.

Implant overdenture (IOD) improves stability, retention and patient-reported outcomes compared to conventional complete dentures. On the other hand, its higher cost causes some concerns, as most of the completely edentulous patients are senior citizens and may have limited financial abilities and would prefer cost-effective procedures.

Up till now, the two (IOD) is the first choice as a standard of care for the edentulous mandible in most cases, but a single (IOD) was raised as a more cost-effective treatment with comparable patient satisfaction levels [1].

In a single (IOD) ball attachments are very commonly used because they are solitary, simple, and easier to use and less technique sensitive. However, ball attachment is known for their susceptibility to wear and their nylon matrix has to be exchanged after a certain time of clinical service, which causes a financial burden on the patients [2].

The Cendres Metaux Locator (CM-LOC) attachment was recently introduced into the market as an alternative to the ball attachment. It is claimed that its new design and materials may significantly reduce wear and subsequently less matrix exchange and less maintenance, which should lead to better cost-efficiency. However, the clinical performance of this

attachment regarding single IOD is not tested yet [3].

This study was conducted to compare the difference in cost-effectiveness between the most commonly used ball attachment and the new CM-LOC attachment in retaining single (IOD).

Material and Methods

Study setting

The randomised clinical trial was conducted in the Removable Prosthodontics Department, Faculty of Dentistry, Cairo University.

Patient selection

The 52 patients fulfilled the following criteria: - Completely edentulous male or female patients between the ages of 50 to 69; - Patients with no systemic or local contraindications for implant placement; - Patients with a mandibular denture height more than 6 mm between the base of the denture and the incisal edge of the central incisors; - Sufficient bone quality and quantity; and - Only compliant and cooperative patients were included.

All patients had a thorough examination including a check on medical and dental history, laboratory investigations, clinical and radiographic examination.

A complete denture was made conventionally and then duplicated into a radiographic stent to be used during the cone-beam C.T. the duplicate was also, used as a guide for implant placement in the midline. An implant fixture was placed conventionally. Three months later, the implant abutment was placed. The patients received either a traditional ball abutment or the novel CM-LOC abutment and direct pick up was performed with the complete denture.

A one year follow up with maintenance was made for every patient after the pick-up. Maintenance included occlusal adjustment, denture relining, denture repair (if fracture) and changing the nylon cap.

Measuring the outcome

Cost analysis was made to the whole study in United States American dollars. The total direct cost was calculated throughout the study by adding the sum of the initial cost and the aftercare cost throughout the follow-up period in an excel sheet.

The statistical analysis

The collected Data were statistically described in terms of mean ± standard deviation (± SD), or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was made using the Mann Whitney U test for independent samples. For comparing categorical data, Chi-square (χ^2) test was performed. Exact test was used instead when the expected frequency is less than 5. P values of less than 0.05 were considered statistically significant. All statistical calculations were done using computer program IBM SPSS (Statistical Package for the Social Science; IBM Corp, Armonk, NY, USA) release 22 for Microsoft Windows.

Results

Cost analysis was divided into initial cost and aftercare cost. The initial cost is concerned with all the cost of the treatment until the end of the prosthetic phase, which is the attachment pick – up. The aftercare cost is concerned with any costs after that phase which is mainly the prosthetic maintenance cost.

The cost of the ball attachment system was used as a standard unit on which other costs were related, and its value was given the mark X. This was done to avoid any currency changes at different times and to have a set value. At the time of the study, the cost of the ball attachment was (75$).

The initial cost

Due to the similarity between the treatment modalities in the initial cost, all common costs were excluded. The initial cost was focused on the attachment system. All other initial costs were completely equal between the two groups (the complete denture construction cost, the radiographic cost, the surgery cost, etc.).

Table 1: initial cost

The ball attachment cost	x
The CM-LOC COST	2.2x

The initial cost of the CM-LOC attachment mechanism was 2.2 more expensive than the traditional ball attachment mechanism, which made the CM-LOC attachment group less cost-effective in terms of the initial cost.

The aftercare cost

The aftercare cost is directly related to the

annual prosthetic maintenance done by each patient. Prosthetic maintenance cost was mainly divided between the attachment system maintenance cost and the denture maintenance cost, which is essentially the lab fees.

The after-care costs are the sum of the attachment system maintenance costs and the denture maintenance costs throughout 1 year follow up for in both groups per patient.

The after-care costs = (the attachment system maintenance cost) + (the denture maintenance costs).

Table 2: The after-care cost

	The aftercare cost of the attachment system maintenance	The aftercare cost of the denture maintenance	The total aftercare cost of the attachment system and denture maintenance
CM-LOC	0.138x	0.255x	0.393x
BALL	0.029x	0.135x	0.164x

The after-care cost of the CM-LOC attachment was 2.39 times more than the ball attachment, which made the CM-LOC attachment group less cost-effective in terms of the after-care cost.

The total cost

The Total cost is the sum of the initial treatment costs and the after-care costs throughout 1 year follow up for in both groups per patient.

Total costs = (initial treatment costs) + (the after-care costs).

Table 3: The total cost

	The initial cost	The aftercare cost	The total cost
CM-LOC	2.2x	0.393x	2.593x
BALL	1x	0.164x	1.164x

The total cost of the CM-LOC attachment was 2.22 times more than the ball attachment.

Discussion

The methods and results for cost analysis are different among multiple studies due to differences between settings in terms of the availability and affordability of dental care and dental supplies, varying pricing policies and type of dental implants and taxes differences. Also, to express the accumulation of costs over the remaining life of the patient about the average life in each country [7].

In literature, Cost is mainly divided into total clinical cost and patient time cost. The patient time cost is very specific for each patient and the large difference between patients. The total clinical cost is divided into initial treatment cost and the aftercare (maintenance) cost. The initial treatment costs include the cost of the implant itself and prosthesis construction-related cost. Maintenance costs includes those associated with remakes, relines, hardware replacement, professional services provided by the Prosthodontists and the required annual recall visits [8].

Ball and ball-like attachments have been used for many years to retain IODs with high success rate and patient satisfaction. They are known to be cost-effective, simple, hygienic and easy to handle [9].

Different studies have reported a loss of retention which required replacement of the matrix (mostly nylon cap) as one of the most common prosthetic maintenance required. With the CM-LOC, it is claimed that multiple improvements have been made in both the design and the materials used to decrease prosthetic maintenance required and subsequently, the after-care cost [11].

There are two main differences between the ball attachment and the CM-LOC attachment, the material of the cap and the design of the attachment and the cap.

In terms of material the manufacturer claim that PEKK material which is used for the cap in CM-LOC attachment is known to have high-quality characteristics such as good dimensional stability, high chemical and mechanical resistance against wear, and high tensile, fatigue and flexural strengths [12]. The difference of resiliency between the PEKK cap and the more resilient polyethylene nylon cap of the ball can be the cause of more PEKK cap change. The PEKK material has a higher ratio and sequence of keto groups which increase the rigidity of the polymer chain. PEKK is a less resilient material may tend to have more wear due to friction during denture movement which may lead to loss of retention and require more frequent cap change [12].

In terms of design, the CM-LOC attachment matrix is similar to the design of the locator attachment but The PEKK matrix has an oval C shaped design which provides a slot in the matrix. This slot is intended to allow expansion of the cap and might act as a buffer, which is claimed by the manufacturer to reduce the deterioration of the matrix surface resulting in a reduced wear of the material [11]. The expansion of the PEKK cap might be questionable due to its rigidity and being surrounded by a metal housing which limits its expansion. Also, multiple expansions in and out from the undercut may increase friction and wear of the cap. Also, the design of the CM-LOC is similar to the design of the locator attachment which is known for the regular replacement of cap due to constant wear and tear [4]. On the opposite side, the nylon cap of the ball attachment has other privileges; being completely round decrease friction during rotational denture movement and having vertical resiliency which decrease friction during tissue ward movement which

decrease wear of the nylon cap [11].

The cost-effectiveness plays the main role in decision making of the treatment plan for both the patient and the prosthodontist. The initial cost and The after the cost of the CM-LOC attachment was higher than that of the ball attachment. So, CM-LOC showed less cost-effectiveness compared to the ball attachment.

The main cause of the cost difference is due to the higher cost of the CM-LOC attachment parts itself than the ball attachment.

There is a great difference in the initial cost in favour of the ball attachment. In addition to that that the CM-LOC group had a slightly higher rate of maintenance compared to the ball attachment which was opposite to what was proposed by the manufacture of the CM-LOC. The rate of change of the CM-LOC cap was generally higher than that of the ball attachment. The manufacture assumed that CM-LOC would need a few maintenances, which would decrease the cost, but this research found the opposite.

In conclusion, the ball attachment system showed better cost-effectiveness compared to the CM-LOC attachment from the beginning of the study and throughout all the treatment period during the first year.

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Global Prevalence of Tobacco Use in Adolescents and Its Adverse Oral Health Consequences

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Keywords: Tobacco use; Oral health conditions; Adolescence; Global prevalence; Cigarette smoking

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Abstract

BACKGROUND: Smoking is associated with various systemic conditions and contributes to a huge financial burden to economies around the world.

AIM: The study aimed to evaluate global data about the prevalence of tobacco use among male and female adolescents and to discuss smoking-related oral complications.

METHODS: The prevalence data of tobacco use among adolescents (13-15 years) was retrieved from the World Health Organization (Global Health Observatory). The World Bank's statistics about gross national income (GNI) per capita were used to categorise low-income, lower-middle-income, upper-middle-income, and high-income countries. PubMed, Web of Science, Scopus, and Embase databases were searched to gather updated evidence about the adverse consequences of smoking on oral health among adolescents.

RESULTS: The prevalence of tobacco use was 19.33%, and there were 23.29% of male and 15.35% female smoker adolescents in 133 countries ($p < 0.001$). The highest prevalence of tobacco use in male (24.76%) and female (19.4) adolescents was found in high-income countries. Significantly higher proportions of male adolescents were smokers than female counterparts in low-income, lower-middle-income, and upper-middle-income ($p < 0.001$). However, there were no statistically significant differences in tobacco use between male and female adolescents in high-income countries. Low-income countries had the lowest prevalence (14.95%) of tobacco use, while high-income countries had the highest prevalence estimates (22.08). Gingivitis (72.8%), gingival bleeding (51.2%), oral malodor or halitosis (39.6%) is common oral conditions among smoker adolescents. Smoking habit is significantly associated with dental caries, periodontal disease, hairy tongue, smoking-related melanosis, and hyperkeratosis among adolescents.

CONCLUSION: There was a high prevalence of tobacco use among male and female adolescents around the globe. Available evidence suggests a strong association between smoking and compromised oral health among adolescents. Globally, measures should be taken to prevent and control the menace of tobacco use to reduce systemic and oral complications.

Introduction

Exposure to tobacco is associated with increased morbidity and mortality among children around the world [1]. Smoking contributes to increased risk of asthma, bronchitis, tuberculosis, inflammatory bowel disease, and leukaemia in children [1]. Moreover, children exposed to second-hand smoke may demonstrate structural and functional impairment in arteries that can lead to premature atherosclerosis and adverse long-term effects on the cardiovascular system [2]. Economic burden of smoking is enormous on individuals and healthcare systems. A recent study found that the economic cost of smoking was US$ 1436 billion around the globe in 2012 (equal to 1.8% of the annual gross domestic product of the world) which accounted for both direct health care expenditures and loss of productivity [3].

Cigarettes contain about 600 ingredients, they produce 7000 chemicals on combustion, and 69 of these chemicals can cause carcinomas. Nicotine is one of the toxic chemicals found in tobacco smoke [4]. It is documented that nicotine exposure among adolescents can affect the central nervous system and can cause cognitive functional and developmental impairment which can be of great concern considering the long-term use of tobacco [5]. Besides, adolescents may develop symptoms of dependence sooner and at

lower levels of tobacco consumption [6]. There is a reciprocal relationship between diminished autonomy and frequency of smoking, and even the consumption of one cigarette can prompt the loss of autonomy in adolescents [7].

The initiation and establishment of smoking behaviour frequently occur during adolescence, and 9 of 10 children start smoking by the age of 18 years [8]. Globally, about 80,000 to 100,000 children use tobacco per day [9]. Several factors are associated with smoking which includes the influence of smoker peers and smoker parents, genetic factors, and low socioeconomic status [10]. Moreover, there is sufficient evidence about the role of tobacco advertisement in causing the onset and continuation of smoking among adolescents [8]. Smoking initiation in the early age is associated with regular smoking in adulthood [11]. This underscores the importance of controlling the habit of smoking during adolescence so that immediate and long-term complications can be prevented in adolescents and adults, respectively.

Oral conditions pose significant public health problems and can negatively affect systemic health and quality of life of individuals [12]. Globally, untreated oral conditions affected 2.5 billion people in 1990 which rose to 3.5 in 2015 [13]. According to the Global Burden of Disease Study (2017), oral conditions accounted for 1.83 million disability-adjusted life-years, an increase of 21.4 % during the past decade (2007-2017) [14]. It was estimated that the cost of dental treatment was $102 billion and 1.6 million school days were missed because of acute dental conditions in 2009 in the U.S. [8].

There is widespread use of tobacco among adolescents which enormously contributes to a high burden of oral and systemic diseases. Therefore, the present review aimed to evaluate global data about the prevalence of tobacco use during adolescence and to discuss oral complications commonly seen among smoker adolescents. The review utilised most updated evidence about the adverse effects of smoking on oral health among adolescents. The information presented in the review is expected to benefit healthcare professionals and other stakeholders so that they familiarise with the global smoking trends and its negative impacts on oral health. Their contribution in raising awareness is critical to preventing smoking-related oral and systemic complications during adolescence and long-term adverse effects in adulthood.

Methods

Data about the prevalence of tobacco use (including smoking cigarettes, oral tobacco, and snuff) among adolescents (13-15 years) were retrieved from the Global Health Observatory, the World Health Organization [15]. Data were collected from 133 countries since 2007. Many of these countries have data collected in multiple years (more than one data), however latest data were used. The countries with data collected in 2006 and beyond were excluded from the study. To evaluate the ecological impact of the income of countries (Gross national income (GNI) per capita) on the prevalence of tobacco use, we compared data among low-income, lower-middle-income, upper-middle-income, and high-income countries. According to the World Bank (2017), the countries with a GNI per capita of ≤ $1,025 are defined as low-income countries; those with a GNI per capita between $1,026 and $4,035 as lower-middle-income countries, those with a GNI per capita between $4,036 and $12,475 as upper-middle-income countries, and those with a GNI per capita of ≥ $12,476 as high-income countries.

For this review, PubMed (Medline) was searched to retrieve studies about tobacco use and its impact on oral tissues. Using the library resources of Imam Abdulrahman Bin Faisal University, Scopus and Web of Science databases were searched. Search through Embase was also carried out to ensure a comprehensive search of related studies. Further, the reference lists of retrieved studies were searched to obtain more information regarding the topic of the review. The literature review gathered recent studies, mostly published during the last ten years, to provide updated information. Data from animal studies, case series, and case reports were excluded. Medical Subject Headings (MeSH)/keywords such as “smoking”, “tobacco”, “nicotine”, “caries”, “decay”, “cavities”, “gingival”, “periodontal”, “halitosis”, “oral malodor”, “cancers”, “carcinoma”, “oral health”, and “oral conditions,” were used to retrieve relevant studies.

Statistical analyses of the data were performed using SPSS Version 22.0 (IBM Corp. Armonk, NY, USA). Mann Whitney U test was conducted to make comparisons between the prevalence of tobacco use in male and female adolescents. Kruskal Wallis test compared the prevalence data among low-income, lower-middle-income, upper-middle-income, and high-income-countries. A significance level of $p < 0.05$ was used.

Results

There were 28 low-income, 76 lower-middle-income, 48 upper-middle-income, and 33 high-income countries included in the present review. The prevalence of tobacco use in adolescents in low-income countries is presented in Figure 1.

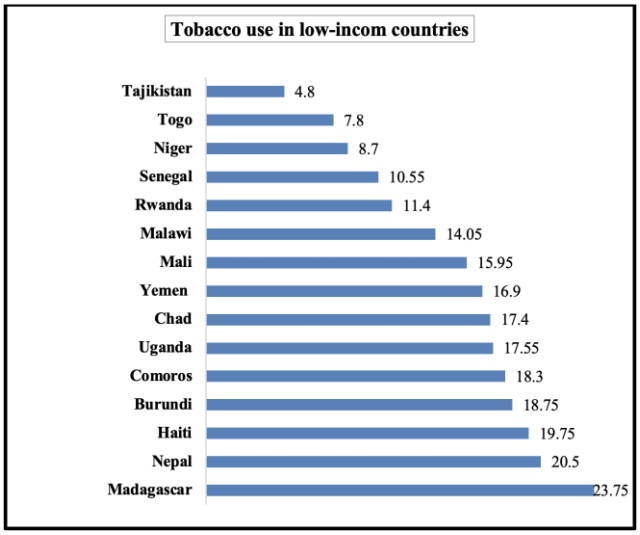

Figure 1: The prevalence of tobacco use in adolescents in low-income countries

Among low-income countries, tobacco use was most frequent in Madagascar (23.75%), followed by Nepal (20.5%), and Haiti (19.75%) whereas 4.8% of adolescents in Tajikistan used tobacco. About half the adolescents (47.85%) in Papua New Guinea consumed tobacco which was highest among lower-middle-income countries. On the other hand, tobacco use was the least common in Vietnam (4%), Bangladesh (6%) and Cambodia (6.45%) (Figure 2).

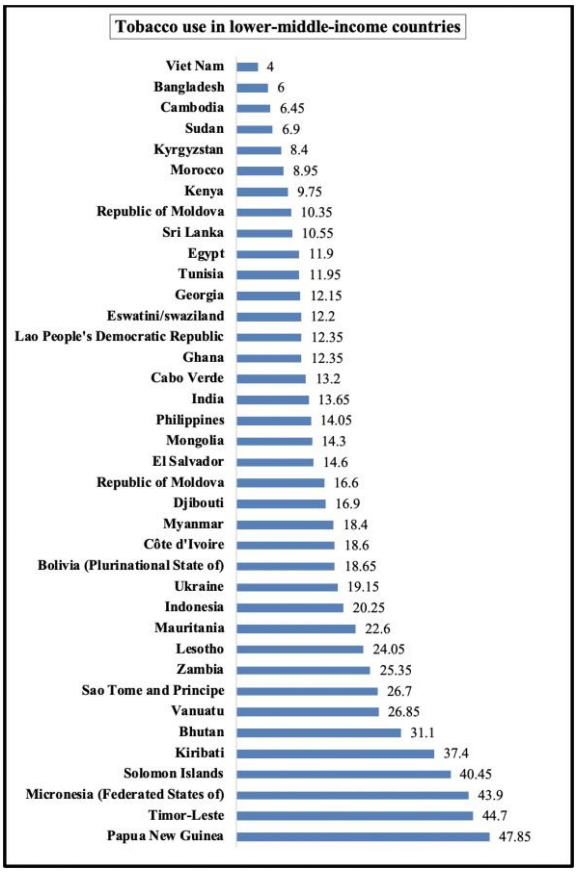

Figure 2: The prevalence of tobacco use in adolescents in lower-middle-income countries

Approximately one-quarter of adolescents in Tuvalu (37.15%), Lebanon (36.65%), and Tonga (36.45%) consumed tobacco in upper-middle-income countries. Montenegro (6.25), China (6.7%), and Azerbaijan (6.75%) had the lowest consumption of tobacco in upper-middle-income countries (Figure 3).

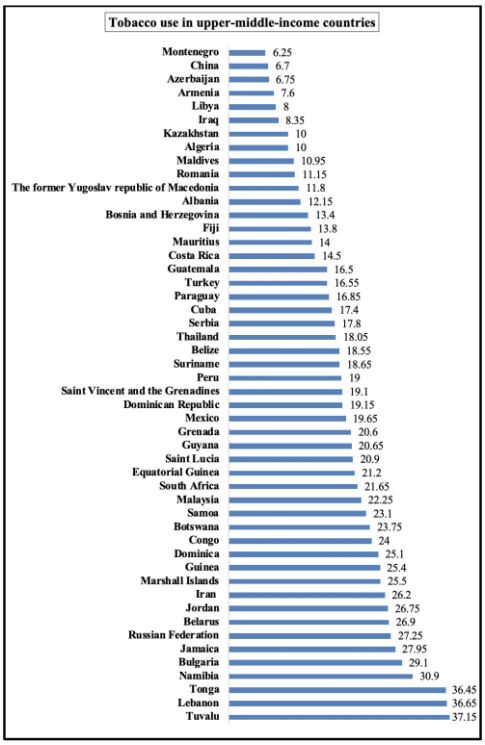

Figure 3: The prevalence of tobacco use in adolescents in upper-middle-income countries

Oman had the lowest prevalence of tobacco use (3.3%), whereas Palau demonstrated the highest distribution of tobacco use in adolescents in high-income countries (Figure 4).

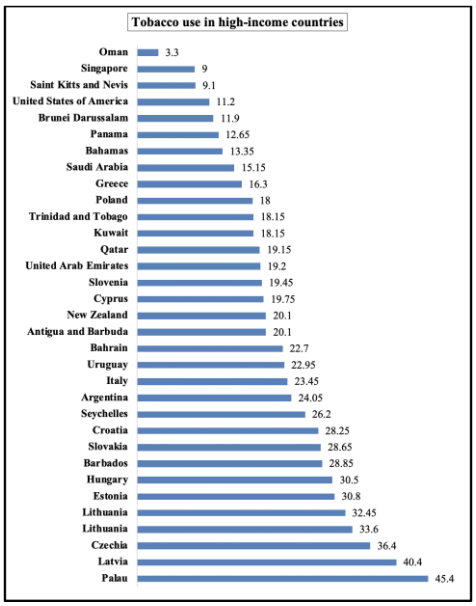

Figure 4: The prevalence of tobacco use in adolescents in high-income countries

The prevalence of tobacco use in adolescents in 133 countries was 19.33%, and it ranged from 1.5% to 65.5%. There were 23.29% male and 15.35% female smoker adolescents in these countries (p < 0.001). In low-income countries, 18.48% of male and 11.41% of female adolescents consumed tobacco (p 0.006). The prevalence of tobacco use was higher in males (24.18%) than females (13.37%) in lower-middle-income countries (p < 0.001). Upper-middle-income countries had prevalence estimates tobacco use in 22.97% of male and 15.29% of female adolescents (p < 0.001). Adolescents in high-income countries showed no statistically significant differences in the prevalence of tobacco use (p 0.052) (Table 1).

Table 1: Tobacco use among male and female adolescents (13-15 years) in 133 countries

Countries based on GNI	Number of countries	Prevalence of tobacco use in male adolescents (Percentages)	Prevalence of tobacco use in female adolescents (Percentages)	p-value
Low-Income Countries	28	18.48 Min = 6.8 Max = 33.2	11.41 Min = 2.8 Max = 19.2	0.006
Lower-Middle-Income Countries	76	24.18 Min = 6.5 Max = 65.5	13.37 Min = 1.5 Max = 40.3	< 0.001
Upper-Middle-Income Countries	48	22.97 Min = 6.6 Max = 44.9	15.29 Min = 2.1 Max = 32.7	< 0.001
High-Income Countries	33	24.76 Min = 4.9 Max = 54.1	19.4 Min = 1.7 Max = 41.4	0.052
Total	133	23.29	15.35	< 0.001

Discussion

The comparison of tobacco use among different countries is shown in Figure 5. Low-income countries had the lowest prevalence (14.95%) of tobacco use, while high-income countries had the highest prevalence estimates (22.08). There were statistically significant differences in the prevalence of tobacco use among low-income, lower-middle-income, upper-middle-income, and high-income countries (p 0.008).

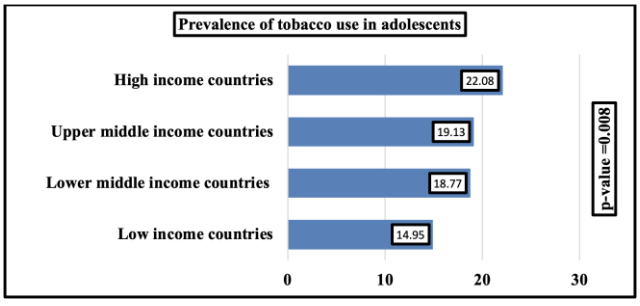

Figure 5: Differences in the prevalence of tobacco use in 133 countries according to their income

Mechanisms of Adverse Effects of Tobacco Use on Oral Health

Several mechanisms are responsible for the adverse effects of tobacco use on oral health. Tobacco smoke is a mixture of thousands of complex chemicals, many of which are poisonous and are responsible for the chemically mediated disease, including cancers [16]. Cigarette smoking is associated with alterations in oral microbial profile**.** A recent study showed that oral microbiome was significantly different in smokers compared with non-smokers. The smokers demonstrated the depletion of *Actinobacillus, Neisseria, Aggregatibacter, Eikenella, Haemophilus, Lautropia, Fusobacterium, Leptotrichia, and Cyanobacteria and abundance of Treponema, and Mycoplasma* [17]. Oral microorganisms accumulate to form biofilm on the tooth surface and subgingival sulcus, and smokers have been shown to demonstrate diverse microbial colonisation of biofilms than non-smokers, thus suggesting the role of smoking in promoting bacterial acquisition and colonisation in the biofilm [18].

Compared with non-smokers, smokers exhibit increased pathogenic species in marginal and subgingival biofilms and greater pro-inflammatory response [19]. It is well recognised that increased susceptibility of smokers to oral infections is because smoking promotes virulence of microorganisms and increases antibiotic resistance [20]. Besides, the adverse health effects of smoking can be related to potential microbial pathogens present in cigarettes. A variety of cigarette brands contain microorganisms which include *Acinetobacter, Bacillus, Staphylococcus species, Clostridium, Serratia, and Pseudomonas* [20].

Health consequences of tobacco consumption are related to its suppressive effects on the immune system. Exposure to tobacco smoke plays an important role in the production of immune and inflammatory mediators [21]. Similarly, provocation of oxidative stress because of smoke can result in dysregulation of the immune system to cause autoimmunity. Smoke can also produce autoantibodies and contribute to autoreactivity in some autoimmune diseases [22]. Smoking is also known to cause temperature variations and alterations in the pH in the oral cavity, which can be attributed to increased incidence of oral cancers. High intraoral temperature variations are associated with certain oral conditions and act as co-carcinogens. Also, chemicals present in tobacco are responsible for high alkaline pH which promotes the absorption of nicotine in the oral mucosal membrane [23]. Tobacco use compromises wound healing by inducing changes in connective and vascular tissues, reducing the supply of oxygen and nutrient to tissues, and decreasing collagen synthesis [24].

Common Oral Conditions among Smoker Adolescents

Smoker adolescents are predisposed to increased risk of oral conditions which include gingivitis and gingival bleeding, oral malodor, staining, dental caries, oral mucosal lesions, periodontal disease, dryness of mouth, and sensitivity.

Gingivitis and bleeding gums

Common adverse oral health consequences among adolescents include gingivitis, including bleeding gums and tooth staining [25]. Gingivitis is dental plaque-induced inflammation of gingiva without periodontal attachment loss or alveolar bone loss. However, persistent gingivitis is a risk factor for attachment loss and tooth loss later in life [26]. A recent study showed a high prevalence rate of gingivitis (72.8%) among adolescents, and male gender, poor oral hygiene, and smoking were significantly associated with gingivitis. It was also found that the smokers were 2.26 times more likely to have gingivitis than non-smokers [27]. A previous study also showed that 51.2% of adolescent smokers exhibited gingival bleeding than 33.1% of non-smokers ($p < 0.02$) [25]. It is documented that poor oral hygiene results in plaque accumulation which causes gingival inflammation and bleeding gums [27].

Oral Malodor

Oral malodor, also known as halitosis has negative effects on self-confidence, self-esteem, professional life, and social interaction. There is a high prevalence of oral malodor among school children. Yokoyama et al. detected oral malodor in 39.6% of senior high school children [28]. Oral malodor prevalence of 23.6% in adolescents was observed by Kim et al., [29]. Tobacco use is considered an extrinsic cause of oral malodor and smoking habit is known to correlate with the presence of oral malodor in youth [30]. Nevertheless, some studies did not find a significant association between smoking and oral malodor in adolescents and youth [29], [31].

Dental caries

The literature consistently shows an association between smoking and increased risk of caries during adolescence [32], [33], [34]. Tobacco consumption (tobacco and smokeless tobacco) is a significant risk factor for caries development. A population-based longitudinal study of 10,068 adolescents (2006-2012) found that tobacco users had significantly higher mean value of decay, missing, and filled surface (DMFS = 1.8) than never tobacco users (mean DMFS = 1.2), and tobacco use was significantly associated with increased caries incidence [32]. Another epidemiological study of 20,703 schoolchildren reported an association between household smoking and increased risk of dental caries. The study also demonstrated a significant dose-response relationship between household smoking and prevalence of decayed and or filled teeth [33]. Passive tobacco exposure also contributes to the development of caries in adolescents. A recent meta-analysis of 21 studies found a moderate association between passive smoking and dental caries in children and adolescents [34].

Periodontal Disease

According to the American Academy of Periodontology, several periodontal diseases affect adolescents, although their prevalence is higher in adults than adolescents [35]. Smoking negatively affects periodontal health by causing degradation of periodontal tissues. A study of adolescents found that smoker participants had significantly more periodontal pockets of ≥ 4 mm than non-smokers. Also, the study identified significantly higher root calculus in smokers than non-smoker adolescents [36]. Available evidence suggests that tobacco consumption can affect inflammatory biomarkers in saliva which have been successfully used as an alternative to evaluating periodontal health. It was reported that smoking increased the risk of periodontal disease among adolescents by lowering salivary biomarkers like matrix metalloproteinase (MMP)-8 and male adolescents demonstrated more susceptibility to periodontitis than female teenagers [37].

Oral Mucosal Lesions

The studies documented high prevalence (26.2%-28%) of oral mucosal lesions in adolescents [38], [39]. Male adolescents were found to have a high prevalence of oral mucosal lesions than females [39]. Aphthous ulcers, traumatic ulcerations, herpes stomatitis, angular cheilitis, geographic tongue, and candidiasis are the most common types of oral mucosal lesions during adolescence [38], [40]. A recent study of adolescents observed a significant association between smoking habit and hairy tongue, smoking-related melanosis, and hyperkeratosis. The authors suggested that smoking can induce alterations in oral microbial profile, thickening of oral mucous membrane, and extravasation of melanocytic pigment which may lead to oral mucosal lesions [41]. A study of 17,027 schoolchildren showed that about 1.5% had smokeless tobacco lesions which were more common in male than female students. The study also demonstrated a dose-response relationship between tobacco use and smokeless tobacco lesions, and snuff was strongly associated with increased odds (odds ratio 18.4) of these lesions [42].

Dryness and hypersensitivity

A significantly higher proportion of smokers were shown to have tooth sensitivity and dryness of the mouth than non-smokers [43]. Evidence suggests that smoking reduces salivary flow which predisposes individuals to dryness of the mouth or xerostomia [44]. A previous study also showed a significant difference in the prevalence of xerostomia among smokers (39%) and non-smokers (12%) [45]. Similarly, it was reported that 37% of smokers had xerostomia compared with 13% of non-smokers [44]. It is well-known that dryness of the mouth can cause inflammation of oral tissues including salivary glands, and can promote caries development, oral malodor, calculus formation, and fungal infection [45], [46].

Oral pre-cancerous lesions and cancers

Tobacco smoking contributes to the formation of oral pre-cancerous lesions, such as leukoplakia and erythroplakia [24]. Oral cancers involve carcinomas of lip, tongue, mouth, and pharynx, and are considered the sixth most prevalent cancers around the world [47]. Globally, 128,000 patients with oral cancer die annually [48]. Men have a higher incidence of oral cancer than women, and tobacco use is a major contributor to oral cancer [49]. The incidence of lip, oral cavity, and pharyngeal cancers was 3.8% (529,500 cases) of all cancers in 2012, and it was predicted that the number of these cancers would increase to 856,000 cases in 2035 representing 62% increase in 23 years [48].

Although childhood cancers are rare, however, there is a rise in the number of new cancer cases of oral cavity and pharynx in adolescents which are associated with human papillomavirus (HPV) infection in addition to tobacco use [50]. Since smoking is widely prevalent among adolescents, and adverse health consequences of oral cancers are huge and far-reaching. Therefore, health professionals should be aware of the signs and symptoms of oral cancers so that early detection and treatment should be started as early as possible to reduce morbidity and mortality later in life. It is the responsibility of healthcare providers to provide counselling to adolescents and their patients about the hazardous consequences of smoking on oral and systemic health in daily practice. A previous study found that the adolescents who were aware of the adverse effects of smoking on oral health had 22% – 47% lesser chances of smoking than those who were unaware of negative smoking consequences [51].

Prevention of smoking

The WHO suggested the 5 A's model that helps individuals avoid exposure to secondhand smoke and prepares individuals to quit smoking, and the 5R's model that increases the motivation of quitting [52]. More than 80% of smokers approach primary health care providers each year, which place them in a central position to advise smokers to quit tobacco use. The 5 A's model (Ask, Advise, Assess, Assist, Arrange) involves using actions and strategies to provide advice to tobacco users [52].

Table 2: Description of 5 A's Model

5 A's	Description
Ask	Health care providers should "ASK" their patients about using tobacco and identify and document tobacco users in each visit.
Advise	They should strongly "ADVISE" tobacco users to quit smoking.
Assess	Smoker's readiness to quitting should be "ASSESSED" health care professionals.
Assist	The tobacco users should be "ASSISTED" by providing them with a smoking quit plan.
Arrange	A follow-up visit or a referral to a specialist should be "ARRANGED" to identify problems and challenges and provide support.

The 5 R's model (Relevance, Risks, Rewards, Roadblocks, and Repetition) provides strategies that can be used in motivational counselling for those individuals who are not ready to quit tobacco use.[52]

Table 3: Description of 5 R's Model

5 R's	Description
Relevance	When using "RELEVANCE", health care providers should describe that quitting smoking is personally relevant to tobacco users concerning diseases, health risks, and family or social concerns.
Risks	Health care providers should help individuals identify negative consequences (RISKS) associated with the use of tobacco, such as acute risks, long-term risks, and environmental risks.
Rewards	The "REWARDS" are potentially relevant advantages of quitting, such as better health outcomes, improved social interactions, and economic benefits. Health care providers should encourage tobacco users to recognize the benefits of quitting.
Road Blocks	They should explain barriers (ROADBLOCKS) to quitting to their patients which may include dependence, fear of failure, and lack of support.
Repetition	"REPETITION" involves repeating motivational counselling intervention if the patient does not demonstrate readiness to quit smoking.

Conclusion and recommendations

The study found a high prevalence of tobacco use among male and female adolescents in 133 countries. High-income countries had the highest prevalence of smoker adolescents, while low-income countries had the lowest prevalence of tobacco use. A significantly higher proportion of male than female adolescents used tobacco in low-income, lower-middle-income, and upper-middle-income countries. However, male and female smoker adolescents in high-income countries showed no significant differences, suggesting that both male and female adolescents were almost equally exposed to tobacco in high-income countries.

The smoker adolescents exhibit a high burden of gingivitis, gingival bleeding, malodor (halitosis), and tooth staining. There is a significant association between smoking and increased incidence of caries, calculus, periodontal pockets, oral mucosal lesions among adolescents. Healthcare professionals, decision-makers in healthcare agencies, and other stakeholders should be aware of the epidemic of

smoking and associated systemic and oral health complications among adolescents. They should play their roles at the individual level and through collaborations in the prevention and control of smoking.

Medical and dental curricula should include strategies on smoking cessation in addition to the epidemiology of tobacco use and pathology of tobacco-related conditions. The health care professionals should use 5A's and 5R's models in their clinical practice to encourage quitting, and they should act as clinicians, educators, opinion makers, role models, researchers, and collaborators to help control tobacco epidemic and to improve the quality of life of adolescents.

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Nurses' Perceptions of Patient Safety Culture in Intensive Care Units: A Cross-Sectional Study

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Abstract

BACKGROUND: Patient safety culture is a relatively new focus where little is known about its current status in Egypt's teaching hospitals, mainly intensive care units (ICUs). Therefore, the authors of this study attempted to assess the patient safety culture dimensions from the nurses' perspective.

METHODS: An exploratory cross-sectional study was conducted in two ICUs (pediatric ICU and adult ICU) at the University Hospital over 3 months from October till December 2018. Sixty nurses were interviewed using the Hospital Survey on Patient Safety Culture.

RESULTS: The current study findings revealed an average positive response to individual items ranging from 6% to 51%. The "Organizational learning" dimension had the highest average percent positive patient safety dimension score (51%) among all respondents, while the "Frequency of events reported" dimension had the lowest one (6%). No statistically significant difference was reported between the pediatric and adult ICUs for all mean scores except for the "Non-punitive response to error" dimension which was reported to be greater in the pediatric intensive care unit (PICU) compared to adult ICU ($P < 0.005$). The overall patient safety grade was rated acceptable by 47.5% of the interviewed nurses.

CONCLUSION: The current study shows that patient safety is fragile in ICUs, and more effort is recommended to increase the awareness of health care providers. Also, hospital managers need to enhance the performance and practices of patient safety within a non-punitive reporting environment.

Introduction

Patient safety is considered to be one of the global health concerns influencing patients in different healthcare settings in both developed and developing countries [1], [2]. In addition to being a substantial economic burden, patient safety causes the expenditure on health to be higher in the developing countries than the developed ones by from 5 to 10%. Fortunately; it is estimated that up to three-quarters of these lapses in health care delivery are preventable [3].

One of the essential steps to improve the patient safety is the promotion of patient safety culture; a culture that supports and allows optimal patient outcomes which are reliant on achieving a culture of trust, reporting, transparency, and commitment to change. Patient safety is critical, mainly in the intensive care unit (ICU) [4], [5]. In ICUs, many incidents threaten patient safety due to the sensitive and complex situations such as conditions of critically ill patients [6], [7]. Farzi et al., [4] indicated that most medication errors were reported in ICUs which could severely threaten patient safety.

Assessing the ICU safety culture will help us to find areas requiring improvement and raise awareness about patient safety [8], [9], [10]. Generation of a safety culture in institutes includes an assessment of the current health care providers' perception of this culture, otherwise, safety precautions implemented may increase costs and cause unpredicted new risks [11]. A study by Verbakel et al., [12] revealed that the patient safety practices improved much after assessing patient safety culture among health care providers.

Studies on patient safety culture mostly come from the developed countries [7]. Literature shows that safety culture differs across hospital organisations depending on the organisation experience, size, and function [13]. In Egypt, patient safety culture is a relatively new focus where little is known about its current status in ICUs. Therefore, authors of this study attempted to assess patient safety culture dimensions from the perspective of nurses who play an important role in providing health care services and are in contact with patients in the pediatric intensive care unit (PICU) and adult ICU. Also, the authors aimed at testing for differences between the PICU and adult ICU regarding the patient safety grade.

Methods

Study design and setting

This is an exploratory cross-sectional study conducted in two intensive care units (ICUs) (Pediatric and Adult ICUs) at the University Hospital in PICU includes 23 beds and receives about 799 patients annually, while adult ICU includes 47 beds and receives about 3000 patients annually. The study extended over 3 months, from October to December 2018.

Study population

All nurses who worked at both ICUs at the time of data collection, were in contact with the patients, worked at this unit for at least one year, and consented to share were included. Thus, based on a population of 72 nurses approached, 60 nurses were apt to share in the research.

Data collection tool

A pre-tested structured interview questionnaire was used to collect data from the study participants. It included two sections: the demographic characteristics (age, marital status, education, nursing experience, and previous training in patient safety) and the Arabic translated version of the Hospital Survey on Patient Safety Culture (HSOPSC) [14]. Psychometric assessment of the Arabic translation of the American HSOPSC version in Palestine and Jordan showed that the HSOPSC is a valid and reliable tool for assessing safety culture in Arabic hospital settings [15], [16]. The HSOPSC covered the followings dimensions: Organizational learning and continuous improvement (3 items), Overall perceptions of safety (4 items), Staffing and workload (4 items), Teamwork across hospital units (4 items), Supervisor/manager expectations and actions promoting patient safety (4 items), Hospital management support for patient safety (3 items), Teamwork within units (4 items), Hospital handoffs and transitions (4 items), Non-punitive response to error (3 items), Frequency of events reported (3 items), Feedback and communication about error (3 items), and Communication Openness (3 items) in addition to two questions; patient safety grade of the ICU (1 item) and number of events reported (1 item).

Items are scored using a five-point Likert scale reflecting agreement (1 = 'Strongly Disagree' to 5 = 'Strongly Agree') or frequency (1 = 'Never' to 5 = 'Always', or 1 = 'Excellent' to 5 = 'Failing) or frequency (No event reports, 1 to 2 event reports, 3 to 5 event report, 6 to 10 event reports, 11 to 20 event reports, 21 event reports or more).

The face and content validities were examined. After collecting the viewpoints of public health experts, required changes were made, and no phrases or words were deleted. Internal consistency/reliability was checked by calculating Cronbach's alpha for each composite to ensure that items within each composite were consistent.

In this study, the Cronbach's alpha for the composites ranged from 0.61 to 0.88. The HSOPSC user's guide indicates that a Cronbach's alpha value of 0.60 or greater is supposed to be acceptable [17].

Operational definition

An “event” is defined as any error, mistake, incident, accident, or deviation regardless of whether or not it results in patient harm [17].

Statistical analysis

Statistical analysis was done using the statistical package for the social science program (SPSS, version 21.0 IBM). The HSOPSC User’s Guide was used to guide data management and analysis [18]. The HSOPSC includes both positively and negatively worded items; all scored using five-point frequency scales. The percentage of positive responses for each item and composite was calculated. An item’s percent positivity was calculated by averaging the total percent positivity for each item. Composite percent positivity was calculated by averaging the percent positivity of all items included in the composite. The 12 HSOPSC composites were then examined to determine areas of strength (percent positive rating > 75%) and those requiring improvement (< 50%), while composites having a percent positive rating from 50% to 75% were considered neutral. Negatively worded items were reversed to compute a percent positive response rate. In addition, descriptive and univariate analyses were conducted to compare between pediatric and adult ICUs. Chi-square test was used to compare percent

positive score between areas. P value less than 0.05 was considered as a level of significance. The positive response for each item was defined as the percentage of strongly agree and agree (or always and most of the time) responses to direct-worded items and strongly disagree and disagree (or never and rarely) to reverse-worded items. In addition, the average percentage of positive responses for each level was defined as the mean of positive responses percent for that dimension's related items. Grouping of the responses was done as follows: Positive responses for (strongly agree and agree on responses), Neutral response for (neither), Negative responses for (strongly disagree and disagree responses) [19].

Ethical considerations

The Ethical Review Committee in the Kasralainy Faculty of Medicine, Cairo University, Cairo revised and approved the study protocol. Written informed consent was obtained directly from the enrolled nurses before data collection and after explanation of the study objectives and importance. All procedures for data collection were treated with confidentiality according to Helsinki declarations of biomedical ethics.

Results

A total number of 60 nurses working in the Pediatric and Adult Intensive Care Units at Cairo university hospitals participated in the current study. The average age of the enrolled nurses was 30.4 ± 5.3, the majority of participants were females (87.0%) and 80.8% were married. About two-thirds of the nurses (67.4%) held a Bachelor degree and 44.6% had a nursing experience of 2-5 years. About half of the nurses (50.3%) reported that they were satisfied with their jobs and a minority of them (16.6%) got training on patient safety (untabulated results).

Tables 1, 2, 3, and 4 show the detailed average percent positive dimension score perceptions regarding all patient safety culture dimensions in both pediatric and adult ICUs. Positive response to individual items ranged from 6 to 51%, with a mean total score of 30% for the positive responses to the 12 dimensions.

As displayed in Table 1, the Organizational learning dimension had the highest average percent positive patient safety dimension score (51%) among all respondents, while the Frequency of events reported dimension had the lowest score (6%). Regarding Organizational learning, Overall perception of safety, and Staffing dimensions, there were no statistically significant differences between pediatric and adult intensive care units. The highest positive responses were for organisational learning (51%), while the least positive responses were for staffing (31%).

Table 1: Average percent positive dimension scores of the enrolled nurses for the Organizational learning, Overall perception of safety, and Staffing dimensions at Pediatric and Adult Intensive Care Units, Cairo University Hospital, Egypt, 2018 (N = 60)

Patient Safety Culture Dimensions	Average percentage-positive response	Average percentage-positive response Pediatric ICU	Average percentage-positive response Adult ICU	p-value
Organizational learning and continuous improvement	51%	53%	49%	0.729
We are actively doing things to improve patient safety	53%	57%	50%	0.796
Mistakes have led to positive changes here	50%	50%	50%	1
After making changes to improve patient safety, we evaluate their effectiveness	50%	53%	47%	0.797
Overall perceptions of safety	36%	38%	34%	0.502
Patient safety is never sacrificed to get more work done	57%	63%	50%	0.435
Our procedures and systems are good at preventing the occurrence of errors	53%	50%	57%	0.796
It is just by chance that more serious mistakes do not take place around here	20%	20%	20%	1
We have patient safety problems in this unit	13%	17%	10%	0.706
Staffing and workload	31%	29%	33%	0.463
We use more agency/temporary staff that is best for patient care	37%	27%	47%	0.18
The staff in this unit work longer hours which is best for patient care	30%	27%	33%	0.779
We work in "crisis mode" trying to do too much, too quickly	30%	33%	27%	0.779
Enough HR to deal with work	27%	30%	23%	0.771

As for the teamwork climate in the ICU, there was no statistically significant difference in the Supervisor/manager expectations and actions promoting patient safety or the Hospital management support for patient safety dimensions between pediatric and adult ICUs as shown in Table 2.

Table 2: Average percent positive dimension scores of the enrolled nurses for the Teamwork across hospital units, Supervisor/manager expectations and actions promoting patient safety, and Hospital management support for patient safety dimensions at Pediatric and Adult Intensive Care Units, Cairo University Hospital, Egypt, 2018 (N = 60)

Patient Safety Culture Dimensions	Average percentage-positive response	Average percentage-positive response Pediatric ICU	Average percentage-positive response Adult ICU	P value
Teamwork across hospital units	30%	32%	28%	0.567
Hospital units work well together to provide the best care for patients	50%	57%	43%	0.439
There is good cooperation among hospital units that need to work together	33%	40%	27%	0.412
It is often unpleasant to work with staff from other hospital units	23%	20%	27%	0.761
Hospital units do not coordinate well with each other	13%	10%	17%	0.706
Supervisor / manager expectations and actions promoting patient safety	27%	26%	28%	0.633
My supervisor/manager seriously considers staff suggestions for improving patient safety.	47%	50%	43%	0.796
Whenever pressure builds up, my supervisor/manager wants us to work faster, even if this means taking shortcuts.	40%	37%	43%	0.792
My supervisor/manager overlooks the patient safety problems happening	17%	13%	20%	0.731
My supervisor/manager says a good word when he/she sees a job done according to established patient 3s	3%	3%	3%	1
Hospital management support for patient safety	26%	24%	27%	0.714
Patient safety is never sacrificed to get more work done	30%	27%	33%	0.779
Hospital management provides a work climate that promotes patient safety	23%	23%	23%	1
Hospital management seems interested in in-patient safety only after an adverse event happens	23%	23%	23%	1

Comparing the results of the pediatric ICU to adult ICU regarding the Teamwork within units,

Hospital handoffs and transitions, Non-punitive response to error, and Frequency of events reported dimensions; all mean scores were not significantly different except for the non-punitive response to error dimension which was reported to be greater in PICU compared to Adult ICU (23% versus 8%) ($p < 0.05$).

Table 3: Average percent positive dimension scores of the enrolled nurses for the Teamwork within units, Hospital handoffs and transitions, and Non-punitive response to error dimensions at Pediatric and Adult Intensive Care Units, Cairo University Hospital, Egypt, 2018 (N = 60)

Patient Safety Culture Dimensions	Average percentage-positive response	Average percentage-positive response Pediatric ICU	Average percentage-positive response Adult ICU	P-value
Teamwork within units	24%	30%	18%	0.229
Work together to finish quickly	27%	37%	17%	0.143
Treat each other with respect	27%	33%	20%	0.382
Others help out when busy	23%	23%	23%	1
Support each other	20%	27%	13%	0.33
Hospital handoffs and transitions	24%	17%	32%	0.103
Shift changes are problematic for patients in this hospital	27%	17%	37%	0.143
Things "fall between the cracks" when transferring patients from one unit to another.	23%	17%	30%	0.36
Important patient care information is often lost during shift changes	23%	17%	30%	0.36
Problems often occur in the information exchange process across hospital units	23%	17%	30%	0.36
Non-punitive response to error	16%	23%	8%	*0.022
The staff feel like their mistakes are held against them	23%	30%	17%	0.36
Staff worry that the mistakes they make are kept in their personnel files	13%	23%	3%	*0.052
When an event is reported, it feels like the person is being written up, not the problem	10%	17%	3%	0.195
Frequency of Events Reported	6%	6%	6%	1
When a mistake is made but caught and corrected before affecting the patient, how often is this reported?	7%	7%	7%	1
When a mistake is made but has no potential to harm the patient, how often is this reported?	7%	7%	7%	1
When a mistake that could harm the patient is made, but it does not, how often is this reported?	3%	3%	3%	1

Statistically significant.

When asked on the frequency of reporting potentially harmful events on patients, even when no harm actually occurred to the patient, only six percent of these events were reported (Table 3).

Table 4: Average percent positive dimensions scores of the enrolled nurses for Feedback & communication about error and Communication openness dimensions at Pediatric and Adult Intensive Care Units, Cairo University Hospital, Egypt, 2018 (N = 60)

Patient Safety Culture Dimensions	Average percentage-positive response	Average percentage-positive response Pediatric ICU	Average percentage-positive response Adult ICU	P value
Feedback & Communication About Error	40%	42%	37%	0.492
We are given feedback about the changes put into place based on event reports.	43%	47%	40%	0.795
We are informed about the errors that happen in this unit.	40%	40%	40%	1
In this unit, we discuss ways to prevent errors from happening again.	35%	40%	30%	0.589
Communication Openness	44%	47%	40%	0.312
Staff will freely speak up if they see something that may negatively affect patient care.	52%	50%	53%	1
Staff feel free to question the decisions or actions of those with more authority.	40%	40%	40%	1
Staff are afraid to ask questions when something does not seem right	38%	50%	27%	0.11

As displayed in Table 5, the overall patient safety grade was rated acceptable by 47.5% of the interviewed nurses with a statistically significant relationship between the patient safety grade and work duration. Regarding the topic of events reporting, the present study revealed that all of the enrolled nurses reported no event during the year previous to data collection (untabulated).

Table 5: Patient safety grade, ICU type, and hospital work duration (N = 60)

Variables		Patient Safety Grade					
		Excellent / Very Good		Acceptable		Poor / Very Poor	
ICU type		N	%	n	%	N	%
	Pediatric ICU	2	6.7	19	63.3	9	30.0
	Adult ICU	6	20.0	19	63.3	5	16.7
	Chi square = 3.143, *P* value = 0.208						
Hospital work duration	Less than 5 years	2	16.7	4	33.3	6	50.0
	From 5 to 10 years	2	7.1	20	71.4	6	21.4
	From 10 to 15 years	2	12.5	12	75.0	2	12.5
	More than 15 years	2	50.0	2	50.0	.0	.0
	Chi square = 12.836, *P* value = 0.046†						

† Statistically significant.

Discussion

The present study revealed a friable safety culture in most dimensions with a mean total positive score of 40% for the positive responses to the 12 dimensions. This is in contrast with other studies conducted in ICUs, which revealed positive responses' percentages of 62% (20) and 55.24% [21]. The scores in the current study are also lower than those in a study conducted in Brazilian neonatal intensive care units with a percentage of 42.58% [21]. A possible explanation for this discrepancy of results is the lack of patient safety culture awareness where a minority of the enrolled nurses in the present study (16.6%) got training on patient safety. Regarding areas of strength, no dimension was classified as such.

However, it is considered that the "Organizational learning-continuous improvement" dimension (51%), being the main ones, demonstrates advances in the safety culture and have the potential to become an area of strength in the ICUs. This is by a previous study conducted in two adult ICUs in Brazilian public hospitals where the "Organizational learning-continuous improvement" dimension was 49% [22]. However, a higher percentage for this dimension (78.2%) was found in Aboul-Fotouh and his colleagues' study that included physicians, technical and administrative staff as well [23].

In the current study, the Non-punitive response to error composite received a low score of 16%. This agrees with Aboul- Fotouh et al., the study [23] in which the Non-punitive response to error score dimension was 19.8%, revealing that healthcare personnel are not comfortable when it comes to reporting errors. On the other hand, higher positive responses were found in other studies for this

dimension such as US hospitals which received a score of 44% [24]. However, the Non-punitive response to error dimension yielded a mean score below 50% in the abovementioned studies that were conducted in a variety of countries, indicating the need for improvement. In the present study, the Non-punitive response to an error reported by nurses working in PICU is higher than that reported by nurses working in Adult ICU. This result highlights the significance of encouraging health professionals to report events in a non-punitive environment for improving patient safety among nurses working in Adult ICU.

Concerning the Frequency of event reporting dimension, a score of 6% was reported. As observed, the reporting of events is not very frequent at the ICU, which ends up not reflecting the actual number of errors, making barriers against these errors hardly effective. In the present study, the dimensions “Non-punitive response to error” and “the Frequency of events reported” are closely related. Many errors in the health care go unreported for numerous reasons including fear, humiliation, the presence of a punitive response to errors, and the fact that reporting would not usually result in an actual change [22]. On the other hand, higher positive responses were found in other studies for these dimensions such as US hospitals where each dimension of them scored an average percent positive score of 44% [24]. The literature shows that safety culture differs across hospital organisations depending on the organisation’s experience, size, and function [13].

Results of the current study revealed that the overall patient safety grade was rated acceptable by 47.5 % of the interviewed nurses. A similar result was revealed by a previously conducted study at Ain-Shams University to assess patient safety culture where 57.3% of the participants found the grade acceptable [23]. However, this result disagrees with the results of a similar study done in Saudi Arabia by Alahmadi in 2010 [25], where 33% of the respondents found the overall patient safety grade to be acceptable. This might be explained by the point that these institutions were variable in terms of size, complexity, and focus on patient safety.

Concerning the issue of events reporting, the present study revealed that all the enrolled nurses reported no event during the year previous to data collection. This result disagrees with another study results conducted in Saudi Arabia where 43% of the subjects indicated that they didn't report any events in the preceding year period, while 10% reported only one or two events [25]. The difference in the obtained results might be because of the conduction of the Saudi Arabian study in sixteen public and private hospitals that have different quality and patient safety initiatives.

The present study findings should be viewed considering the following limitations. As the data collected is based on participants' self-report, probably, the nurses were not honest enough in completing the questionnaire because of their fear of penalties. This was lessened by anonymous questionnaires.

In conclusion, the current study shows that patient safety is fragile in ICUs, and more effort is recommended to increase health care providers’ awareness of this culture. Patient safety culture needs to be incorporated into the education of health care providers across the spectrum of healthcare. A blame-free environment should be created to detect threats to patient safety, share information, and learn from events.

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The Analysis of Health Index Development Factor in Aceh Province, Indonesia

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Abstract

BACKGROUND: Development of Health Index becomes important in measuring development. The development of the Health Index in Aceh Province is influenced by several internal and external factors.

AIM: The purpose of this study was to analyse the factors that influence the health index as well as formulate a health index development strategy for Aceh Province

METHODS: The method used is quantitative with a survey approach. This research was conducted from January to March 2019 in Aceh Province. Participants were determined as many as 50 stakeholders using the purposive sampling method. Data was collected in the form of primary and secondary data. Primary data were obtained through questionnaires and interviews with key stakeholders such as the Aceh Provincial Government, regional authorities within the Aceh Province (Agency, Bureau, Office, Hospital, etc. Secondary data was obtained from relevant research reports, government regulations, and others. The SWOT analysis was used to identify internal and external factors, strategies for developing the health index of the Aceh Province.

RESULTS: Sixteen factors influence the development of a health index in the province of Aceh. Analysis of internal and external factors has identified five strength factors, six weakness factors, five opportunity factors, and five threat factors. The internal factors (strength) that influence the development of the health index are the increasing role of the provincial government in improving health (0.437). Internal factors (weaknesses) that influence the development of health indices in the province of Aceh are the low quality of human resources in the health sector (0.336). External factors (opportunities) that influence the development of health indices in Aceh province are support from the central government with a value (0.399). External factors that pose a threat to the development of health indices in Aceh province are the ability of the community to finance health with a value (0.437). The results of the SWOT analysis of the condition of the development of the health index are currently in squared II (Strategy Diversification). This condition shows that the development of the health index faces a big threat. Alternative strategies for the development of health indices can be done by increasing the allocation of health spending, increasing partnerships with the private sector in implementing health programs, cooperation between the central, provincial and district governments in improving policies and improving service quality.

CONCLUSION: Some factors influence the development of the Health Index in Aceh Province. Internal factors are the role of the provincial government in improving health, and the low quality of human resources in the health sector. While external factors are the existence of support from the central government, and the ability of the community to finance health.

Introduction

Development' is to improve the quality of people's lives by creating an environment for them to engage in a wide range of activities, to be healthy and well-nourished, to be knowledgeable and to be able to participate in the community life. The Human Development Index (HDI) is a multi-dimensional index of development as it is the combination of three development indices-health index, education index and income index [1]. As Indonesia moves to provide health coverage for all citizens, understanding patterns of morbidity and mortality are important to allocate resources and address inequality [2]. Indonesia faces significant challenges in the health sector despite notable progress in the past decades, especially about improved life expectancy. Underpinning these problems are significant disparities in access to quality health services across geographic regions and socioeconomic groups [3], [4]. For example, health outcomes are lower in many Eastern Indonesian provinces as well as in rural areas and among people from the lowest wealth quintile [5]. Indonesia faces critical challenges about human resources for health (HRH). These relate to HRH

policy, planning, the mismatch between production and demand, quality, renumeration, and mal-distribution [6].

Health development is an investment to improve the quality of human resources by Law Number 36 of 2009 concerning Health. Health development aims to increase awareness, willingness, and the ability to live healthy as to realise the degree of public health [7]. The success of health development is determined by the sustainability of the program, cross-sector cooperation, and health financing. One of the factors that affect the level of health is the level of financing for the health sector [8]. The amount of health expenditure is positively related to the achievement of public health. Also, government tax revenues have a positive influence on the effectiveness of public policy in improving health community [9]. To achieve this goal, local governments have a large role in the development of the health index. One of the policies of the regional government that is seen as being able to improve the health index in the regions is the expenditure of the local government in the health sector. The low value of the health index in Aceh Province contributed to the slow pace of growth in the Human Development Index in the Aceh Province. Based on data from the central statistical agency (BPS), in the 2016-2017 period, Aceh Province's HDI grew by 0.79 percent [10]. Growth in the period was lower compared to the increase in the 2014-2015 period, growing by 0.93 percent. With the increase in government budget allocations in the health sector, it is expected to encourage the growth of the Human Development Index. Aceh Province as one of the health benchmarks in Indonesia, must be able to catch up with the increase in the growth of the Human Development Index from other regions in Indonesia. Many factors influence the health index in Aceh Province. Therefore, the purpose of this study is to identify factors that influence the development of the health index and formulate a strategy for developing the health index in Aceh Province.

Material and Methods

The research method used in this study was the survey technique. This research was conducted from January to March 2019 in Aceh Province. Respondents were determined to be 50 using the purposive sampling method. Data was collected in the form of primary and secondary data. Primary data were obtained through questionnaires and interviews with key stakeholders such as the Aceh Provincial Government, regional authorities within the Aceh Provincial Government (Agency, Bureau, Office, Hospital, etc.). Secondary data was obtained from relevant research reports, government regulations, and others. The SWOT analysis is used to determine internal and external factors that influence the Health Index Development Factors in Aceh Province. The use of SWOT analysis to identify strengths and weaknesses (Weakness) of internal factors, as well as opportunities (Opportunity) and threats (Threat) of external factors [11], [12]. Furthermore, a strategy for the development of the Aceh Province health index was formulated and outlined in the SWOT matrix.

Results

SWOT Analysis

The SWOT analysis focuses on the strategy of developing health index in Aceh province. The interviews, questionnaires, and observations in the field were analysed into internal and external factors. Based on the results of the analysis identified several internal and external factors that influence the health index. Internal factors consist of strengths and weaknesses. While the external factor consists of opportunities and threats.

Internal Factors Affect Development of Health Index

The internal analysis aims to harness the power possessed to overcome weaknesses in the development of a health index in Aceh province. There are eleven internal factors (strengths and weaknesses) that influence the development of the health index.

Table 1: Internal Strategic Factors Analysis Summary (IFAS)

No	Internal factors Strengths	Weight	Rating	Score
S1	The role of the provincial government in improving health.	0.115	3.8	0.437
S2	The commitment of government in improving health	0.113	3.8	0.4294
S3	The coordination between government and other private sectors	0.102	3.8	0.3876
S4	The coordination between provincial and district government	0.109	3.7	0.4033
S5	The coordination between the health department and community clinic centre.	0.102	3.8	0.3876
	Sub Total			2.0449
No	External factors Weakness	Weight	Rating	Score
W1	Allocation of health expenditure 10% of total regional expenditure	0.089	3.3	0.2937
W2	Quality of health infrastructure	0.082	3.4	0.2788
W3	Ease of community towards health facilities and medicines	0.056	3.7	0.2072
W5	Availability of human resources in the health sector.	0.069	3.4	0.2346
W6	Quality of human resources in the health sector.	0.096	3.5	0.336
	Sub Total			1.3503
	Total = Strength + Weakness			3.3952

Source: processed primary data.

The internal factors (strength) that influence the development of the health index are the increasing role of the provincial government in improving health (0.437). Therefore, the importance of strengthening of

public, private and community health systems has been emphasised time and again. In most of the developing countries, certain weaknesses and gaps in the government health systems have been hampering the achievement of improved health outcomes [13].

Whereas internal factors (weaknesses) that influence the development of health indices in the province of Aceh are the low quality of human resources in the health sector (0.336). Human resources, when about health care, can be defined as the different kinds of clinical and non-clinical staff responsible for public and individual health intervention [14] Proper management of human resources is critical in providing a high quality of health care. Effective human resources management strategies are greatly needed to achieve better outcomes and access to health care around the world [15]. Based on the Internal strategic Factors Analysis Summary (IFAS), it is known that the development of the health index in Aceh province has an IFAS score of 3.395, meaning that its internal position is at a good level.

External factors that influence the development of the health index

The external analysis aims to see opportunities that can be used to develop the development of health indices and prepare strategies to minimise threats. There are ten external factors (opportunities and challenges) that influence the development of the health index.

Table 2: External Strategic Factors Analysis Summary EFAS)

No	External Factors	Weight	Rating	Score
	Opportunities			
O1	Support from center government	0.105	3.8	0.399
O2	District role in province	0.111	3.5	0.3885
O3	District commitment to improving community health	0.113	3.4	0.3842
O4	The involvement of another stakeholder in health sectors	0.101	3.3	0.3333
O5	Community participatory in planning health development	0.043	3.7	0.1591
				1.6641
No	Threats (T)	Weight	Rating	Score
T1	The ability of community in health finance	0.115	3.8	0.437
T2	The low of community ability in using government health facilities	0.112	3.5	0.392
T3	The financial regulation management is ambiguous	0.113	3.4	0.3842
T4	The economic growth rate in district and province	0.109	3.7	0.4033
T5	The low community ability to make a healthy environment.	0.113	3.5	0.3955
	Sub Total			2.012
	Total = opportunities + threats			3.6761

Source: processed primary data.

External factors that influences of health index development in Aceh are the support from central government with point (0,399). One of the government's supports in health development is the issuance of Jaminan Kesehatan Nasional (JKN). The JKN brings together all major health insurance schemes (Askes, Jamkesmas, Jamsostek and Jamkesda) under a single agency-the Social Security Management Corporation for the Health Sector (BPJS Kesehatan) [16]. A key response by the Government has been the development of a compulsory national health insurance scheme designed to pave the way for the achievement of universal coverage [17]. This scheme, known as Jaminan Kesehatan Nasional (JKN), seeks to make comprehensive care available to the entire population by 2019 [2]. An external factor that becoming development health index threat in Aceh is community ability to finance health with a score (0,437). People's ability to pay is a serious challenge facing the government now. Out-of-pocket health expenditure exposes households to the risk of incurring large medical bills that can push households into financial catastrophe [18]. This is of major concern to countries such as Indonesia where more than 28 million people currently live below the poverty line and around 100 million remain vulnerable to falling into poverty, as their income hovers marginally above the national poverty line [19], [20]. For the poor, this translates into high out-of-pocket payments that in turn, limits access to health care and pushes many into poverty [16].

Based on External Strategic Factors Analysis Summary (EFAS), it was found that health index development in Aceh has score EFAS as (3,676). This internal position gives a good. Strategy for Developing a Health Index in Aceh Province. The analysis of the level of development of the health index uses IFAS and EFAS metrics, then graphs the results of the SWOT analysis. The total value of IFAS, which is the difference between strength and weakness is 0.694. This indicates that the positive IFAS value means that the strength factor is greater than the weakness factor. The total value of EFAS which is the difference between opportunity and threat is (- 0.347). It is negative EFAS value which means the threat factor is greater than the opportunity factor. The thing that needs to be done is to strengthen internally to anticipate threats.

Strategy for Developing a Health Index in Aceh Province

The analysis of the level of development of the health index uses IFAS and EFAS metrics, then graphs the results of the SWOT analysis. The total value of IFAS, which is the difference between strength and weakness is 0.694. This indicates that the positive IFAS value means that the strength factor is greater than the weakness factor. The total value of EFAS which is the difference between opportunity and threat is - 0.347. It is negative EFAS value which means the threat factor is greater than the opportunity factor. The thing that needs to be done is to strengthen internally to anticipate threats.

Based on Figure 1 above, the current condition of developing the health index is in quadrant II (Strategy Diversification). The position in quadrant II implies that the position of health index is still good. However, it faces many big challenges. A big challenge has the potential to hamper the

development of a health index. Alternative strategies for developing health index in Aceh by using strengths, weaknesses, opportunities and threats. Based on this approach, alternative strategies such as SO, ST, WO, and WT were created as follows.

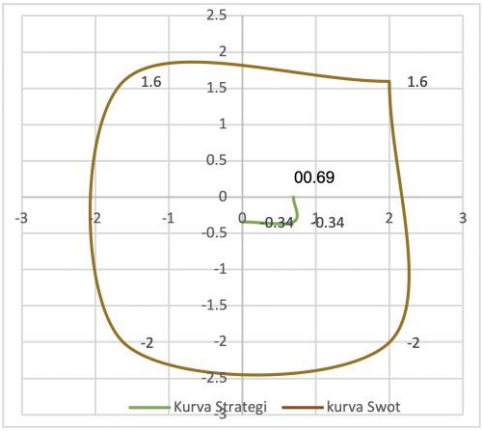

Figure 1: SWOT diagram

SO-strategy: Strategy in utilising the power (S) maximally to seize the opportunity (O) ie: - Improving the Quality of Health Development Planning; and - Enhancing partnerships with the private sector as well as cooperation between the central, provincial and district/city governments.

ST-strategy: Strategy in utilising the power (S) maximally to anticipate and overcome the threat (T): - Carry out promotions and education related to awareness of maintaining health; and - Preparation of policies related to the distribution and development of health workers and improving service quality.

WO-strategy: a strategy in minimising weakness (W) to seize the opportunity (O) ie: - Improvement of policies and budgeting in the health sector, policy formulation and development of health personnel; and - Increased proportional and responsible allocation of health spending in the Regional Expenditure Anngaram.

WT-strategy: Strategy in minimising weakness (W) to avoid threat (T), i.e., - Improve coordination with the central government, district/city government, and the community in implementing health programs; and - Synchronize government programs in the health sector with the central government and district/city government.

Discussion

Sixteen factors influence the development of a health index in the province of Aceh. Analysis of internal and external factors has identified five strength factors, six weakness factors, five opportunity factors, and five threat factors. The strategy to improve the Health Index through the allocation of Government Expenditures for Health in Aceh can be done by increasing the role of the provincial government, and support from the central government. Moreover, like many other countries in the region Indonesia has a thriving private-sector with two-thirds of health financing and more than half of all health services in private hands [21].

The role of the provincial government in increasing income can reduce dependency between districts. Districts/cities that have low income has a high dependence on the province in determining expenditure health [22], [23]. One of the factors affecting the level of health is how much the level of funding for the health sector [7]. The amount of health expenditure is positively related to the achievement of public health degrees. The greater the health expenditure issued by the government, the better the achievement of public health status. There is a positive relationship between government health spending in Life Expectancy [24]. The government plays an important role in public health services. Government support in developing the health index is the provision of primary health insurance (Askes, Jamkesmas, Jamsostek and Jamkesda) under a single agency-the Social Security Management Corporation for the Health Sector (BPJS Kesehatan). These roles include giving licenses, carrying out various actions to control the spread of disease in the community, supervision, hospital operators, and providing support for the implementation of research in the health sector [15], [26].

The quality of human resources in the health sector affects the development of health indices in the province of Aceh. Human resources implementing health services must have basic competencies in health education, such as the ability to make changes, support health, mediate partnerships, communication, leadership, assessment, planning, implementation, and evaluation and health research [27]. Also, the low quality of resources affects the quality of public services and ownership of health insurance for the community. The low quality of public services, limited health services, and the lack of service providers are also major factors in the low registration of health insurance [28]. Support from the central government affects the development of the health index in Aceh. Support from the central government in the form of co-administration funds in the health sector, operational health assistance, poor people's health funds, and assistance in the form of debt or grants.

Government support through the formulation of policies related to the development of health workers and improving the quality of health services. Government participation in the health sector is carried out by providing funding support to the health sector [29]. However, the implementation of the health funding policy made by the central government may not necessarily go according to plan, because there

are differences in values, there is no frame of reference, and there is a lack of support from the regional government. This is an impact of the delegation of obligations and responsibilities to the region [30], [31].

Health service policies will be difficult to support if policymakers and implementers still prioritise curative and rehabilitative health services rather than promotive and preventive [32]. The ability of the community to finance health affects the development of health indices in the province of Aceh. The ability of the community to finance health is still very low and depends on the government through the Aceh health insurance program (JKA). The low ability to reach health facilities and health information provided by the government is one of the causes of the uneven development of health in the province of Aceh.

In conclusion, sixteen factors influence the development of a health index in the province of Aceh. Analysis of internal and external factors has identified five strength factors, six weakness factors, five opportunity factors, and five threat factors. The internal factors (strength) that influence the development of the health index are the increasing role of the provincial government in improving health (0.437). Internal factors (weaknesses) that influence the development of health indices in the province of Aceh are the low quality of human resources in the health sector (0.336). External factors (opportunities) that influence the development of health indices in Aceh province are support from the central government with a value (0.399). External factors that pose a threat to the development of health indices in Aceh province are the ability of the community to finance health with a value (0.437). The results of the SWOT analysis of the condition of the development of the health index are currently in squared II (Strategy Diversification). This condition shows that the development of the health index faces a big threat. Alternative strategies for the development of health indices can be done by increasing the allocation of health spending, increasing partnerships with the private sector in implementing health programs, cooperation between the central, provincial and district governments in improving policies and improving service quality.

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Public Health

Victims of Physical Violence Have a Higher Risk to Be Perpetrators: A Study in High School Students Population

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Abstract

BACKGROUND: Violence experienced by a person can trigger mental disorders. It was reported that 1 in 4 children in the world had experienced severe and sustained physical violence. The form of violence varies from various bullying actions that seem mild, to deadly physical violence.

AIM: This study aimed to see the association between the victim and perpetrator of violence in high school students.

METHODS: A Hundred and Forty-Four high school students were chosen by stratified random sampling. They were assessed using Hurt, Insulted, threatened with harm, and Screamed (HITS) assessment tool. Univariate, bivariate, and multivariate analysis were used in this analysis, along with a cross-tabulation table.

RESULTS: Out of the 144 subjects, 66 (45.8%) admitted that they experienced violence in different gradations. A total of 34 (47.2%) respondents revealed that the perpetrators of the most frequent acts of violence were either one or both of their parents. The odds ratio (OR) of the subjects who experienced violence to commit violence is 3.571 (Confidence Interval (CI) 95% = 1.792-7.120).

CONCLUSION: More than 60% of high school students who experienced acts of violence turned out to experience more than one type of violence. The most frequent perpetrators of violence are the parents (47.2%), followed by friends and siblings. Those who experienced violent acts are 3.5 times more likely to become a perpetrator of violence.

Introduction

The problem of child abuse has become a global concern, as many children have become victims of mistreatment by adults, who eventually become perpetrators of violence. The United Nations Children's Fund (UNICEF) says that 1 in 4 children in the world has experienced severe and sustained physical violence [1]. Furthermore, UNICEF also said that this mistreatment could cause dangerous consequences in childhood, adolescents as well as adulthood, including mental disorders and become perpetrators of violence.

Child abuse occurring from 1999-2002 in 7 major cities in Indonesia found 3,969 cases with details of sexual abuse (65.8%), physical abuse (19.6%), emotional abuse (6.3%), and child neglect (8.3%) [2]. Among high school students, this form of violence varies significantly from various bullying actions that seem mild, to acts of violence that are fatal. Some cases have to be reported to the authorities and processed according to the law in Indonesia. In the past decade, there has been an increase in the number of people who commit crimes and violence, which is 83% increase in adults and 50% increase in youths [3].

Human aggressiveness is often compared with aggressive behaviour in animals. There are various types of aggressive behaviour in mammals: efforts to look for food (predatory), self-defence (defensive), protecting their territory, dominance in a

relationship, competition, looking for sexual relations, protecting their couples, protecting their children (maternal), and infanticide [1]. Aggressive behaviour presented as acts of violence in humans in part or whole often also has reasons like those of mammals. However, it is more complex and usually occurs in different social contexts and is governed by social norms [4].

Until today there is no exact data on long-term effects of violence committed by students. The goal of this study was to see the association between the victim and the perpetrator of violence in high school students.

Methods

The present study is a cross-sectional, case-control study, conducted in 2017. The research subjects were students from seven selected high schools in Denpasar. The study protocol was approved by the institutional review board of the Faculty of Medicine, Udayana University.

The tool used to assess violence was the Hurt, Insulted, threatened with harm, and Screamed (HITS) assessment tool [2]. The tool includes four questions that can be asked both written and verbally. The HITS screening tool has been proven to be useful as a diagnostic tool [2], [3]. HITS is a valid, consistent scale/screening tool, making it an excellent way for primary care physicians to identify victims of abuse.

A questionnaire-based on HITS screening tool was distributed to 500 respondents. All subjects agreed and signed an informed consent to be included in this study. Out of the 500, we recruited 144 subjects for this study by stratified random sampling. We used SPSS 20.0 software to analyse the data. Univariate, bivariate, and multivariate analysis were used in this analysis along with a cross-tabulation table. A p-value of < 0.05 was considered significant.

Results

The 144 subjects were analysed, and their characteristics are presented in Table 1. There were more female compared to male students (78 vs 66) in our study. They are between the ages of 15 to 19 years, with the largest population aged 17 years (36.1%). The mean age of the subjects is 16.69 ± 0.94 years and the median age was 17 (15-19) years old.

Table 1: Subjects' characteristic

Characteristics	N	%
Total subjects	144	100
Gender		
Male	66	45.8
Female	78	54.2
Age (years old)		
15	13	9.0
16	50	34.7
17	52	36.1
18	26	18.1
19	3	2.1
HITS score		
4	78	54.2
5	31	21.5
6	17	11.8
7	8	5.6
8	7	4.9
9	2	1.4
10	1	0.7

As many as 54.2% (78 people) of these high school students stated that they had never experienced an act of violence as asked in the HITS tool. The other 66 (45.8%) admitted that they experienced violence in different gradations. The highest score here is 10, found in one subject.

It turned out that the perpetrators of violence were people around their victims, as seen in Table 2. A total of 34 respondents admitted that the perpetrators of the most frequent acts of violence were either one or both of their parents.

Table 2: Violence perpetrators

Violence perpetrators of the victims	N	%
Father	11	15.2
Mother	9	12.6
Both parents	14	19.4
Sibling	11	15.2
Boyfriend / girlfriend	5	7.0
Others	22	30.6
Total	144	100

Table 3 tries to prove the causal relationship between being a violent-conduct victim and the perpetrators of violence. It turns out that there is a significant relationship between them.

Table 3: Relationship between the victim of violence and violence perpetrator

	Violence perpetrator	Not a violence perpetrator
Victim of violence	44 (61.1%)	28 (38.9%)
Not a victim of violence	22 (30.6%)	50 (69.4%)

The odds ratio (OR) of the subjects who experienced violence to commit violence is 3.571 (Confidence Interval (CI) 95% = 1.792-7.120). The risk of a perpetrator of violence to commit a subsequent act of violence is 2-fold (CI95% = 1.349-2.966), as seen in Table 4. While the risk of perpetrators of violence not to commit a subsequent act of violence was 0.56 (CI95% = 0.404-0.777).

Table 4: Risk estimation analysis derived from Table 3

Analysis	OR	CI95%
Victim to become a perpetrator	3.571	1.792-7.120
Perpetrator to repeat his/her violent act	2.000	1.349-2.966
Perpetrator not to repeat his/her violent act	0.560	0.404-0.777

Discussion

Male and female students turned out to have the same opportunity to commit acts of violence. This result is similar to another study reporting that boys and girls have equal opportunities to be a victim and a perpetrator of violence [5]. That is why parents must continue to learn and understand many things, including understanding the media used for the act of violence. When parents understand the media, they will more easily supervise their children [6].

More than 60% of those who experienced violence said that they experienced more than one type of violence in their lives, according to Sherin's predictions that stated that when someone experiences sexual violence, at the same time, they also get various threats (verbal violence) and physical violence [7]. The incident was certainly very painful for the victims, even in their minds, often for life. Male victims often externalise, one of which appears to be the change in their behaviour that becomes naughtier and more aggressive. While women are just the opposite, they are more internalised and appear with various symptoms of depression and self-harm [8], [9].

Among the seventy-two respondents who experienced acts of violence before, turned out that 61.1% became perpetrators of violence in the future. This is by the theory of intergenerational violence, which continues to be repeated as a learning process in one's household [10]. In this study, the risk of someone who has experienced acts of violence to become a perpetrator of violence is twice as high, so it is necessary to think of an intervention for the recovery and therapy of victims so that the inter-generational cycle no longer occurs.

More than half of the respondents to this study claimed that the perpetrators of the most frequent acts of violence were the fathers and mothers of the children. Several studies reported that perpetrators of violence against children were either one or both of their parents [11], [12], [13]. Another study reported that the perpetrators of violence against children lived together in their home [14]. The other perpetrators, for example, their friends (30.6%), including their boyfriend and girlfriend (7%). That is why it is necessary to consider the existence of an instrument that can be used as an initial assessment of the presence or absence of the risk of violence in a group, including domestic violence [15].

In conclusion, more than 60% of high school students who experienced acts of violence turned out to experience more than one type of violence. The most frequent perpetrators of violence are the parents (47.2%), followed by friends and siblings. Those who experienced violent acts are 3.5 times more likely to become a perpetrator of violence.

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Challenges and Barriers to Providing Care to Older Adult Patients in the Intensive Care Unit: A Qualitative Research

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Abstract

BACKGROUND: Enhancing the quality of care for elderly patients needs an understanding of the challenges and obstacles experienced by the intensive care unit (ICU) staff in providing care.

AIM: To explore the most challenging issues experienced by ICU staff, in particular, nurses, in the care of elderly patients in the general adult ICU.

DESIGN: A qualitative research design was employed. The Standards for Reporting Qualitative Research (SRQR) were followed.

METHODS: Based on theoretical sampling, we carried out 34 in-depth semi-structured interviews from two medical adult ICUs. Data analysis was carried out using qualitative conventional content analysis.

RESULTS: Data analysis led to the identification of three interrelated categories and 12 subcategories. Three main categories were factors related to nurses' attitude in elderly care, factors related to the system of care, and factors related to the models of patient care delivery. These categories came under the main theme of "Inappropriate and unfair system for elderly care".

CONCLUSION: The findings of this study increase scholarly understanding of challenges and barriers to providing care to elderly patients in the general adult ICU. We found that the provision of care to elderly patients is inappropriate and unfair. Various obstacles must be overcome to improve the care of these patients. For example, negative attitudes toward elder care, inappropriate environments, lack of resources, lack of knowledge and skills, a specialized model of care delivery, respect for humanity, care without considering patient age, and separating professional conflicts from patient care. These findings may be used by ICU's caregivers and managers to improve the quality of care.

IMPLICATIONS FOR PRACTICE: Various obstacles were documented that need to be overcome by hospital administrators, nursing managers, clinical nurses, nursing educators, nursing researchers to improve the care of elderly patients admitted to ICU.

Introduction

Recent advances in the treatment of diseases have led to an increase in human life expectancy and an increase in the population of older adult people with multiple chronic diseases [1]. According to an official World Health Organization (WHO) report, Iran's population aged 60 and over in 2015 was about 10 per cent of the total population, which is expected to increase to more than 33 percent by 2050 [1]. According to the Iranian Census Bureau, the age distribution of the country's population is changing very fast from youth to old age [2]. Given the rapid growth rate of the elderly population in the coming years, there is a growing need for future planning to control the health of this population. However, the evidence suggests that due to the inadequacy of care processes and structures, the current healthcare systems in most countries of the world, including Iran, are unable to meet the complex needs of elderly patients suffering from various disabilities [3]. Regarding the process of care, the results of previous studies show that healthcare providers, including nurses, do not have the required and sufficient abilities to manage the physical and psychological

needs of hospitalized elderly patients [4].

To provide specialised care to elderly patients, it is necessary for the healthcare system to make appropriate changes in the structure and process of providing services to these patients [5]. Also, it is imperative that the nursing services system, which is primarily responsible for patient care, undergo fundamental changes in the care of this vulnerable group [6].

One of the hospital wards, where elderly patients are frequently admitted, is the intensive care unit (ICU), which is one of the expensive, invasive, and intensive settings in hospitals [7]. Care of elderly patients admitted to ICU is fundamentally different from younger patients because the care of them requires specialised knowledge and skills [8], [9]. They need more complex care than other patients because of the coexistence of several chronic diseases [10]. Evidence suggests that advanced age is associated with worsened outcomes and increased mortality in critically ill patients admitted to ICU [11]. Also, the length of hospitalisation of elderly patients in ICU is longer than in other wards [12], [13]. Prolonged hospitalisation is associated with the occurrence of new complications and disorders; therefore, it requires advanced care, which can increase treatment costs in the absence of clear benefits [14], [15]. Because of the futility of care, there is some evidence that ICU caregivers do not pay enough attention to the care of elderly patients in some cases, and most of the care they provided to elderlies is routine and palliative [8], [16]. Furthermore, due to the high mortality rates of elderly patients in ICU, admission, hospitalization, and care of them face challenges [17]. For these reasons, there have been considerable debates about whether to admit patients with limited life expectancy to ICU, such as elderly patients with significant chronic diseases [18].

Among ICU caregivers, nurses play a key role in maintaining and promoting the health of various groups of critically ill patients, including the elderlies, by providing advanced care [19]. However, the barriers to the care of elderly patients admitted to the ICUs have not yet been fully elucidated. Enhancing the quality of care for elderly patients needs an understanding of the challenges and obstacles experienced by the ICU staff in providing care. It is necessary to explore the ICU caregivers' perspective and experience regarding barriers to care for elderly patients. Understanding these experiences can help develop interventions to improve the provision of care for elderly patients and improve their quality of life. Therefore, the aim of this qualitative study was to explore the most challenging issues experienced by ICU staff, in particular, nurses, in the care of elderly patients in the general adult ICU.

Methods

Research design and participants

This study was conducted by a qualitative research method. Using purposive sampling, nurse participants with at least one year of work experience in the ICU were eligible to participate in the study. Maximum variations in sampling in terms of various socio-demographic and educational status were considered during the recruitment of the participants. Also, patients' family members and healthcare professionals who worked at ICU were interviewed using theoretical sampling. The number of interviews was guided by reaching data saturation. Finally, data saturation was reached with 34 participants (26 initial interviews and 8 additional interviews as member check). The study was conducted between June 2017 and June 2019 within two ICUs of Emam-Reza hospital, a principal referral center in northeast Iran, which is affiliated to the Mashhad University of Medical Sciences, Mashhad, Iran.

Data collection

In-depth semi-structured interviews were conducted by the first author (HSH) who is a fourth-year doctoral student in nursing and was highly trained in qualitative interview processes and techniques. Participants were directly contacted and informed of the purpose of the study. All the interviews took place face-to-face at a date and time that was most convenient to participants, conducted in Persian and were audio taped. Interviews lasted between 25 and 85 minutes (mean: 46 minutes). The interviews began with the general open-ended question: "What factors affect the provision of care to elderlies admitted to the ICU?" The interviews continued with the question "How do these factors affect treatment and care outcomes?" Participants were asked to share their experiences regarding main barriers to and facilitators of caring for elderlies in the ICU. The interviewer used probing questions to clarify a situation or to provide detail to an answer (would you explain further? could you give an example of what you mean?). In addition, in-field notes were taken to cover untold aspects of the elderly care.

Data analysis

All the interviews were immediately transcribed verbatim. The transcripts were read by the authors several times to get insight into the participants' experiences. Thereafter, they were analyzed using conventional content analysis according to the technique described by Hsieh and Shannon [20]. Data were coded, and related codes were finally grouped under certain categories by using the constant comparative analysis. After each new interview, the previous categories were either revised,

combined, or a new category was shaped. All of the authors contributed to categories development through face-to-face meetings. The MAXQDA (Version 10) was used to organise, code, and manage the data.

Ethical considerations

Ethical approval for the study was obtained from an institutional ethics committee affiliated with Mashhad (decree number: IR.MUMS.REC.1396.111). Participation was voluntary, and the participants provided their consent to participate. All of the interviews were audiotaped with the consent of the participants. The participants were assured that their information would remain confidential and they had the right to withdraw from the study at any time.

Methodological rigour

Lincoln and Guba's criteria for trustworthiness, including credibility, transferability, dependability and confirmability, were used to ensure the trustworthiness of this study [21]. These criteria were established through a 24-month engagement period in the research setting, providing thick descriptions illustrating the participants' lived experiences, peer debriefing, member checking (the participants were invited to attend a peer review to establish the initial coding of initial interviews), recording the decision trail throughout the data analysis process, recording the interviews and transcribing them immediately after each interview. Furthermore, to expand the transferability of the data, the participants' quotes were presented verbatim.

Results

As shown in Tables 1, 26 participants with maximum variation were interviewed, 16 participants were female and 10 were male, 8 participants were re-interviewed (member check). Due to the gender distribution of ICU nurses, the majority of nurses were female, and therefore, the majority of participants in this research project were female. The mean age of the participants was 36 years (ranged from 26 to 52 years). ICU nurses were the main participants in this study. However, data analysis led to planning for interviews with other caregiver members. Accordingly, the participants included the staff involved in elderly patient care including nurses (at least Bachelor of Science in Nursing degree), physiotherapists, intensive care specialists, anaesthesia residents, and patient family members. The mean duration of the interview was 47 minutes (25 to 85 minutes).

Table 1: Participants profile in the initial interviews

Row	Interview time(min)	Gender	Age	Profession	License	Work experience (year)	
						ICU	Total
1	37	Female	31	Nurse	Master	1	5
2	37	Female	34	Nurse	BSN	3	7
3	44	Female	37	Nurse	BSN	5	13
4	54	Female	36	Nurse	BSN	8	13
5	45	Female	35	Nurse	Master	7	10
6	33	Female	36	Nurse	Master	8	10
7	52	Female	44	Nurse	BSN	7	12
8	85	Male	32	Nurse	BSN	6	9
9	55	Male	38	Nurse	BSN	10	14
10	50	Female	33	Nurse	BSN	7	7
11	57	Female	33	Nurse	Master	3.5	8
12	44	Female	39	Nurse	BSN	7	7.5
13	47	Female	35	Nurse	Master	10	12
14	40	Female	33	Nurse	BSN	10	10
15	45	Female	36	Nurse	BSN	8	8
16	50	Female	34	Nurse	BSN	2	11
17	45	Female	37	Nurse	BSN	8	8
18	55	Male	29	Nurse	BSN	3	7
19	57	Male	32	Nurse	BSN	9	11
20	45	Male	52	Nurse	BSN	24	27
21	25	Female	42	Physiotherapist	BSN	10	15
22	35	Male	26	Nurse	BSN	7	8
23	42	Male	34	Nurse	BSN	8	10
24	45	Male	46	Nurse	BSN	11	12
25	40	Male	37	Doctor	Anesthesiologist	2	5
26	45	Male	38	Nurse	BSN	6	7
Specifications of participants in the re-interview							
Row	Interview time(min)	Gender	Age	Profession	License	Work experience (year)	
						ICU	Total
1	25	Female	31	Nurse	Master	1	5
2	20	Male	46	Nurse	BSN	11	12
3	30	Female	37	Nurse	BSN	5	13
4	34	Male	36	Nurse	BSN	8	13
5	24	Female	35	Nurse	Master	7	10
6	20	Male	29	Nurse	BSN	3	7
7	15	Male	37	Doctor	Anesthesiologist	2	5
8	35	Male	38	Nurse	BSN	6	7
Statistics							
Mean	42	-	36	-	-	7	10
SD	14	-	5	-	-	4	4
Min	15	-	26	-	-	1	5
Max	85	-	52	-	-	24	27

According to the participants' experiences, several challenging factors influenced the care of elderly patients in the general adult ICU. These factors were organised into three interrelated categories and 12 subcategories as follows: factors related to nurses' attitude in elderly care; factors related to the system of care; factors related to the models of patient care delivery. We put these categories under the main theme of "Inappropriate and unfair system for elderly care". Table 3 shows the main theme, categories and their subcategories regarding the challenges of elderly care in the ICU. Table 2 shows the challenging factors affecting the care of elderly patients in general adult ICU.

Table 2: Challenging factors affecting the care of elderly patients in the general adult ICU

Main theme: Inappropriate and unfair system for elderly care	
Factors related to nurses' attitude in elderly care	
A negative bias towards the elderly	Priority for non-elderly patient care
	Poor response to care and treatment
	Complexity and time-consuming care
	Prolonged hospitalisation
	Higher mortality rate
	Crossing the "life expectancy" limit
The unfavourable working atmosphere for elderly care	Decentralisation of care for the elderly
	The futility of care
	Useless discharge of the elderly
	Delayed and incomplete care
Factors related to the system of care	
Stressful environment	The stressful, emotional environment
	Stressful physical environment
Improper management policies	Nurse isolated from the patient's bedside
	Resource constraints (human/financial)
	Double nurse pressure
Non-constructive inter-professional communication	Improper interdisciplinary cooperation
	The passive role of the nurse in the care team
Intra-professional conflict	Lack of intra-professional differentiation
	Professional disappointment and dissatisfaction
Factors related to the models of patient care delivery	
Inappropriate patient assignment method	
Inappropriate nursing handover styles	
Inappropriate model of care delivery	
Lack of specialised model of elderly care	
Task-oriented nursing care	
Lack of specialised knowledge of elderly care	

Factors related to nurses' attitude in elderly care

A negative bias towards the elderly care

The attitude and belief of the intensive care unit's caregivers towards elderly patient care are biased and prejudiced.

Priority for non-elderly patient care

Based on the participants' experiences, care of elderly patients, particularly those with complicated vital conditions, is not a high priority in the ICU. In this regard, a participant stated, "in the ICU, many elderly patients have an irreversible vital condition and are unlikely to survive. That's why they are not a priority in care." (P4).

Poor response to care and treatment

Inadequate response of these patients to the treatments and care provided by the medical and nursing staff has led to a decrease in the quantity and quality of care for them. A participant stated, "although standard care and treatments are provided for the elderlies, they do not respond to treatments. For this reason, as the length of hospitalisation increases, the quantity and quality of care gradually decrease." (P7).

Complexity and time-consuming care

Elderly patients suffer from multiple diseases at the same time, which makes it difficult and time-consuming to care for patients. This has made the nurse reluctant to care for these patients. A participant stated, "I don't want to care for these patients at all because they have complex diseases, the care of these patients is very heavy and frustrating, and in the end, you will not get any results." (P11).

Prolonged hospitalisation and higher mortality rate

Long-term hospitalisation of these patients is associated with the occurrence of several complications that can ultimately increase the rate of patients' mortality. A participant stated, "these patients have been hospitalised for a long time without significant improvement. Because of organ failure and irreversible complications, most of these patients die and the resources spent on caring for these patients are wasted." (P1) Another participant stated, "as most of the elderly patients have multiple chronic diseases, they face a higher mortality rate in spite of receiving standard care." (P5).

Crossing the "life expectancy" limit

Based on the participants' experiences, patients who live longer than average life expectancy and have multiple concurrent disabling diseases are not in a high priority for care. A participant stated, "in the ICU, I have to say some age boundaries have occurred in the care of patients. Usually, patients over eighty years of age are not much considered. Even their admission to ICU is negligible. These patients have passed their useful life and the limited equipment should be used to save the lives of non-elderly patients." (P13).

The unfavourable working atmosphere for elderly care

A persistent gap between ICU bed supply and demand has led to the creation of the unfavourable working atmosphere for elderly care in terms of quality and quantity of care. There is a hidden working atmosphere assumes that caring for terminally ill elderly patients in the ICU is a futile task. This hidden working atmosphere has also created the attitude that the continuity of care for these patients is a factor for further discomfort and distress.

Decentralisation of care for the elderly

Based on the participants' experiences, care of the elderly with a complex or terminal life condition is not at the centre of care and it is insignificant. A participant stated, "here, fewer persons care about the critically ill elderly with a terminal illness. All staff are focused on improving young patients and those with reversible vital status." (P19).

The futility of care

The attitude of most participants was the futility of intensive care for the elderly. They believed that intensive care would only increase the suffering of elderly patients while not improving the outcomes and quality of life of these patients. Also, the continuity of care for the elderly with terminal illness imposes substantial costs on the family and the healthcare system. A participant stated, "when the patient can never come back to life, continuing care is useless and only causes the patient to suffer." (P2).

Useless discharge of the elderly

Elderly patients are admitted to ICUs for a long time. During prolonged hospitalisation, new and irreversible diseases and complications occur. Among those elderly, a limited number of them will be discharged, of course, along with severe disabilities. Participants believed that the prolonged hospitalisation of these patients would only lead to continued suffering and increased economic costs to the family. This underlying belief has led to a decline in the quantity and quality of care provided to the

elderly patient. A participant stated, “If an elderly patient can survive ICU, he / she will be discharged with severe neurological and skeletal complications that make life very difficult.” (P9).

Delayed and incomplete care

Perception of the futility of elderly care has led to a delay in providing care, which is also done incompletely. A participant stated, “sometimes care is delayed because they are repeated and only routinely delivered to the patient. No one is looking for results. For example, if a blood sample is needed, it is delayed because the test result is not followed up.” (P12).

Factors related to the system of care

Stressful environment

Stress can have agonizing effects on both elderly patients and ICU staff. Based on the participants' experiences, there are several sources of stress in the ICU setting, which can alter the health outcomes of elderly patients, who are a very vulnerable population. Both the elderly patient and the nurse are exposed to these environmental stressors. The nurses stated that the quality of care in such a stressful environment would not be optimal.

The stressful, emotional environment

There are several factors in the ICU that cause emotional stress to nurses, which in turn can affect the quantity and quality of care provided to the elderly patient. Main emotional stressors were feelings of hopelessness following frequent deaths of patients, mental exhaustion from working in the ICU, endless compassion for patients and their families, verbal tension with nursing colleagues and managers, nursing managers' neglect to the high working tension in the ICU. For instance, nurses' mental exhaustion caused them to become impatient and being frustrated during the working shift, and thus, to provide incomplete care for elderly patients. In this regard, a participant expressed, “when you are very tired and frustrated, you do not care about a terminal elderly patient” (P6).

Stressful physical environment

Elderly patients are vulnerable, and their admission to the ICU increases the severity of the vulnerability. Based on the participants' experiences, several physical and structural factors contribute to increased vulnerability of elderly patients. Continuous lighting, annoying noise pollution, and inadequate physical environment for care were among those factors, which can affect all aspects of mental and physical health of elderly patients.

Improper management policies

Based on the participants' experiences, numerous management policies have reduced the quantity and quality of care provided to patients, including the elderlies.

Nurse isolated from the patient's bedside

Most participants deeply expressed their concern that nurses were shifted from patient’s bedside to the bureaucratic paperwork, such as frequent documentation of routine events into the hospital information system that focuses mainly on the administrational needs of hospitals. A participant stated, “according to the inappropriate policies of the nursing authorities, most of what we do is paperwork. During the shift, we visit the patient two or three times; then our job is to keep track of events in multiple registries.” (P17).

Resource constraints (human/financial)

According to the participants' experiences, the presence of multiple resource constraints, either human or financial, negatively affected the provision of care to all patients in all hospital settings, and elderly patients are also no exception.

Double nurse pressure

Nurse shortage or the non-employment policy has led to additional pressure on nursing staff. The experiences of the participants indicated that too much nursing work has led to physical and mental fatigue, which negatively affected the quantity and quality of care. A participant stated, “we have to do a lot of overtime to compensate for the shortage of nursing staff. Working overtime made us mentally and physically fatigued, and thus has reduced the quantity and quality of care provided to elderly patients.” (P10). Another participant stated, “nursing is a low-paid job; thus, nurses need to working overtime to get paid more. That is why they are more stressed and tired.” (P3).

Non-constructive inter-professional communication

Improper interdisciplinary cooperation

Many nurse participants complained that they had not been actively involved with patient care by physicians. They stressed that an unexplained absence of nurses could lead to a decline in the quality and quantity of care provided to elderly patients. A participant stated, “some physicians visit the patient alone and do not coordinate new orders with the patient’s designated nurse.” (P8). Another participant stated, “physicians' neglect to the presence

of nurses and nursing's reports indirectly lead to a decline in the quality of care for elderly patients." (P14).

The passive role of the nurse in the care team

Based on the participants' experiences, the passive role of nurses in the care team has a significant impact on the quality of care provided to complex patients in ICU, including elderlies. A participant stated, "the quality of care provided to the patient will improve if our role becomes more active in the healthcare team. At present, we are only sheer obedient to the physicians' orders." (P15).

Intra-professional conflict

The intra-professional conflict within nursing was also one of the factors that indirectly affected the quantity and quality of care provided to the patient.

Lack of intra-professional differentiation

If the positive personal and occupational characteristics of a dutiful nurse are ignored by their authorities, the nurse realises that the quality and quantity of care provided to the patients are of no value to the nursing authorities and therefore, she no longer attempts to improve the quality of care. A participant stated, "in our nursing profession, there is no difference between an experienced nurse and a novice nurse, between a dutiful nurse and an irresponsible one. There is no significant differentiation. No more motivation to work hard. Why should I try to take better care of elderly patients with end-stage disease?" (P16).

Professional disappointment and dissatisfaction

Nurses were dissatisfied and disappointed with their profession for many reasons, including income, social status and professional autonomy. Dissatisfaction had indirectly reduced the quantity and quality of care provided to elderly patients in the ICU setting. A participant stated, "hardship and difficulty of nursing and its low income have produced strong dissatisfaction among nurses. The prerequisite for quality patient care is to provide the welfare of nurses first." (P20).

Factors related to the models of patient care delivery

Inappropriate patient assignment method

The patient-to-nurse assignment was another factor that affected the quantity and quality of care. In the ICU, the principal criteria for assigning a patient to a nurse were the proximity of the two beds and the complexity of care. In the next shifts, the patient was cared for by a new nurse. Thus, the continuity of care was disrupted. A participant stated, "the assigned patient to a nurse changes every shift, and we do not have a fixed patient that we can fully care for. This makes us unable to do the best for the patient." (P26).

Inappropriate nursing handover styles

One of the important things to maintain the continuity of care is a detailed handover of care responsibility of a patient to another nurse at the end of a working shift. Most participants expressed that there is no appropriate nursing handover style between nurses' staff in the ICU. A participant stated, "we do not have a style for patient handover to the next shift nurse. Patient handover is conducted orally and arbitrarily. It is unclear how nurses coordinate patient care across different shifts." (P24).

Inappropriate model of care delivery/lack of a specialised model of elderly care

The main model of care delivery was 'case method' (total patient care), in which one nurse was responsible for all aspects of the care of two patients during one particular working shift. That nurse worked directly with the patient, family and other healthcare staff in implementing a plan of care. According to the participants' experiences, this model (case method) is inappropriate for elderly patients hospitalised in the ICU. They stressed that these patients need a more specific model of care delivery in the ICU such as, 'nursing case management' or 'primary nursing' or 'patient-centred care'.

Task-oriented nursing care

Task-oriented and passive nursing care has led to the tedium of everyday nursing care in the ICU. A participant stated, "The nursing care we provide to elderly patients in ICU is often routine-based interventions and is more technical. We do not implement the nursing process for elderly patients because we do not have a dedicated system for the care of elderly patients." (P18).

Lack of specialised knowledge of elderly care

Elderly patients hospitalised in the ICU have advanced diseases. Caring of them requires specialised knowledge and skills. However, currently, these skills and knowledge have not been taught to nurses. A participant stated, "at the Faculty of Nursing, we have not been taught the knowledge of intensive care for the elderly patients and we have learned some important skills ourselves, of course, individually." (P23).

Discussion

In this qualitative study, we explored the most challenging issues experienced by nurses in the care of elderly patients in the general ICU. We organised these issues into three main inter-related categories and 12 subcategories as the following: “factors related to nurses’ attitude in elderly care (negative bias towards the elderly care, unfavourable working atmosphere for elderly care); factors related to the system of care (stressful environment, improper management policies, non-constructive inter-professional communication, intra-profession conflict); and factors related to the models of patient care delivery (inappropriate model of care delivery, inappropriate nursing handover styles, lack of specialised model of elderly care, inappropriate patient assignment method, task-oriented nursing care, and lack of specialised knowledge of elderly care).

About the staff nurses, our findings support the need for a significant shift in the attitude of ICU staff in providing care for elderly patients, particularly those with end-stage disease. As most of the participants in our study reported, we found that the experience of caring for elderly patients over the past years has led to some implicit bias and prejudices against older adults in the provision of care by professional caregivers. These prejudices have diminished the quantity and quality of care provided to this group of patients. The most important factor underlying this negative attitude was the ineffectiveness of treatments and care for the elderly patient concerning survival and future quality of life. Although all elderly patients received standard care after being admitted to the ICU, many responded poorly to the interventions and eventually died. The frequent occurrence of these consequences over the years has tended to foster a negative attitude towards admission and hospitalisation of elderlies with advanced diseases. In line with our findings, previous studies found that the perception of futile care hurts caring behaviours of ICU caregivers toward elderly patients [22], [23], [24], [25].

According to the participants’ experiences, the system for delivering intensive care to elderly patients is inappropriate in terms of environment, management policies, inter-professional communication, and intra-professional conflict. The ICU environment involves various dimensions of care to save patients' life by facilitating interpersonal communication among healthcare professionals [26]. Similar to our findings, previous studies found that the environment of ICU is very hostile for staff and the vulnerable critically ill elderly patients in terms of physical and mental stressors [27], [28], [29], [30]. In particular, the intensive care setting can be an obstacle to providing intensive care for long-stay elderly patients who have complex vital conditions [31].

Similar to our findings, due to the nature of the intensive care unit, numerous sources of conflict cause inconsistency between different members of the care team. Among these resources were uncertainty about the continuity of care for the elderly and the occurrence of ethical challenges in the resuscitation of elderly patients [26]. Poor interprofessional communication has negative impacts on patient outcomes [32].

Nurse participants expressed that because of the lack of professional autonomy, they excluded from direct patient care. They no longer have an active and autonomous role in the clinical team to make decisions about improving patient outcomes. In this regard, previous studies have shown that limited autonomy of nurses is a barrier to nurse-physician collaboration, which, in turn, negatively affects patient outcomes [33], [34].

We found that several factors made nurses dissatisfied with ICU work, including stressful work environments, increased work pressure, human resources constraint, poor nurse-physician relationships, inequality in pay, mandatory overtime and inadequate professional autonomy. Along with numerous factors related to the attitudes of staff and the system of care, as stated by participants, job dissatisfaction has negatively affected the quantity and quality of care provided to elderly patients. Similar to our findings, several studies have indicated high levels of job dissatisfaction and burnout among ICU nurses [35], [36]. Also, evidence suggests that organisational policies are linked with undesirable consequences in workforces [36], [37].

As our participants experienced, the model in which nursing care was delivered to elderly patients was inappropriate in terms of patient assignment method and nursing handover style between different working shifts. These issues negatively affected the quality and quantity of elderly care in the ICU. Previous studies in ICUs have emphasised that an optimal patient flow between different working shifts is critical to ensure a high quality of care and maintain care continuity [38]. However, similar to our findings, a recent Cochrane review revealed that the effectiveness of current nursing handover styles for ensuring continuity of care is unclear and uncertainty about the optimal handover style remains [39]. Regarding the model for delivering care to critically ill elderly patients, our participants highlighted a need for developing a specific care delivery model for elderly patients; because they had experienced that the ‘case method’ model was not suitable for the elderly patients. Based on the participants' experiences, a care model that maintains the nurse-patient relationship as much as possible is appropriate for elderly patients. In the literature, the study that examined the best model of nursing care delivery for the elderly in ICU was not found.

Another challenging issue for the care of the

elderly in ICU was that participants noted a lack of specialist knowledge of elderly care. They needed continuing education courses about issues related to intensive care for the elderly, including advanced care training for pressure ulcers, prevention of extremity deformities, mental relaxation of elderly patients, and management of elderly delirium.

There are some limitations to the present study that need to be addressed. In this study, all participants were enrolled from two ICUs within one hospital; therefore, the results may not be generalised to all ICUs who are admitting elderly patients. Also, the responses of participants regarding elderly care in the ICU might have been influenced by environmental factors, organisational culture and social acceptability bias, although an attempt was made to minimise this bias during interviews.

In conclusion, the findings of this study increase scholarly understanding of challenges and barriers to providing care to elderly patients in the general adult ICU. The challenging issues experienced by general adult ICU staff in the care of elderly patients were explored and organised into three main categories as follows: factors related to nurses' attitudes in elderly care, factors related to the system of care, and factors related to the models of patient care delivery. Ultimately, we found that the provision of care to elderly patients in the general adult ICU is inappropriate and unfair. Various obstacles must be overcome to improve the care of these patients. For example, negative attitudes toward elder care, inappropriate environments, lack of resources, lack of knowledge and skills, a specialized model of care delivery, respect for humanity, care without considering patient age, and separating professional conflicts from patient care. These findings may be used by ICU's caregivers and managers to improve the quality of care.

Summary statement of implications for practice

What does this research add to existing knowledge in gerontology?

- The attitude and belief of the intensive care unit's caregivers towards elderly patient care are biased and prejudiced.

- There is a hidden working atmosphere assumes that caring for terminally ill elderly patients in the intensive care unit is a futile task.

- The current system for the provision of care to elderly patients is inappropriate and unfair.

What are the implications of this new knowledge for nursing care with older people?

- The findings of this study increase scholarly understanding of challenges and barriers to providing care to elderly patients in the general adult intensive care unit.

- The challenging factors in the care of elderly patients were related to nurses' attitude, the system of care, and the models of patient care delivery.

How could the findings be used to influence policy or practice or research or education?

- Various obstacles must be overcome by hospital administrators to improve the care of elderly patients, including inappropriate environments and lack of resources.

- Nursing educators should design and implement training courses for intensive care nurses and senior nurse students regarding the specific care of elderly patients.

- Nursing managers need to manage intra- and inter-professional conflicts and enhance the quality and quality of elderly patients care by creating a favourable environment.

- Nursing researchers need to work to find new models of care delivery for elderly patients admitted to the intensive care unit.

Authors' contributions

Study conception and design: HSH, AH, and ABM. Acquisition of data: HSH. Interpretation of data: HSH, AH, and ABM. Drafting of the manuscript: HSH, AH, and ABM. Critical revision of the manuscript for important intellectual content: HSH, AH, and ABM.

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Public Health

World Health Organization Surgical Safety Checklist with Addition of Infection Control Items: Intervention Study in Egypt

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Abstract

BACKGROUND: Surgical team is in command of the operating room (OR) and takes decisions regarding various patient care procedures. Educational programs directed to them, should be creative, provocative and tailored to their specific needs and the expected outcomes.

AIM: This study aims to design and conduct an educational program of patient safety and infection control for the OR team based on the WHO surgical safety checklist and to assess their post-intervention knowledge and practices.

METHODS: This interventional study was conducted at the ORs of Port-said general hospital. It passed through three stages; baseline assessment of knowledge and practice regarding patient safety and infection control among OR team (surgeons, anaesthetists and nurses), intervention stage in which an educational program based on the WHO surgical safety checklist with modifications and additions of more infection control items was conducted, then re-assessment of their post-intervention knowledge and practices.

RESULTS: All the studied participants showed improvement in both knowledge and practices of patient safety and infection control after the educational program based on the WHO surgical safety checklist with modifications and additions of more infection control items and including not only practices but also knowledge as well, than before.

CONCLUSION: The modification of the WHO surgical safety checklist to fit local knowledge and practices created a comprehensive tool that led to an improvement in both knowledge and practices of patient safety and infection control among the OR team.

Introduction

Operating room (OR) is a critical facility within the hospital where surgical operations are carried out in an aseptic yet stressful environment. This stress coupled with the performance of various surgical patterns is rarely admitted by the surgical community since accentuation of leadership and self- confidence is very exalted that stress is often realised as a stamp of weakness or failure [1].

Surgical team members highly contrive their efforts to attain their patients' full care and safety. Culture of patient safety and infection control needs to be established first of all, especially in OR [2], [3]. World Health Organization (WHO) published the surgical safety checklist which comprises 19 items that must be checked at 3 points all the time of surgery; sign in, time out and time out [4].

A mechanism needs to be developed for capturing data relating to knowledge of patient safety and patient safety practices [2], [3]. Infection prevention and control(IPC) seizes an exquisite place in patient safety framework because it is universally pertinent to health workers and patients at whatsoever health-care station. The conveyance of essential health services and the recovery phase of any health system should include IPC as a vital constituent, not just a response-specific intervention [4].

If health care personnel (HCP) realise the evocation of infection control program, they will presumably carry out any exposure-control plan. Congruity, proficiency, and applicable coordination of IPC activities can be attained through unmistakably written policies, procedures, and guidelines. Infection-control training should be received by HCP minimally three times; on the initial appointment when exposed to new tasks or procedures, and at a minimum, annually. Occupational education and training should take hold of the assigned duties [5], [6].

Providing high-quality care should include

organisational atmosphere cohesive to commended patient safety and infection control practices created by hospital administrators. Sufficient resources and visible support in the form of continuous education programs must be provided by hospitals to reach this high-quality care concept [7].

This study aims to design and conduct an educational program of patient safety and infection control for operating rooms OR team at Port-Said general hospital and to assess their post-intervention knowledge and practices changes.

Materials and Methods

Study design

A quasi-experimental study (Pre-post intervention design) was used to assess the effects of a comprehensive educational program about patient safety and infection control in OR on the following main outcome measures: knowledge and practice of OR team (surgeons, anaesthetists, and nurses).

Study Setting

This study was conducted at the ORs of Port-said general hospital, which is 1st Egyptian hospital to implement the new universal health coverage system in Egypt.

Study population

A purposive sampling technique was used. All OR team members composed of 48 surgeons, 8 anaesthetists and 16 nurses, of a total of 72 participants on duty at the time of research were included.

Data collection

The (WHO) recommends: "routine use of a surgical safety checklist before all surgical operations. This checklist, WHO surgical safety checklist is not intended to be concise. Fitting local practice by additions and modifications are encouraged. It is originally branched into three domains (Sign In, Time Out and Sign Out)." [8] It comprises 19 items that need to be checked at three points around the time of surgery as the practice of OR team (Figure 1): 1. During "Sign In" before induction of anaesthesia (7 items for only anaesthetists and nurses); 2. For “Time Out” before skin incision (7 items for all OR team); and 3. For the “Sign Out” before the patient leaves the operating room (5 items for all OR team).

This study added 3 items of knowledge of patient safety as was encouraged by the WHO [9], [10], [11], [12], [13], [14], [15], [16], [17]: 1. Factors inside OR could lead to medical errors including (bad conditions of hospital infrastructure, shortage of staff and work overload and lack of communication between OR team); 2. Types of medical errors inside OR including: (operation at the wrong side/the wrong site, wrong procedure and wrong patient (WSPE); and 3. Reporting medical errors when happening (to both the patient and the administration).

Also, our study added 3 items of infection control inside the OR as knowledge and practice :1. Surgical hand washing technique including (when scrubbing and after scrubbing); 2. Techniques of wearing and removing personal protective equipment (PPE) including (gowning, gloving and maintaining the sterile surgical field); and 3. Techniques for prevention of bloodborne pathogens (BBP) including: (infection prevention and universal precautions).

So, this study not only added more infection control items but also assessing and improving the knowledge of OR team through an educational program was added. All of these additions to the original WHO surgical safety checklist was obtained from WHO best practice protocols for clinical procedures safety [18], WHO guidelines on hand hygiene in health care and their consensus recommendations [19] and the Centers for Disease Control (CDC) 2007 guideline for isolation precautions preventing transmission of infectious agents in healthcare settings [20]. These protocols included items of patient safety and infection control specially designed for OR.

Our data collection tools were a questionnaire and an observational checklist. Knowledge of OR team was assessed using the interview questionnaire, while practices were assessed using the observational checklist. One of the researchers was the coordinator to manage and follow up the application of the checklist in OR for practice attending the duties with the OR team and also interviewing them for their knowledge.

The questionnaire also included demographic characteristics OR team including name, age, gender, medical profession (surgeons, anaesthetists and nurses), surgical speciality, Years of working at OR, No of days/week at OR, Average hours at OR/day and No of operations/month.

Each item in both the questionnaire of knowledge and the checklist of practices was assessed through a group of questions and observations respectively. Then the right choices for answers or practices were calculated and divided by total answers and practices for that item, so each item had a percentage from 0% to 100% according to the right choices for each participant, these percentages were then compared for each item before and after the intervention to estimate its effect.

Finally, all the components of knowledge and practice of both patient safety and infection control were collected, so we have four totals, which also compared before and after the intervention for each participant, as follows: 1. Total of knowledge of patient safety; 2. Total of practices of patient safety; 3. Total of knowledge of infection control; and 4. Total of practices of infection control. After all, the change difference percentage of all the components of knowledge and practice of both patient safety and infection control was compared between the 3 studied groups: surgeons, anaesthetists, and nurses.

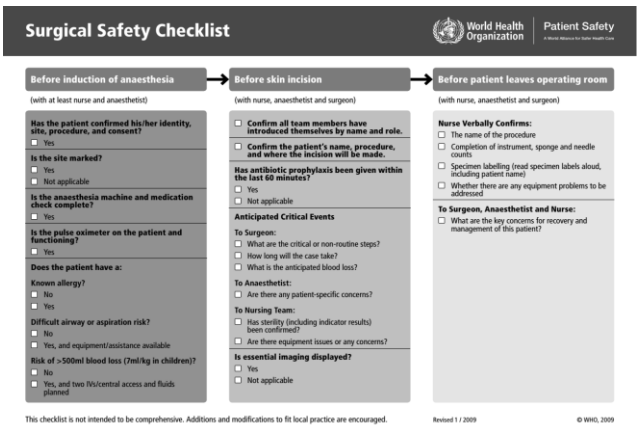

Figure 1: Copy of World Health Organization Surgical Safety Checklist [8]

The Intervention program

Implementation of the program was done by the researcher with the help of hospitals administration as well as the head nurse of each OR. The program was passing through three phases: planning, implementation, and evaluation phases. The Planning Phase was of 6 months duration and included obtaining the official permissions, arrangements with the quality department of the hospital which is responsible for the patient safety, as well as the infection control department and preparation of the educational program for the OR team.

The implementation Phase was of 9 months duration. The educational program was designed according to the baseline assessment data in the following designs: Booklets designed for each speciality (surgeons, anaesthetists and nurses), Powerpoint presentations, Brochures, and Videos. It was tested before actual implantation, then revised, edited and finally conducted. The lectures contained several activities to engage learners such as videos and discussions as well as groups to work to answer some questions that had been answered wrongly in the pre-intervention questionnaire. The practical part was designed to apply patient safety and infection control practices. This part included a simulation of these practices inside the OR.

Finally, the evaluation Phase was of 6 months duration. After the implementation of the educational program by 3 months a post-test questionnaire and observational checklist were used to assess the change in knowledge and practices of participants respectively regarding patient safety and infection control, the results were then analyzed to determine the impact of the intervention program.

Ethical Considerations

The study was approved by the research ethics committee of the Faculty of Medicine, Cairo University in November 2016. Agreements from the responsible authority (Port-Said General Hospital) were obtained. Confidentiality of the collected data was guaranteed. Participants were informed that responding is voluntary and that they can withdraw without stating any reason. Aims of the research were achieved without disturbing the harmony of work rhythm. Feedback about the results was given to the responsible authority at the end.

Data Management and Statistical Analysis

All collected data were assorted into pre- and post-intervention. Statistical analyses were performed using the SPSS program, IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp. Analytic statistics was applied to compare the general characteristics of the 3 studied groups: surgeons, anaesthetists, and nurses. Each item was assessed through a group of questions. Then the right answers were calculated and divided by total answers of the item, so each item had a percentage from 0% to 100% according to the right answers for each participant, before and after the intervention.

Fisher's test was used for comparing categorical variables, Kruskal-Wallis test for unrelated numerical variables and Wilcoxon test for related numerical variables. P values below 0.05 were considered statistically significant. Box and whisker plots were used to compare totals of knowledge and practice scores before and after the intervention. The change difference percentage was calculated as follows: the pre-intervention number was subtracted from the post-intervention number, and then the outcome was divided by the pre-intervention number and multiplied the answer by 100. The change difference percentage was compared between the 3 studied groups: surgeons, anaesthetists, and nurses, using the Kruskal-Wallis test.

Results

The general characteristics of the OR team revealed that the average age was 33±5 years for

surgeons, 43±11 years for anaesthetists and 31±9 years for nurses. The anaesthesia team had significantly older age, more hours of working inside ORs as well as the number of operations per month than the rest of OR teams (Table 1).

Table 1: General characteristics of the OR team (n = 72)

General characteristics		Surgeons (n = 48)	Anasthesiatists (n = 8)	Nurses (n = 16)	*P-value*
Age(years)	Mean ± SD	33 ± 5	43 ± 11	31 ± 9	*0.01**
Gender Freq. (%)	Male	39 (81.3)	7 (87.5)	2 (12.5)	*0.001**
	Female	9 (18.8)	1 (12.5)	14 (87.5)	
Surgeon speciality Freq. (%)	General		9 (18.8)		
	Orthopaedic		18 (37.5)		
	Gynaecology		15 (31.3)		
	ENT		2 (4.2)		
	Neurosurgery		4 (8.3)		
OR working experience(years)	Mean ± SD	6 ± 5	17 ± 12	8 ± 9	*0.055*
OR days/week	Mean ± SD	4 ± 1	5 ± 2	4 ± 6	*0.172*
OR hours/day	Mean ± SD	6 ± 2	10 ± 3	5 ± 2	*0.001**
Operations No/month	Mean ± SD	32 ± 24	49 ± 6	15 ± 8	*0.001**

* *P*-value statistically significant at < 0.05.

All the OR team showed a statistically significant increase in knowledge and practices of different items of patient safety after the intervention (P-value < 0.05) except for anaesthetists of item of knowledge: factors inside OR could lead to medical errors, as their knowledge of this item was 100% before as well as after the intervention (Table 2).

Table 2: Comparison of Patient safety Knowledge and practices before and after the intervention

Knowledge of patient safety		Surgeons (n = 48)		Anesthetists (n = 8)		Nurses (n = 16)	
		† Pre-	†† Post-	† Pre-	†† Post-	† Pre-	†† Post-
Factors inside OR could lead to medical errors	Median (Min-Max)	100 (25-100)	100 (100-100)	100 (100-100)	100 (100-100)	87.5 (25-100)	100 (100-100)
	P-value	*0.011**		*1*		*0.009**	
Types of medical errors inside OR	Median (Min-Max)	57.1 (0-100)	100 (71.4-100)	93 (85.7-100)	100 (100-100)	78.5 (28.6-100)	100 (85.7-100)
	P-value	*0.001**		*0.046**		*0.001**	
Reporting medical errors when happen	Median (Min-Max)	0 (0-100)	100 (25-100)	0 (0-100)	100 (50-100)	50 (0-100)	100 (50-100)
	P-value	*0.001**		*0.02**		*0.026**	
Practices of patient safety		† Pre-	†† Post-	† Pre-	†† Post-	† Pre-	†† Post-
Before inducing anaesthesia (sign in)	Median (Min-Max)	---- ----	---- ----	92.5 (90-95)	97.5 (95-100)	75 (75-75)	100 (75-100)
	P-value	----		*0.011**		*0.001**	
Before skin incision (time out)	Median (Min-Max)	0 (0-25)	50 (12.5-87.5)	50 (50-50)	100 (50-100)	0 (0-57)	57 (28.6-86)
	P-value	*0.001**		*0.014**		*0.001**	
Before patient leaves OR (sign out)	Median (Min-Max)	0 (0-0)	50 (50-66.6)	33.3 (33.3-33.3)	66.7 (66.7-66.7)	0 (0-50)	66.7 (66.7-66.7)
	P-value	*0.001**		*0.005**		*0.001**	

† Pre-: Pre-intervention; †† Post-: Post-intervention; * *P*-value statistically significant at < 0.05.

The total knowledge of patient safety (Figure 2), as well as the total practices of patient safety (Figure 3), showed a statistically significant increase after the intervention (P-value < 0.05).

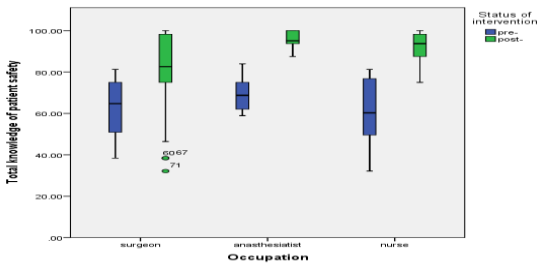

*Figure 2: Total of patient safety knowledge before and after the education program among OR team; * P-value is statistically significant at < 0.05*

All the OR team showed a statistically significant increase in knowledge of different items of infection control after the intervention (P-value < 0.05) except for nurses of item: prevention of transmission of blood-borne pathogens (BBP).

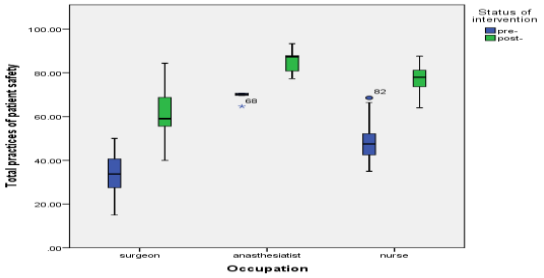

*Figure 3: Total of patient safety practices before and after the education program among OR team; * P-value is statistically significant at < 0.05*

There was no statistically significant increase in their knowledge for this item (P-value > 0.05) (Table 3).

Table 3: Comparison of infection control knowledge and practices before and after the intervention

Knowledge of infection control		Surgeons (n = 48)		Anesthetists (n = 8)		Nurses (n = 16)	
		† Pre-	†† Post-	† Pre-	†† Post-	† Pre-	†† Post-
Surgical hand washing	Median (Min-Max)	37.5 (25-75)	62.5 (50-87.5)	56.3 (50-75)	81.3 (62.5-87.5)	37.5 (12.5-62.5)	62.5 (37.5-87.5)
	P-value	*0.001**		*0.009**		*0.001**	
Personal protective equipment (PPE)	Median (Min-Max)	44.4 (22-66.6)	55.6 (11-66.6)	61.1 (55.5-66.6)	50 (44.4-66.6)	44.4 (0-55.6)	55.5 (11-66.6)
	P-value	*0.001**		*0.014**		*0.001**	
Prevention of blood borne pathogens (BBP)	Median (Min-Max)	83.3 (0-100)	100 (16.7-100)	0 (83.3-83.3)	100 (100-100)	83.3 (50-100)	100 (33.3-100)
	P-value	*0.001**		*0.005**		*0.617*	
Practices of infection control		† Pre-	†† Post-	† Pre-	†† Post-	† Pre-	†† Post-
Surgical Hand Washing Techniques	Median (Min-Max)	38.5 (38.5-38.5)	76.9 (38.5-92.3)	30.8 (0-30.8)	50 (0-85)	33.4 (38.4-38.4)	77 (38-92)
	P-value	*0.001**		*0.039**		*0.001**	
PPE: Gowning Techniques	Median (Min-Max)	80 (80-80)	80 (80-80)	80 (80-80)	80 (80-80)	80 (80-80)	80 (80-80)
	P-value	*1*		*1*		*1*	
PPE: Gloving Techniques	Median (Min-Max)	100 (100-100)	100 (100-100)	100 (100-100)	100 (100-100)	100 (100-100)	100 (100-100)
	P-value	*1*		*1*		*1*	
Techniques of Prevention of BBP	Median (Min-Max)	0 (0-100)	100 (0-100)	0 (0-0)	100 (0-100)	66.6 (33.3-100)	83.3 (66.6-100)
	P-value	*0.001**		*0.025**		*0.002**	

† Pre-: Pre-intervention; †† Post-: Post-intervention; * *P*-value statistically significant at < 0.05.

The total knowledge of infection control (Figure 4) showed a statistically significant increase after the intervention (P-value < 0.05).

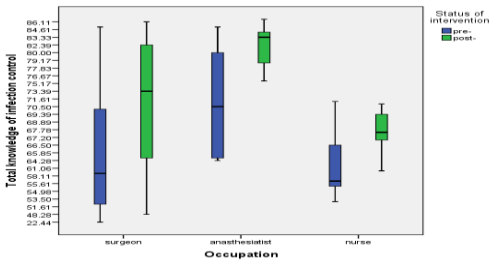

*Figure 4: Total of infection control knowledge before and after the education program among OR team; * P-value is statistically significant at < 0.05*

The total practices of infection control (Figure 5) showed a statistically significant increase after the

intervention (P-value < 0.05).

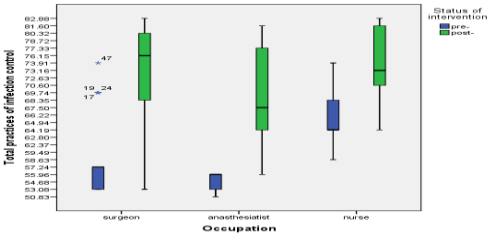

*Figure 5: Total of infection control practices before and after the education program among OR team; * P-value is statistically significant at < 0.05*

The change difference percentage of Factors inside OR could lead to medical errors was statistically significantly higher among nurses than the other OR team as well as before patient leaves OR (sign out) among anaesthesiologists and the total practices of patient safety among surgeons (P-value < 0.05). The knowledge of surgical handwashing and personal protective equipment (PPE) were higher among nurses, while the total knowledge of infection control was higher among surgeons (P-value < 0.05) (Table 4).

Table 4: Comparison of change difference percentage in knowledge and practices of patient safety and infection control among the studied participants

Knowledge of Patient safety *Percent Change difference (%)*		Surgeons (n = 48)	Anesthetists (n = 8)	Nurses (n = 16)	*P-value*
Factors inside OR could lead to medical errors	*Median (min-max)*	0 (0-300)	0 (0-0)	16.7 (0-300)	*0.002**
Types of medical errors inside OR	*Median (min-max)*	50 (16.7-250)	8.3 (0-16.7)	18.3 (0-250)	*0.112*
Reporting medical errors when happen	*Median (min-max)*	0 (0-100)	0 (0-0)	0 (0-100)	*0.668*
Total	*Median (min-max)*	27 (4-160)	45 (8-59)	38 (8-191)	*0.78*
Practices of Patient safety Percent Change difference (%)					
Before inducing anaesthesia (sign in)	*Median (min-max)*	------	5 (0-11)	33.3 (0-33.3)	*0.085*
Before skin incision (time out)	*Median (min-max)*	250 (50-300)	100 (0-100)	50 (0-100)	*0.08*
Before patient leaves OR (sign out)	*Median (min-max)*	0 (0-0)	100 (100-100)	33.3 (33.3-33.3)	*0.003**
Total	*Median (min-max)*	87 (21-270)	25 (11-32)	66 (9-87)	*0.001**
Knowledge of Infection Control Percent Change difference (%)					
Surgical hand washing	*Median (min-max)*	66.7 (33-250)	40 (16.7-50)	66.7 (20-600)	*0.033**
Personal protective equipment (PPE)	*Median (min-max)*	20 (17-25)	20 (20-20)	20 (20-33)	*0.006**
Total	*Median (min-max)*	23 (1.96-115)	12 (4.5-25)	14 (2.89-27)	*0.004**
Practices of Infection Control Percent Change difference (%)					
Surgical Hand Washing Techniques	*Median (min-max)*	100 (0-140)	150 (0-175)	120 (0-140)	*0.156*
Total	*Median (min-max)*	29 (0-48)	31 (2-46)	13 (8-23)	*0.071*

* *P-value* significant at < 0.05.

Discussion

This quasi-experimental study (Pre-post intervention design) was conducted to assess the effects of a comprehensive educational program about patient safety and infection control in OR on the following main outcome measures: knowledge and practice of OR team**.** All the OR team in our study showed improvement in both total knowledge and total practices of patient safety and infection control after the educational program than before. This finding goes in conformity with several previous studies which investigated surgical team performance before and after implementation of educational interventions [4], [5], [21], [22].

The checklist is a framework on which attitudes toward teamwork and communication can be encouraged and improved [4]. Besides, encouraging customisation of the checklist to fit the needs of the team identified from the baseline assessment, may promote a feeling of ownership over the checklist, increasing compliance along the way, that's how our newly modified checklist showed a high compliance and improved adherence among the OR team [23], [24].

Optimal training of OR team requires a program that focuses on the cognitive elements as well as the technical skills, that are essential to providing safer patient care [25]. Our comprehensive educational program about patient safety and infection control that included knowledge rather than only practices, could, therefore, achieve the requisite knowledge and skills, with the resulting improvement.

This improvement after educational programs could be more explained by the following facts: 1) educational visits combined with other complementary interventions including; booklets, brochures, and videos, are more effective than no intervention, 2) a personal visit by a trained person to a health provider in his or her own set is better than inviting them to other settings like educational halls and on times rather than work time with such burden of extra time and effort, 3) using more than one educational outreach visit is more effective than using only one visit and 4) the support obtained from the hospital's administration as well as IC and quality teams, and finally the intense help of OR head nurse in implementing the checklist items into the routine work inside ORs. In addition to the WHO, surgical safety checklist, which is designed in a concise to the point built and facilitated the compliance with most of the items [21], [26].

Our educational program included also training sessions on patient safety and infection control practices in the practical part. This part included a simulation of these practices inside the OR. The usefulness of simulation in OR team training is recognised, with proven validity and noticeable transfer of skills to the clinical setting. Simulation can prepare the OR team for actual practice, so it has the potential to improve both patient safety and the whole OR environment. Generally, the simulation technique of the OR environment can help the development of non-technical skills and aid in preparing OR teams for uncommonly faced situations and emergencies [27], [28].

However, certain individual items of patient safety and infection control showed various degrees of improvement than the total. OR nurses showed an

increase in knowledge after the intervention of all the items except for the prevention of BBP. They believe that prevention of BBP is the responsibility of central sterilisation unit (CSU), and they only transmit and properly handling the sterile instruments as much as they can [29]. This conflict in knowledge as regarding the prevention of BBP is the result of that studying the responsibility of central sterilisation unit was beyond the scope of this study, and we only studied all the patient safety and infection control when only the patient is inside OR ready for the planned procedure.

All the OR team showed improvement in infection control practices, mainly of surgical handwashing techniques. However, before the intervention, many of the OR team declared of the complexity of sterile gowning techniques special precaution of not exposing their hands outside the traditional sterile field; outside the cuff of gown allowing just fingertips to touch the proximal boundary of the cuff. Scrubbing cannot completely sterilise the skin but will decrease the bacterial load and risk of wound contamination from the hands. So, during gowning, it must be ensured that no part of the hand protrudes out of the cuff [18], [30].

Our study revealed that surgeons improved more as regarding the component of before patient leaves OR (sign out), the total practices of patient safety and the total knowledge of infection control. Surgeons are the ablest members to give orders to all the team members, and they are tasked with correcting any error that may occur and prevent it during surgery [31]. They are in an exquisite situation to empower distinctive behaviours to ameliorate teamwork in the intraoperative setting and eventually, patient outcomes. Moreover, the fear of surgical site infection is the main motivation for the study of infection control in surgical procedures which makes surgeons of a better knowledge more than others in this matter. Prevention remains of utmost importance [5].

The component of before patient leaves OR (sign out) showed more improvement among anaesthesiologists. This part of the checklist is to be completed before removing the patient from the operating room with the aim is to facilitate the transfer of important information to the care teams responsible for the patient after surgery [8]. Intraoperative transfers of patient care and responsibilities among anaesthesia caregivers, that is, handovers, are relatively frequent. Lost critical information during handovers may result in delays, inefficiencies, suboptimal care, or patient harm [32].

Improvement of factors inside OR could lead to medical errors, knowledge of surgical handwashing and personal protective equipment (PPE) were higher among nurses than the other OR team. Nurses are responsible for the day-to-day smooth running of OR activities and management. Therefore they are better in observing medical errors inside OR and the factors could lead to them [32]. Operating room management including not only patient safety and medical errors, but also operative considerations of infection control [33].

In conclusion, the educational program based on the WHO surgical safety checklist with modifications and additions of more infection control items to fit local practice, being given according to the occupation and role inside OR, together with intervening knowledge too, have led to improvement in both total knowledge and total practices of patient safety and infection control among the OR team; surgeons, anesthesiologists and nurses.

Conducting similar studies but with assessing OR team compliance about the rate of surgical complications in the form of postoperative morbidity and mortality. Also, conducting similar studies to improve special practices of patient safety and infection control in other critical areas like critical care units.

Study Limitations: All the OR team were not gathered except for the time of their scheduled operations, so the educational program was delivered individually not in groups.The enhancement in performance due to participants' knowledge of being observed that modified their behaviour from what it would have been without that knowledge, Hawthorne effect. Data collection was restricted to major surgical operations and didn't include emergency surgical operations. To implement the checklist; it had to be introduced as a formal document and compulsory step to go forward in the surgery which was not available authority for us.

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Public Health

The Cost Prediction for Chromium Detox Using Foods Intake Containing Glutathione at the Leather Tanning Industry in Magetan, Indonesia

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Abstract

BACKGROUND: Chromium was an organic compound which was used in metal alloys such as stainless steel, chrome plating, metal ceramics, leather tanning, etc. To reduce and eliminate toxin of chromium from the human body could be using detoxification process, one of them was using foods.

AIM: The aim was to calculate the foods intake contain glutathione to improve chromium detoxification and calculate the cost of foods intake.

METHOD: The type of research was a descriptive study. The subjects were 10 workers. Inclusion criteria of subjects were workers who had worked for more than or equal to 10 years. Variables were body weight, duration of work, and chromium concentration. After getting all variables above, breathing rate and intake non-carcinogen per respondent can be calculated. Then, the effective dose of foods rich in glutathione and costs of foods will be obtained.

RESULTS: The results of this research indicated that the highest cost of foods intake containing glutathione was 5948 idr of broccoli per week and the lowest cost of foods intake was 535 idr of avocado per week.

CONCLUSION: Intake of foods containing glutathione (avocado, broccoli, carrot, tomato, and grape) was expected to increase detoxification of chromium. Each individual had a different amount of cost. This depends on the effective dose, chromium concentration, weight, and duration of work per respondent.

Introduction

The development of the industry is quite rapid in recent times. The industry has various work activities such as the use of equipment, the use of machinery, the use of chemicals as raw materials, and the production of chemical emissions, and so on. The use of chemicals, especially hazardous chemicals, can certainly provide a threat at work. This can be a potential source that can trigger danger to the health and safety of workers [1]. One of the chemicals that are dangerous or carcinogenic is chromium. Chromium is one of the chemicals that use in the leather tanning industry. The leather tanning industry is an industry that produces finished leather that can be used as an ingredient in making sandals, shoes, bags, jackets and more. One of the processes in making leather is the tanning process. In this process, it aims to change raw skin that is easily damaged due to the activity of microorganisms into tanned skin that is more resistant, using chemicals. Chemical substances are often used in the tanning process, one of which is chromium sulfate ($Cr_2(SO_4)_3$). Chromium is chosen because it provides advantages and eases in the process, is better, and more durable [2]. Also, tanned skin using chromium has several advantages because it is suitable for the production of a variety of leather goods and has better compatibility with chemicals for the process of retaining and fatliquoring

[3]. Chromium tanned leather also has high tensile strength, is weaker, more durable to high temperatures, and gives good results on painting and has high hydrothermal stability.

Mineral skin tanning consists of 3 stages, namely: Beam House, Tanning, and Finishing. The raw materials used are animal skin (cows, buffaloes, goats, etc.), especially the results of slaughterhouses. Beam House Process (Pre Tanning), which includes dyeing skin into the water for one night to remove blood, dirt, salt solution, and protein. Then remove hair and skin parts that are not needed by immersion in lime, and sodium sulfide as a skin swelling material. Shaving and removing extra tissue from the flesh and skin, then separating using lime 2/3 the top layer from the bottom. Then the lime is removed using lactic acid and eradication uses auxiliary chemicals to remove leftover hair and broken proteins. Preservation uses saline and sulfuric acid to a certain pH to prevent precipitation of chrome salts on the skin fibres. Second is the Tanning Process. Chrome tanning is done using chrome sulfate. This process is useful for stabilising protein (collagen) tissue from the skin. Tanning aims to change the raw skin that is clean, which has an unstable nature into tanned skin that has a stable nature. The last is the Finishing Process (post tanning). This process includes pressing (slamming) to remove moisture from fresh skin, shaving, colouring and softening tanned skin using oil emulsion (fat liquoring), final drying and shaving, and surface coating and buffing (finishing) [4].

Chromium (Cr) is a metal that is grey, shiny, hard and brittle that requires high polishing, durability, and has a high melting point. This metal is one of the toxic heavy metals. Its toxicity depends on the valence of the ion. By its nature, chromium metal has an oxidation number of +2, +3, and +6. At Cr^{+2} it forms an alkaline compound, the compound formed from Cr^{+3} ions has an importer, and Cr^{+6} ions are more acidic. In the case of chronic ionic acid (CrO_4^{-2}), it can cause a very strong reduction event. Chromium is widely used for industrial activities such as electroplating, leather tanning, and painting of chromate and dichromate compounds [5]. And the toxicity of Cr^{+6} is equal to one hundred times the toxicity of Cr^{+3}. Besides, Cr^{+3} is also highly corrosive and carcinogenic [6].

There are many types of chromium ions, but chromium (III) sulfate is used in this study. The chrome compound (Cr) in the tannery industry wastewater comes from the leather tanning production process, in which tanning using chromium sulphate compounds between 60% -70% in the form of chromium sulfate solution can not all be absorbed by the skin during the tanning process so that the remainder is released in the form of liquid as liquid waste [7]. Hexavalent chrome (Cr^{+6}) from the leather tanning industry is usually in the form of chromate (CrO_4^{2-}). Chromatic poisoning can cause irritation to the skin, accumulate in the liver, and systemic poisoning. Chromate vapour when inhaled can cause infection (inflammation) in the respiratory tract and lung cancer, and skin damage by chrome salts [8]. Chromium has a role in the body. In humans and animals, chromium in the lower concentration is an essential micronutrient, but in high concentrations, it can cause carcinogens. Chromium can be called heavy metal because, in the long run, it can cause a health disorder such as allergic to cancer in humans. Accumulation of heavy metals can cause interactions between heavy metals can result in interactions between heavy metals with cells or body tissues. The toxic nature of chromium can cause acute poisoning and chronic poisoning. These metals and their compounds can interfere with the function of organs that work in metabolic processes when they enter the human body.

To reduce and even eliminate toxins in chemical compounds in the body, a biotransformation process is needed. Biotransformation is a change in the toxin catalysed by certain enzymes in living things. The purpose of biotransformation is to convert non-polar to polar, then to become hydrophilic so that it can be excreted out of the body. Biotransformation occurs in two phases. The first phase is the functional phase where the functional group matches the oxidation, reduction and hydrolysis reactions. Then the second phase is the conjugate reaction phase involving several types of endogenous metabolites in the body in the endoplasmic reticulum [9].

Research using foods approach as chromium detoxification is still very limited. Foods containing glutathione such as avocado, broccoli, carrot, tomato, and grape [10], [11], [12]. But there has never been researching that explains how much intake of these foods is needed to improve chromium detoxification, especially in a population that exposed to chromium in a long time. Based on the background above, this research aims to calculate the intake of foods containing glutathione (avocado, broccoli, carrot, tomato, and grape) are needed (effective dose) to detoxify chromium at leather tanning industry workers in Magetan and to calculate the cost of foods intake containing glutathione to detoxify chromium.

Material and Methods

The type of research was a descriptive study. Subjects were workers in the leather tanning industry in Magetan. The inclusion criteria were all workers who had worked in this industry for more than or equal to 10 years, workers who worked on the tanning process using chromium, and willing to be used as research respondents. The sample of this research was 10 respondents. The research location was in the Technical Implementation Unit of the Leather Products Industry in Magetan.

Research technique started from collecting secondary data such as work processes that include the number of workers involved. While the primary data includes the worker's weight, duration of working (years), working time per week (days), an average of working every day (hours), the concentration of chromium in the blood of workers. Measurement of chromium concentration was carried out at 10 respondents. Measurement of respondents weight using manual measurement method with body scales. Measurement of the duration of work, working time per week, and an average of working every day were obtained with an in-depth interview with respondents. Then, measurement of chromium concentration in the blood of workers using method by Atomic Absorption Spectrophotometer method. Blood collection of respondents was carried out by skilled medical personnel from a laboratory. Blood is taken through a vein that has been adopted using alcohol. Blood is taken as much as 5 ml and put in a tube containing Ethylene Diamine Tetra Acetate (EDTA) as a coagulant material so that the blood does not clot when going to the laboratory. Blood in the laboratory will be analysed using Atomic Absorption Spectrophotometer method.

After getting all variables above, it can be found breathing rate and intake non-carcinogen of chromium per respondent. Then, an effective dose of foods rich in glutathione would be obtained by manual calculating used the formula below:

$$dose\ effective\ of\ food\ intake = \left\{\left(intake\ nc\ x\ \frac{Mr\ enzyme}{Mr\ toxin}\right) - (C\ enzyme\ x\ 70)\right\}\ x\ \frac{100}{A}$$

Explanation:

Intake NC (non-carcinogen) = $\frac{C\ x\ R\ x\ tE\ x\ fE\ x\ Dt}{Wb\ x\ 30\ x\ 365}$

C: Chromium concentration (mg/m^3)

R: Breathing rate (m^3/hour)

Dt: Duration of working (years)

fE: Working time per week (days)

tE: Average of working time per day (hours)

Wb: Weight (kg)

C enzyme = $C\ enzyme\ (normal) x\ Mr\ enzyme$ (Tualeka, 2018)

Glutathione = $\frac{0.00000099\ mmol/ml}{307.32}$

A = Content of enzyme in 100 grams of the food

Glutathione

- Avocado: 31.2 mg
- Broccoli: 7.8 mg
- Carrot: 5.9 mg
- Tomato: 10.9 mg
- Grape: 14.6 mg

After getting all variables above, the cost of meeting food requirements containing glutathione released by each respondent to detoxify chromium can be calculated. The calculation of the cost of each food is calculated using the formula below:

$$cost\ of\ food\ intake = dose\ effective\ x\ cost\ of\ food/kg$$

Results

Distribution of Chromium Concentration at Workplace

Figure 1 shows that most locations of the workplace have chromium concentration above the threshold limit value (TLV). The TLV of chromium concentration at the workplace is 0.5 mg/m^3. The highest chromium concentration at the workplace is 1.7 mg/m^3, while the lowest is 0.32 mg/m^3. The average of chromium concentration at the workplace is 1.01 mg/m^3.

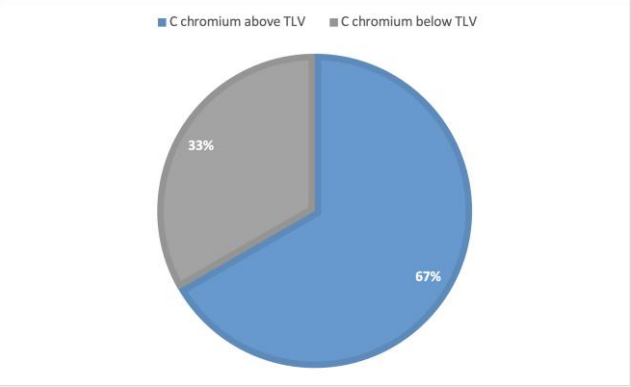

Figure 1: Distribution of Chromium Concentration at Workplace

Distribution of Chromium Concentration in the Blood of Workers

Figure 2 shows that all respondents have chromium concentration above the threshold limit value (TLV) in the blood. The TLV of chromium concentration in the blood is 1.6-5.1 mg/m^3. The highest chromium concentration in the blood of workers is 48.3 mg/m^3, while the lowest is 23.6 mg/m^3. The average of chromium concentration in the blood of workers is 36.15 mg/m^3.

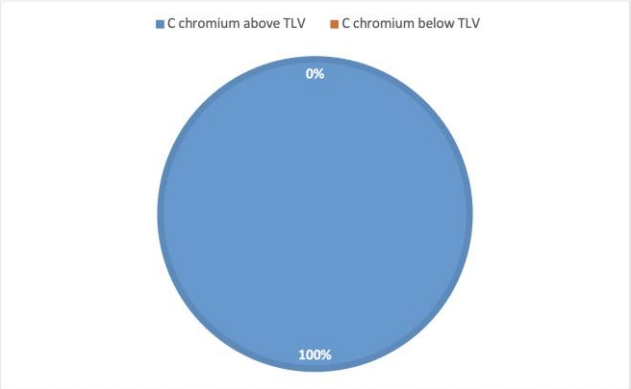

Figure 2: Distribution of Chromium Concentration in the Blood of Workers

Effective Dose of Foods Intake Containing Glutathione to Chromium Detox per Week

In Figure 3 shows that the highest effective dose of avocado, broccoli, carrot, tomato, and grape to chromium detox are 297.42 mg, 1190 mg, 1572.82 mg, 851.34 mg, and 635.6 mg (respondent 3). While the lowest effective dose are 89.18 mg, 356.75 mg, 471.63 mg, 255.28 mg, and 190.6 mg (respondent 1). The average of an effective dose of avocado, broccoli, carrot, tomato, and grape are 186.92 mg, 747.7 mg, 988.5 mg, 535 mg, and 399.5 mg.

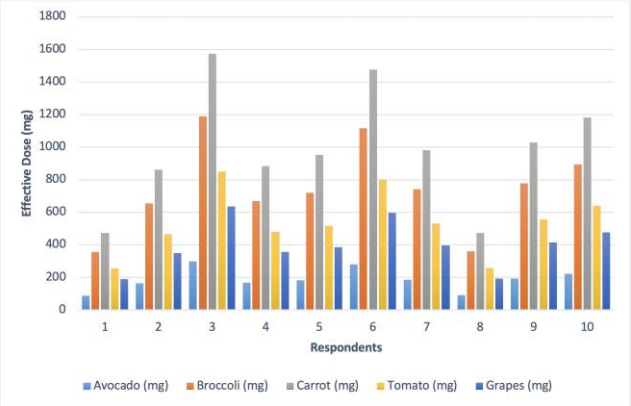

Figure 3: Effective Dose of Foods Intake Containing Glutathione to Chromium Detox per Week

The Cost of Effective Dose of Foods Intake Containing Glutathione to Chromium Detox per Week

A. *The Cost of Effective Dose of Avocado to Chromium Detox per Week*

Figure 4 shows that the highest cost of an effective dose of avocado to chromium detox is 1784 idr (respondent 3), while the lowest is 535 idr (respondent 1). The average the cost of an effective dose of broccoli to chromium detox on respondents is 1186 idr.

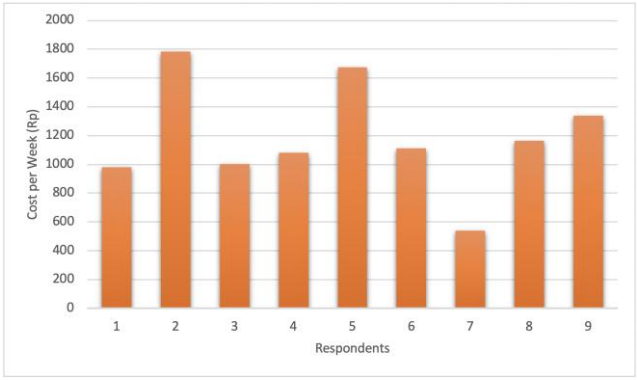

Figure 4: The Cost of Effective Dose of Avocado to Chromium Detox per Week

B. The Cost of Effective Dose of Broccoli Chromium Detox per Week

Figure 5 shows that the highest cost of an effective dose of broccoli to chromium detox is 5948 idr (respondent 3), while the lowest is 1783 idr (respondent 1). The average the cost of an effective dose of broccoli to chromium detox on respondents is 3738 idr.

C. The Cost of Effective Dose of Carrot to Chromium Detox per Wee

In Figure 6 shows that the highest cost of an effective dose of carrot to chromium detox is 3932 idr (respondent 3), while the lowest is 1179 idr (respondent 1). The average of the cost of an effective dose of carrot to chromium detox on respondents is 2471 idr.

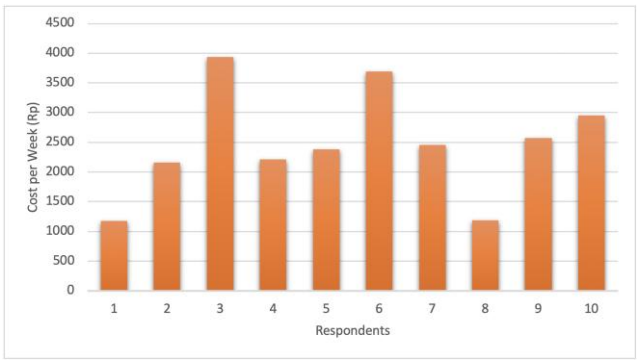

Figure 6: The Cost of Effective Dose of Carrot to Chromium Detox per Week

D. *The Cost of Effective Dose of Tomato to Chromium Detox per Week*

Figure 7 shows that the highest cost of effective dose of tomato to chromium detox is 2979 idr (respondent 3), while the lowest is 893 idr (respondent 1). The average of cost of effective dose of tomato to chromium detox on respondents is 1872 idr.

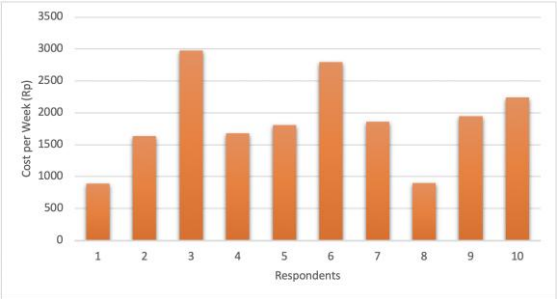

Figure 7: The Cost of Effective Dose of Tomato to Chromium Detox per Week

E. *The Cost of Effective Dose of Grape to Chromium Detox per Week*

Figure 8 shows that the highest cost of an effective dose of grape to chromium detox is 5084 idr (respondent 3), while the lowest is 1524 idr (respondent 1). The average cost of an effective dose of grape to chromium detox on respondents is 3195 idr.

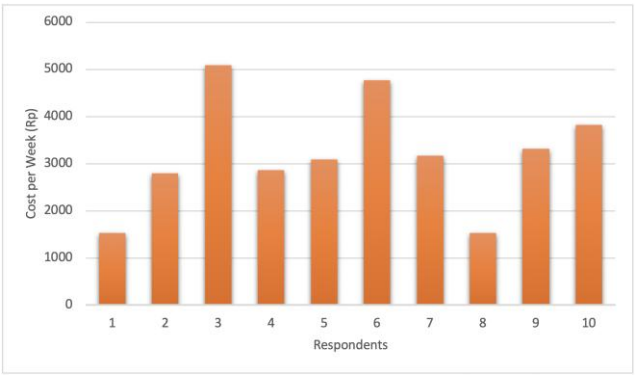

Figure 8: The Cost of Effective Dose of Grape to Chromium Detox per Week

Discussion

Detoxification is the process which toxic compounds to less toxic. The detoxification process becomes non-toxic eliminated through urine and bile [13]. Toxin detoxification is important in modern times. Many toxins are spread on this earth, such as in the sea, rivers, food, drinks, and objects around us. Detoxification is very important to be done to remove harmful or toxin chemicals in the body. Detoxification can be done foods approach. Food-based nutrition continues to be investigated for its role in modulating the metabolic pathways involved in the detoxification process. Several publications that have used cells, animals and clinical studies show that food-based components and nutrients can modulate the process of conversion and excretion of toxins from the body [14]. In this research, chromium detox can be done foods approach with foods that rich in glutathione, such as avocado, broccoli, carrot, tomato, and grape (Dhivya, 2012) [10], [11], [12]. Glutathione is a potent antioxidant compound and detoxifying agent that is produced in the cytoplasm of every cell of the human body. In broad terms, these studies have found glutathione to protect against oxidative stress, detoxify chemicals and toxins, boost immune function, and support healthy ageing. One of the toxins that can be detoxified is chromium [15].

The main function of Cr is to increase insulin activity inside glucose metabolism and to maintain the speed of glucose transport from the blood into the cell. Cr also plays a role in activating the work of several enzymes. Cr deficiency causes impaired glucose tolerance (Glucose Tolerance). More severe deficiencies will result in disrupted growth, hyperglycemia (hyperglycemia), glycosuria (glycosuria) and increased levels of serum cholesterol. The GTF structure is composed of a complex between Cr^{3+} and 2 nicotinic acid molecules and 3 amino acids contained in glutathione, i.e. glutamate, glycine and cysteine. Chromium is biologically active as a component of GTF which increases cell and tissue sensitivity to glucose and insulin use, in the absence of inactive chromium GTF [16].

Based on the diagram analysis, the results show that each individual has a different cost. This is because each individual has a different effective dose of foods. The effective dose of foods can also depend on the amount of chromium concentration, weight and length of work of workers. The higher the concentration of chromium in the body, the greater the mass of detox for the foods is needed. This is consistent with the formulation that has been made in previous studies which states that it has a synergistic relationship with substance concentration [17]. Bodyweight, length of work, and chromium concentration can affect the intake of non-carcinogens in each respondent which can affect the effective dose of foods. This is following previous research which mentions that genetic variances, gender, and maybe body weight can play a role in biotransformation enzymes [18]. By knowing foods that can be used to detoxify chromium exposure from the body, leather tanning workers who have a high risk of exposure of chromium can prevent this exposure. Also, knowing the estimated costs that will be incurred to prevent chromium exposure through these foods, workers can choose foods that can detoxify chromium in the body with foods that are in line with the income of the leather tanning workers in Magetan.

In summary, intakes of foods containing glutathione (avocado, broccoli, carrot, tomato, and grape) were expected to increase detoxification of chromium. Each individual had a different amount of cost. This depends on the effective dose, chromium concentration, weight, and duration of work per respondent.

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Thoracic Kyphosis Angle in Relation to Low Back Pain among Dentists in Iran

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Abstract

BACKGROUND: Non-specific low back pain (LBP) has a direct impact on the quality of life, active days at work and health care costs.

AIM: This study was conducted to determine the relationship between LBP and thoracic kyphosis angle among dentists.

MATERIAL AND METHODS: This cross-sectional and descriptive-analytical study carried out in the form of census among 84 dentists employed in a specialised clinic in Iran. Dentists LBP prevalence and intensity and thoracic kyphosis angle were evaluated respectively with the self-administered body map questionnaire, visual analogue scale and flexicurve ruler. Statistical data analysis was done using SPSS software, version 22.

RESULTS: The data showed that the prevalence of LBP in dentists was 44.9% and intensity of LBP was reported about 71.9 ± 19.34. Pearson correlation coefficient between thoracic kyphosis angle and dentist's characteristics was not significant except for work experience. The single linear regression model showed that 1.3% of thoracic kyphosis angle changes was positively dependent on LBP. Also, the present study proved that thoracic kyphosis angle changes were positively dependent to 2.6%, 10.8% and 5.7 percent of age, work experience and Body Mass Index (BMI), respectively.

CONCLUSION: Despite the lack of significant statistical relation between LBP and thoracic kyphosis angle, there is a high prevalence and intensity of LBP among Dentists. To reduce the risk of work-related LBP among dentists, managing factors such as BMI, work postures and exercises during work should be taken into consideration.

Introduction

Musculoskeletal disorders are the most common and most costly occupational injuries; because one-third of work-related injuries in each year and approximately half of all work-related illnesses account for musculoskeletal disorders as these disorders are considered being the hidden epidemic in the today world [1]. It is estimated that the direct and indirect costs of musculoskeletal disorders account for about of 1% the gross national product (GDP) of industrial countries [2], and are considered as the main causes of lost working time, increasing costs, and workforce damages [3], [4]. As well as, these disorders are one of the main causes of absenteeism in the workplace, so that according to previous studies, about of 40% of the compensation paid to workers was related to musculoskeletal disorders [5], [6].

Musculoskeletal disorders are prevalent among different occupations, and dentists are not excluded from this norm. In the dental profession, due to repetitive movements, long-term work in static situations, awkward postures, use of excessive force and inappropriate working tools, there is the probability of musculoskeletal discomfort in the form of pain in various areas of the body. So that, Shrestha et al., reported the highest prevalence of

musculoskeletal disorders among dentists in the lumbar (80%), neck (58.8%) and shoulder (47%) [7], and also according to Tim and colleagues study, the highest prevalence of these disorders was reported in the neck and shoulder with range of 37 to 72% and 11 to 35%, respectively [8]. Based on studies, more than 90% of dentists postures during work have moderate and high-risk levels, as they have the worst postures while performing some of their tasks, such as surgery, endometrium, etc., which require corrective actions [9], [10].

Thoracic kyphosis is the main curvature of the vertebral column, which consists of twelve vertebrae [11]. The increase in this curvature (arch) is due to changes in the intervertebral disc and height of the vertebra by reducing the anterior height of the vertebral body and the imbalance between the soft tissues and the anterior and posterior supporting muscles. Thoracic kyphosis is the increase of the thoracic spine curvature in the sagittal plane. Psychological agents such as sadness, depression, weakness and excitement can increase the kyphosis arch [12], [13]. Based on biomechanical information, thoracic kyphosis may be associated with increased pressure on muscles and muscle strength and accelerates the degeneration process, dysfunction, and pain [14]. Increased thoracic kyphosis is associated with decreased body function [15], impaired pulmonary function [16], and increased neck pain, headache and subacromial impingement pain syndrome [17], [18]. This abnormality is most commonly seen in women, for reasons such as muscle weakness, increased fat percentage, and repetition of false habits [19], [20]. Accurate, early and timely identification of these abnormalities can be effective in preventing and correcting them. Various instruments have been used to evaluation and diagnosis of these abnormalities, which include the spinal mouse, pantograph spinal, inclinometer, flexible ruler and kyphometer [21]. The standard method for measuring the thoracic kyphosis is Cobb's radiographic method, but because of exposure to radiation and costly, this method is not appropriate [22], [23]. Therefore, in this study, a flexible ruler was used to evaluate the thoracic kyphosis. The validity of the flexible ruler in measuring thoracic kyphosis has been proven in many studies abroad and inside our country [24], [25]. Considering the importance of prevention and treatment of musculoskeletal disorders among dentists, this study aimed to measure the thoracic kyphosis and its association with low back pain (LBP) intensity.

Methods

This cross-sectional study was conducted among all 84 dentists employed in a specialized dental clinic in Tehran, Iran, in 2018. The criteria for inclusion in the study were the lack of a history of spinal surgery and traumatic orthopaedic problems such as acute back and nerve problems, inflammatory diseases such as Ankylosing spondylitis involving the spine, congenital diseases such as scoliosis and hemivertebrae. Due to the inclusion criteria, 6 dentists were excluded from the study. In the first phase of this study, the prevalence and intensity of dentists LBP were evaluated with the self-administered body map questionnaire along with a visual analogue discomfort scale. In the second phase, the thoracic kyphosis angle was measured using the flexicurve ruler in dentists with LBP. All participants agreed to participate in this study, read and signed an informed consent form.

Body Map Questionnaire

To evaluate the prevalence of LBP, body map questionnaire was used [26]. In this questionnaire to facilitate understanding and position musculoskeletal disorders, the body was divided into 13 areas, including low back in the form of a map.

Visual Analog Discomfort Scale

To indicate the level of discomfort, the subjects were asked to mark the degree of subjective of LBP on a horizontal line of 100 mm long. The intensity of LBP was recorded numerically from zero (without discomfort) to 100 (severe discomfort) using a millimetre ruler. LBP intensity divided into mild (0-20), moderate (21-40), severe (41-60), disabling (61-80) and severe disabling (81-100).

Thoracic Kyphosis Angle Measurement

Thoracic kyphosis angle was measured in each subject using a flexicurve ruler (Ghamat Pooyan Co., Iran) based on Hoppenfeld's method, according to the distance of the spinous process of two reference bones, i.e. C7 and T12. The Hoppenfeld method was employed to find the two bone landmarks [27]. Finally, the angle between these two bone landmarks (C7 and T12) was calculated and reported as the thoracic kyphosis angle. Besides, the sensitivity and specificity of the flexicurve method for diagnosing hyper thoracic kyphosis were 85% and 97% respectively [28]. Also, the ICC of the flexicurve method obtained by Iranian studies, Khalkhali et al., [29] and Kargarfard et al., [30] was 0.97 and 0.99, respectively.

Statistical data analysis was done using SPSS (version 22.0, IBM Corporation, Armonk, NY, USA). Kolmogorov-Smirnov test was used to determine the normality of the data. Besides, we used the t-test, Mann-Whitney, Spearman correlation

coefficient, single and multiple linear regressions.

Results

In this study, a total of 84 dentists participating in this study, six dentists were excluded due to lack of inclusion criteria, and 78 dentists were examined. 55.1% of participants were male and 44.9% female. 24.4% of them were single and 75.6% married.

The mean age, work experience, weight, height, and body mass index (BMI) of dentists were equal to 38.47 ± 6.54 years, 13.29 ± 7.24 years, 75.12 ± 17.66 kg, 26 172.41 ± 9.9 cm and 24.98 ± 4.16 kg/m^2, respectively. Furthermore, demographic variables distribution of the studied dentists was reported in Table 1. There was no significant difference between demographic variables in two groups with and without LBP(P > 0.05).

Table 1: Distribution of dentist's demographic characteristics (n = 78)

Variable	Low Back Pain		P-value
	Yes	No	
Age (years)	38.7 ± 6.6	38.3 ± 6.5	0.772
Work experience (years)	13.9 ± 7.2	12.8 ± 7.3	0.471
Weight (kg)	78.4 ± 18.4	72.4 ± 16.7	0.136
Height (cm)	173.2 ± 9.5	171.7 ± 9.1	0.455
BMI (kg/m^2)	25.8 ± 4.3	24.3 ± 3.9	0.104

The prevalence of LBP in dentists was reported at 44.9%. The mean of LBP intensity in dentists was 71.9 ± 19.34. The results of the classification of the LBP intensity obtained from the visual analogue discomfort scale (Table 2) showed that the highest relative frequency among the subjects was related to disabling pain and severe pain, with 34.3% and 31.4%, respectively. The relationship between LBP intensity and angle of thoracic kyphosis was investigated among dentists with LBP by gender and marital status. The results of the Mann-Whitney U test indicated that there was no significant correlation between LBP intensity, gender, and marital status among dentists (P > 0.05). Also, independent sample t-test did not show a significant relationship between the mean angle of thoracic kyphosis and gender and marital status of the dentists (P > 0.05).

Table 2: Percent distribution of low back pain among dentists using the visual analogue scale (n = 35)

Low back pain (%)				
Mild	Moderate	Severe	disabling	Severe disabling
0	5.7	31.4	34.3	28.6

Table 3 illustrated the relationship between factors affecting on LBP in dentists using a single logistic regression. There was no significant correlation between LBP and demographic variables (P > 0.05). However, men had more the risk of LBP than women did. Likewise, the risk of LBP was higher in married than single. Dentists with high age or experience over 10 years had more LBP than others. The odds ratio of LBP was lower in dentists with exercise activity. With the increase in BMI, the odds ratio of LBP was increased. People with more daily work hours had a higher chance of LBP.

Table 3: Odds ratio and CIs for predictive factors of Low Back Pain in dentists (n = 78)

Variable		Frequency (%) of the total sample	Frequency (%) affected by LBP	Odds Ratio	95% Confidence intervals	P-value
Gender	Female	35 (44.9)	14 (40)	1	Reference	
	Male	43 (55.1)	21 (60)	1.432	0.581-3.531	0.436
Marital Status	Single	19 (24.4)	8 (22.9)	1	Reference	
	Married	59 (75.6)	27 (77.1)	1.160	0.408-3.298	0.781
Age (years)	< 40	53 (67.9)	24 (68.6)	1	Reference	
	40-50	22 (28.2)	10 (28.6)	1.655	0.141-19.386	0.688
	51-60	3 (3.8)	1 (2.9)	1.667	0.131-21.195	0.694
Work Experience (years)	< 10	27 (34.6)	9 (27.7)	1	Reference	
	10-20	35 (44.9)	18 (51.4)	2.118	0.749-5.986	0.157
	> 20	16 (20.5)	8 (22.9)	2.00	0.564-7.087	0.283
BMI (kg/m^2)	< 18.5	5 (6.4)	2 (5.7)	1	Reference	
	18.5-25	30 (38.5)	11 (31.4)	0.868	0.125-6.026	0.886
	25-30	36 (46.2)	17 (48.6)	1.342	0.200-9.019	0.762
	> 30	7 (9)	5 (14.3)	3.750	0.331-42.467	0.286
Exercise activity	No	30 (38.5)	15 (42.9)	1	Reference	
	Yes	48 (61.5)	20 (57.1)	0.714	0.285-1.788	0.472
Work habit	Left	12 (15.4)	6 (17.1)	1	Reference	
	right	66 (84.6)	29 (82.9)	0.784	0.229-2.686	0.698
Daily work hours	< 8 hours	21 (26.9)	8 (22.9)	1	Reference	
	≥ 8 hours	57 (73.1)	27 (77.1)	1.462	0.526-4.067	0.466
Weekly work hours	< 40 hours	23 (29.5)	10 (28.6)	1	Reference	
	≥ 40hours	55 (70.5)	25 (71.4)	1.083	0.406-2.888	0.873

Correlation between age, work experience and BMI of dentists was surveyed with the angle of thoracic kyphosis using the Pearson statistical test (Table 4), and the results showed that there is a significant relationship between work experience and thoracic kyphosis (P = 0.045).

Table 4: Correlation between Thoracic Kyphosis and dentist's characteristics (n = 35)

Variable	Age		Work experience		BMI	
	p-value	r	*p*-value*	r	*p*-value	r
Thoracic Kyphosis	0.101	0.282	0.045	0.340	0.170	0.237

*Pearson correlation test.

Regression models of the correlation between LBP and thoracic kyphosis exhibited in Table 5. Based on the linear regression equation, 1.3% of changes in thoracic kyphosis are dependent on the LBP. The correlation between LBP and thoracic kyphosis is a positive relationship, and somewhat LBP increases the thoracic kyphosis. Based on the multiple regression equation, 2.6%, 10.8%, and 5.7 percent of the thoracic kyphosis changes are positively correlated with age, work experience, and BMI, respectively, and 36.6 percent of changes in thoracic kyphosis is negatively related to gender.

Table 5: Linear regression models for correlates of low back pain and Thoracic Kyphosis

Variable	Adjusted R^2	P-value	Linear Regression Equation
LBP	0.686	0.702[a]	Thoracic Kyphosis = 38.293 + (0.013 × LBP)
LBP with age & work experience & BMI & gender	0.008	0.405[b]	Thoracic Kyphosis = 38.293 + (0.006 × LBP) + (0.026 × age) + (0.108 × work experience) + (0.057 × BMI)-(0.366 × gender)

[a] Single linear regression; [b] Multiple linear regression.

Discussion

The results of this study showed that dentists have a high LBP prevalence and pain intensity so that these results are consistent with the LBP prevalence results of similar studies among dentists, including Nadri et al., (LBP 34.5%) [31], Varmazyar et al., (LBP 42.9%) [32], Motemayel Ahmadi et al., (LBP 16.9%) [33], Askaripoor et al., (LBP 46%) [9], Pourabas et al., (LBP 22.9%) [34], Vakili et al., (40.6%) [35], Samat et al., (LBP 44.9%) [36] and Gaowgzeh et al., (LBP 70%) [37].

Investigating the factors affecting on LBP in dentists using single logistic regression showed that despite the lack of significant correlation between LBP and demographic variables; odds ratio of LBP in men were more than women and as well as married than single. Furthermore, with increasing the age and/or work experience (over 10 years), daily working hours, increase BMI and lack of exercise activity, the odds ratio of LBP has increased in dentists.

The result of this study is consistent with the result of Nadri et al., [31]. In a study by Mohseni-Bandpei et al., the higher LBP was reported among people with the work experience more than 20 years [38]. Also, Aasa et al., reported the higher prevalence of LBP in men than women [39], while Leijon and Mulder reported a higher prevalence of LBP in women than men [40].

Assessing the discomfort along with the LBP prevalence, is a useful tool to determine the effectiveness of ergonomic interventions before and after study. The mean LBP intensity was 70.5 ± 24, which was consistent with the previous results by the researcher [31], [41]. In the Nield-Gehrig study, more than 80% of dentists complained of upper trunk and back pain, which was a direct consequence of posture and movements of dentists in daily work [42]. As well as, in Gaowgzeh et al., 90.5% of dentists had mild-to-moderate LBP intensity, and 9.5% had severe LBP intensity [37].

In the sitting position (on the seat during work), the low back arch decreased, and it can prevent from LBP [43]. Dentists should change their working positions based on the muscles group involved during different activities [44]. So that changing sitting and standing situations can be an effective strategy. Dentists, who are working only in sitting position, experienced more LBP (due to static posture) than those with standing and sitting rotation position [45].

Based on the results of the Pearson test, thoracic kyphosis presented a significant correlation with work experience. Despite the lack of significant correlation between thoracic kyphosis with LBP and demographic variables in both single and multiple linear regressions, according to the results of single linear regression, 1.3% of changes in thoracic kyphosis were related to LBP. In multiple linear regression, changes in thoracic kyphosis have increased with increase work experience and BMI, and thoracic kyphosis in women has been higher. However, in multiple linear regressions, changes in thoracic kyphosis are not approximately related to the LBP.

Previous studies have revealed that gender is a risk factor for the intensity of LBP and thoracic kyphosis [46]. In the study of Kargarfard et al., the average degree of thoracic kyphosis was higher in boys than girls, and there was a significant difference [30]. The difference in reporting the results may be due to the demographic characteristics of individuals or studied population.

According to the results of previous studies, the use of preventive strategies such as proper desk, regular and tensile exercises, use of the helpful instruments, use of the assistant, suitable posture can be associated with a reduction in LBP intensity as well as thoracic kyphosis [38]. In Kazemi et al., study, the reason of the reduction of thoracic kyphosis angle in the subjects after a period of exercise with the physioball can be attributed to strengthen the muscles of the posterior region of the spine (spinal extensor muscles) through strength and endurance exercises; as well as flexural exercises in the thoracic area [47]. Hrysomallis and Goodman [48] and Choi et al., [49] showed that the thoracic kyphosis is reduced by increasing the strength of the back extensor muscles through exercise. There also is a negative correlation between the back extensor muscles strength and thoracic kyphosis [50], [51]. The positive effects of physical activity with Physioball on the physiological and physical factors are well known.

Moreover, exercise with physio ball is a reliable way to achieve relaxation and happiness. So ergonomic training courses, back support programs, and exercise programs before and during work recommended to strengthen the musculoskeletal system and improve the musculoskeletal disorders in the work environment of dentists. It can be noted that the being cross-sectional and small sample size are the limitations of this study.

In conclusion, despite the lack of significant statistical relation between LBP and thoracic kyphosis angle, there is a high prevalence and intensity of LBP among Dentists. To reduce the risk of work-related LBP among dentists, managing factors related to LBP such as BMI, work postures and relaxation and stretching exercises during dentists' work should be taken into consideration. In addition to the above measures, effective training and ergonomic interventions to correct inappropriate work, postures can play an important role in preventing and managing MSDs. Additional research is required to determine the thoracic kyphosis angle about LBP in dentists.

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Epidemiological Study of Cutaneous Leishmaniasis in Neyshabur County, East of Iran (2011-2017)

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Abstract

BACKGROUND: Cutaneous Leishmaniasis (CL) isn't a lethal disease, but it has always been taken into consideration due to more involvement of patients with skin ulcers and its long-term treatment. Various factors can play an intervening role in increasing the rate of disease. The present study aimed to evaluate the prevalence and associated factors of disease from 2011-2017 and provide appropriate control strategies for reducing its incidence in Neyshabur county.

METHODS: All patients with CL, who had medical records in the health centres of Neyshabur from 2011 to 2017, were examined for conducting this cross-sectional analytical study. Data were analyzed by descriptive statistics and chi-square test at a 0.05 using Statistical Package for the Social Sciences (SPSS V.22).

RESULTS: Findings indicated that the highest annual incidence was in 2016 (229 patients), and the least incidence was in 2014 (100 patients). The majority of patients were under 10 years of age and 51.7% of patients were male. About 59.5% of patients were living in cities and 35% of them were living in North of Neyshabur city. Hands were the most affected part of the body (56.0%) followed by trunk (1.3%). Most patients (69.9%) were treated with topical regimens.

CONCLUSION: This study showed that CL was hypo-endemic in Neyshabur. Also, the disease was more prevalent in urban areas. Therefore, appropriate health measures to improve environmental conditions, public health educations, and the public awareness of the positive impact of early diagnosis of disease in the success of treatment (especially for inhabitance suburbanite) are essential.

Introduction

Leishmaniasis is a vector-borne and zoonotic disease [1] and is considered a neglected illness [2]. This disease is caused by a protozoan parasite called Leishmania from the Trypanosomatidae family and Leishmania genus [3]. This disease is transmitted to humans or animals by the bite of a female insect called sandflies from the Psychodidae family [4]. Dogs and rodents are the main reservoirs of this disease [5]. Leishmaniasis has three main clinical forms, namely Visceral leishmaniasis (VL) (Kala-azar), Cutaneous Leishmaniasis (CL) and Mucocutaneous leishmaniasis (MCL) [6], [7]. In this regard, the CL is a very common form and has the highest prevalence in the world [8], So that the World Health Organization (WHO) has introduced this disease as an important neglected tropical disease. About 350 million people per year are exposed to this disease, and new cases are emerging annually [9]. In 2017, the WHO reported 94% of new cases from seven countries, Brazil, Ethiopia, India, Kenya, Somalia, Sudan, and South Sudan. The highest number of CL are also reported in Afghanistan, Algeria, Brazil, Colombia, Pakistan, Peru, Saudi Arabia, Syria and Iran [10]. In Iran, the CL is found in two types, namely Anthroponetic Cutaneous Leishmaniasis (ACL) and Zoonotic Cutaneous Leishmaniasis (ZCL) [9], [11] that are transmitted mainly by parasites, namely Leishmania (L.) major and L. tropica, Phlebotomus (Ph.) papatasi and ph. sergenti sandflies as the main vectors of this disease [12]. The main reservoirs of urban form are humans with CL and occasionally dogs; and rural form reservoirs are mainly desert rodents such as Rombomis opimus, Meriones lybicus, and tatera indica [13]. CL which is popularly known as Salak in

Iran.

According to reports of the Center for Disease Control and Prevention, the number of people with Leishmaniasis in Iran is more than 20,000 per year [14]. CL is an important health problem in more than 15 provinces of Iran. Nearly 88 cities in Iran have been diagnosed with this disease, and the most frequent reports are related to ZCL [14]. The disease is endemic in many regions of Iran. Due to the increased prevalence of the disease in Iran, northeastern regions including Khorasan Razavi such as Neyshabur and Mashhad have had the emergence of new foci of this disease. The eastern regions of Iran, including the vast Neyshabur region with its numerous villages, have long been considered as a focus of the CL [15]. Although CL is no lethal disease, it has always been taken into consideration due to the more involvement of patients with skin lesions and its long-term treatment [16].

Various factors contribute to the incidence of disease, including climatic changes, environmental conditions, occupation, gender, and reduced vegetation cover. Furthermore, agricultural expansion, urban migration, and marginalisation contribute to the transmission of vector and parasitic of disease to new areas and increase the risk of this disease [17]. The incubation period of ACL is up to 8 months, and it is less than 4 months for ZCL. After the incubation period, painless red papules appear in the sandfly bite location; and the lesion grows after a few weeks to several months, and dimples at a depth of 1 mm appear with the secretion of liquids [18]. Treatment of leishmaniasis is based on the national protocol using Antimony (Sb5+) compounds such as Glucantime (intramuscular injection of 50 mg/kg of body weight, 10 ml per day for two to three weeks).

Patients should be examined and monitored by physicians for up to three weeks after the start of treatment to three months after healing. The injection of Glucantime into the lesion should be performed three times a week until the complete recovery [19]. Since CL treatment needs a lot of time and it is associated with several adverse effects such as arrhythmia, increasing of liver enzymes, anaemia, thrombocytopenia and leukopenia [20], Even if left untreated, the disease leads a fatality rate 95%-100% within 2 years [21], therefore the use of effective measures to prevent the disease is important and requires the identification of vulnerable groups and epidemiologic agents that are effective in the disease in these areas. Given the increased cases of the disease in northeastern villages of Iran, especially in Neyshabur and its villages, the present study sought to evaluate the incidence of disease in this county in recent years, so that more effective control strategies could be adopted to reduce disease by identifying related factors of disease.

Material and Methods

Geographical area

Neyshabur County is a city in the central part of Razavi Khorasan and is located between 58° 19' and 59° 30' longitude and 35° 40' to 36° and 39' latitude in the eastern margin of the central desert of Iran [22]. The vast majority of this city is located in a relatively large plain that is limited to Chenaran and Quchan counties from the north (by the Binalud Mountains); Mashhad County from the east; Torbat Heydarieh and Kashmar from the south; Sabzevar from the west; and Farooj County from the northwest in North Khorasan province. Neyshabur is located 110 kilometres west of Mashhad (Center of the Khorasan Razavi province) and 768 kilometres east of Tehran (Capital of Iran). The size of Neyshabur county is 8,722 square kilometres, which is equal to 2.9 percent of the area of Khorasan and 53 percent of the total area of Iran. Due to the population of Neyshabur with 451,780 people in the last census in 2016, it is the second-most populous city in Khorasan Razavi province [23].

Figure 1: Geographical location of Neyshabur county, Khorasan Razavi province, East Iran

Data collection and analysis

In this cross-sectional analytical study, the statistical population consisted of all individuals (971 cases of CL) who who had medical records in the health centers of Neyshabur and were diagnosed with CL according to the laboratory confirmation in Neyshabur county and its villages during 2011-2017; and their data were recorded in a health record by health care workers. Diagnostic methods were smear, culture. The necessary data including age, sex, place of residence (urban and rural), month and season of disease, incidence, number and location of lesions and some other important factors were extracted from their cases and then centres. The records that did not have the necessary data such as age, sex, job, diagnosis date and place of residence were removed from the study. To observe ethics in research, their full name and, father name and national code of all patients were removed from information files. For data analysis, data were first categorised into appropriate

groups. After grouping and preparing data, the whole data was analysed using SPSS V.22. Chi-square test was used to analyse data, and the alpha value less than 0.05 was considered as the significance level.

Results

The research results indicated that during the 7 years (2011-2017), a total of 971 cases of CL were reported. The highest incidence of CL was estimated in 2017 (44.7 per 100,000) and the least in 2015 (19.5 per 100,000) according to the population of Neyshabur in 2016 census (Fig. 2).

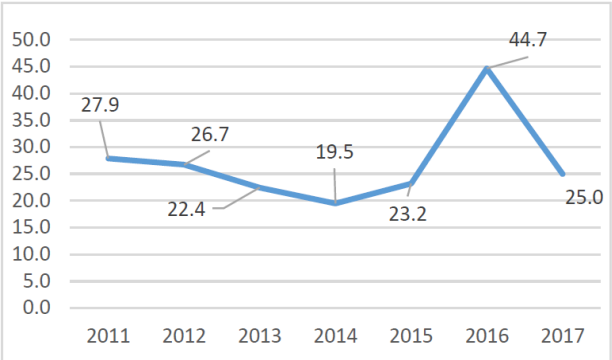

Figure 2: Time trend of incidence of CL per 100,000 populations in Neyshabur from 2011-2017 according to 2016 census

Of these, 469 (48.3%) were female, and 502 (51.7%) were male. The chi-square test showed that there was no significant association between sex and the disease (P > 0.05). In Neyshabur villages, the CL was more common among men 246 (56.7%) versus 188 (43.3%) females but unlike villages, more women were affected in Neyshabur city 281 (52.3%) women versus 256 (47.7%) males, however, there was no significant difference between men and women in the incidence of CL in the city (P > 0.05), while the difference was statistically significant in the village (P < 0.001).

The mean and standard deviation of patients' age was 29.4 ± 19.3. The youngest patient was under the age of one, and the oldest was 90 years old. Less than a quarter of patients were above 42 years of age, and more than half of the patients were under the age of 28. The prevalence of CL was statistically significant in different age groups (P < 0.001) (Table 1).

Exactly 393 (40.5%) and 578 (59.5%) patients were rural and urban, respectively. There was a decreasing trend in the number of people with CL in villages and the increasing trend in the city from 2011 to 2017, and numbers of patients in the city and village were significantly different (p < 0.001) in all years except for 2013 and 2014; and number of patients in cities was always higher than the villages. Considering the number of the rural population (N = 201,492) and the urban population (N = 311,256) in Neyshabur in 2017, the prevalence of the disease in the city (31 per hundred thousand) was about twice as high as the village (16.6 per hundred thousand).

In terms of geological division, people living in the north of the city (n = 340 patients) and southern villages (n = 195 patients), were more susceptible to CL, but those living in Northern villages (n = 35 patients) and southern city (n = 42) were less prone to CL (Fig. 3).

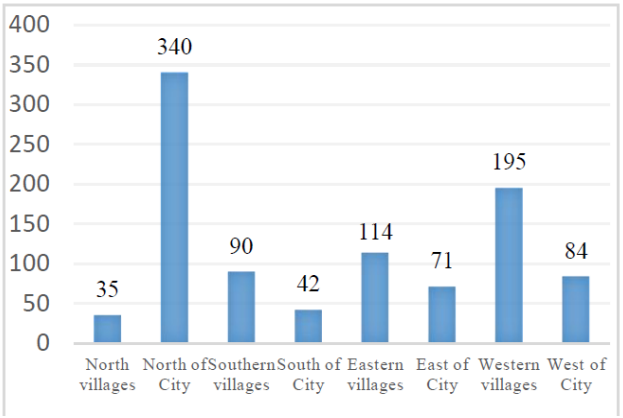

Figure 3: Distribution of CL disease in the city of Neyshabur according of Geoghraphic Area from 2011-2017

Regarding the seasonal tendency, the highest and lowest number of patients with CL belonged to autumn 308 (31.7%) and summer 183 (18.8%), and there was a significant different incidence in different seasons during 2011, 2012 and 2015, but no difference was seen in the rest of years, but generally there was a significant difference in the incidence of disease in different seasons (P < 0.001). The highest prevalence was seen in April, October, November, and December in rural areas, and November, December, January, and February in urban areas.

According to research findings, in all examined years, the incidence of CL among those, who had travelled to other cities during the last year, was 510 (52.5%) compared to those who had not traveled 461 (47.5%) and they were not significantly different (P = 0.062), while the incidence of disease was greater and significantly different among non-travellers in 2011, 2013, 2016 and 2017.

Most of the patients with CL were housewives 287 (29.6%), children 193 (19.9%) and the least of them had military occupation 18 (2.0%). There was a statistically significant difference between the incidence of disease and multiple occupations in all years (P < 0.001).

In this study, the location of the lesion was different in patients' bodies. The most commonplace of the lesion was on hands (56.0%), legs (22.0%), face (20.7%) and trunk (1.3%). The size of lesions

was 2.7 ± 1.6 centimetres on average, and 47.0% were less than 1 cm, and 38.9% (1-4 cm); and only 14.0% of lesions were greater than 4 cm. The greatest lesion size was 11 cm (2.0%).

Table 1: Distribution of CL disease characters in 971 patients in the city of Neyshabur, in 2011-2017

Characters/Years		*2011*	*2012*	*2013*	*2014*	*2015*	*2016*	*2017*	*Total (%)*
Age Groups	0-10 Year	18	19	18	10	34	64	38	201 (20.7)
	11-20 Year	26	25	18	22	14	26	20	151 (15.6)
	21-30 Year	36	33	42	15	17	25	17	185 (19.1)
	31-40 Year	26	22	22	17	23	47	22	179 (18.4)
	41-50 Year	12	18	9	13	16	37	7	112 (11.5)
	>50 Year	25	20	6	23	15	30	24	143 (14.7)
	Total	143	137	115	100	119	229	128	971 (100)
	P-Value	0.016	0.237	0.000	0.170	0.012	0.000	0.000	0.000
Sex	Male	76	75	75	49	62	105	60	502 (51.7)
	Female	67	62	40	51	57	124	68	469 (48.3)
	P-Value	0.452	0.267	0.001	0.841	0.647	0.209	0.480	0.290
Habitat	Rural	92	77	61	60	30	39	34	393 (40.5)
	Urban	51	60	54	40	89	190	94	578 (59.5)
	P-Value	0.001	0.146	0.514	0.057	0.001	0.001	0.001	0.001
Session	Spring	29	26	27	25	13	65	30	215 (22.1)
	Summer	28	28	20	26	12	43	26	183 (18.8)
	Autumn	54	59	41	29	30	56	39	308 (31.7)
	Winter	32	24	27	20	64	65	33	265 (27.3)
	P-Value	0.005	0.000	0.044	0.641	0.001	0.129	0.421	0.000
Treatment Type	Systemic Glucantime	43	39	15	0	0	0	0	97 (10.0)
	Topical Glucantime	99	69	38	0	0	0	0	206 (21.2)
	Topical Glucantime & Cryotherapy	1	9	48	0	0	0	0	58 (6.0)
	Cryotherapy	0	20	14	2	1	4	1	42 (4.3)
	Cryotherapy & Topical Antimoniate	0	0	0	63	52	122	60	297 (30.6)
	Topical Antimoniate	0	0	0	10	48	71	47	176 (18.1)
	Systemic Antimoniate	0	0	0	25	18	32	20	95 (9.8)
Duration of systemic treatment	7-10	10	10	1	2	0	0	1	24 (2.5)
	11-14	5	6	2	4	2	0	6	25 (2.6)
	15-18	1	3	5	14	3	0	1	27 (2.8)
	19-21	27	20	7	5	13	32	11	115 (11.8)
Duration of Topical & Cryotherapy treatment	1-4	2	12	4	27	31	46	2	124 (12.8)
	5-8	85	75	81	48	45	81	60	454 (48.9)
	9-12	13	11	15	0	25	70	47	154 (18.6)
Occupation	Driver	4	2	1	4	1	4	3	19 (2.0)
	Worker	14	7	11	6	4	6	4	52 (5.4)
	Employer	3	7	5	2	5	23	11	56 (5.8)
	Farmer	22	20	17	10	5	6	7	87 (9.0)
	Student	24	21	19	19	26	46	33	188(19.4)
	Military	1	1	10	0	0	0	6	18 (1.9)
	housewife	49	46	21	38	34	64	35	287 (29.6)
	Baby	13	14	10	19	42	74	21	193 (19.9)
	self-Employment	13	19	21	2	2	6	8	71 (7.3)
	P-Value	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Lesion Site	Hand	95	88	80	76	78	146	71	634 (56.0)
	Feet	30	35	31	23	37	73	20	249 (22.0)
	Face	36	29	15	17	28	68	41	234 (20.7)
	Trunk	5	1	3	0	2	0	4	15 (1.3)
	Total	166	153	129	116	145	258	136	1132 (100)
Travel history	Haven't	89	62	40	42	60	133	84	510 (52.5)
	Have	54	75	75	58	59	96	44	461 (47.5)
	P-Value	0.003	0.087	0.000	0.450	0.929	0.002	0.000	0.062

Most of the patients (80.2%) were treated by topical method, and 192 (19.8%) patients were treated using the systemic method. The duration of treatment in 608 (62.7%) of patients, who were treated by topical and combination of cryotherapy and topical methods, was 5-8 weeks or more. Patients treated with systemic methods had a shorter duration of treatment (Table 1).

Discussion

The present study aimed to provide and analyse epidemiological information about CL in Neyshabur county during the last 7 years (2011-2017). During this period, a total of 971 cases of CL referred to health centres of the Neyshabur and were treated. Over the years, the overall incidence of CL did not change significantly in Neyshabur, and it also seemed to be a public health problem. Medium prevalence of CL in Neyshabur county (27.05 per 100,000) was almost equal to the average prevalence in Iran (27.5 per 100,000) based on the results of studies [24].

In this study, both sexes were susceptible to CL disease; and the results of the study did not show any significant relationship between gender and disease (P = 0.29). Studies in Iran in cities, such as Kermanshah, Kashan, and Fars province reported similar findings [25], [26], [27] but studies in Fasa city of Fars province and Jahrom different reports [28], [29].

Based on findings of the study in terms of age, the minimum age of 7 months and the highest age of 90 years had CL; the highest frequency (20.7%) was observed in the age group of 0-10 years probably due to the higher prevalence among the students. Our findings were inconsistent with studies in Kashan, Kermanshah and Fars [25], [26], [27]. Based on analyses, prevalence in age groups depended on the residence ($p < 0.04$, ρ (Spearman Correlation) = -0.031), gender ($p < 0.001$, $\rho = 0.105$) and occupation ($p < 0.001$, $\rho = -0.091$).

The incidence of disease in urban regions (59.5%) was higher than in rural regions (40.5%), and a significant difference was found between habitat in terms of the prevalence of CL ($P < 0.001$). Results of our study were inconsistent with a study by Doroodgar in Kashan [26] and Mohammadi's study in Marvdasht [30] and a study by Rahmanian in Jahrom; there was a higher prevalence in villages [29]. Due to its increasing prevalence in urban areas relative to rural areas, there was a need for health and education measures in these regions.

In terms of geographical division, the majority of patients lived in the north of the city (35%) and western villages (20%) of Neyshabur; and rates of CL were in statistically different in various geographical regions ($p < 0.001$), probably due to amounts of building waste and public health conditions in those areas. Since the rate of disease increased in the north of the city and western villages during 2011-2017, it indicated the source of infection and appropriate growth conditions for cause of CL.

Seasonal trend study indicated that the highest prevalence of the disease was in autumn, especially November/December in Neyshabur and its villages. It seems that the incubation period of disease begins due to cool weather in autumn, and sandflies' peak activities and sucking the victims' blood reach their peak in the winter, and then their activity is slowly decreasing. Our results were consistent with findings in Kashan, Qom, Fars, and Yazd in terms of more prevalence in autumn [26], [31], [32], but they were inconsistent with findings of Kermanshah in terms of more prevalence of the disease in winter [25].

In the field of occupational groups, the

highest prevalence was seen in housewives (27.1%), students (18.9%) and infants (19.3) due to staying more at home and their clothing styles, and more activity of sandflies in wet places [33]. Also it may be due to higher number of housewives group in population. In men, the prevalence of disease was higher in farmers (8.5%), workers (5.4%) and self-employment (7.3%) due to their location in endemic regions, inadequate health information, and a significant difference in the incidence of disease and type of occupation ($P < 0.001$). In a study by Doroodgar et al., (2018), the prevalence of disease was higher in housewives [26] and in a research by Rahmanian et al., [2018] in the southwest of Iran, the highest prevalence was seen in students (28.8%), and there was a significant statistical difference between occupations in terms of prevalence of CL ($p < 0.001$) [29].

Based on findings of the present study, 95% of lesions were dry and without secretion, and were seen in all organs of the body such as (hands, arms, feet, trunk, face, forearm, legs, head and neck). In this study, hands (56%) had the highest bite reporting; and the location of the lesion was probably associated with the body cover. Face (32%) in women and hands and arms (31%) in men were the most involved locations. The average size of lesions was (2.2 ± 1.6 cm), and the smallest one was 1 cm, and the biggest one was 11 cm, and the average number of lesions was 2 ± 1.9 and the highest was 18. Rahmanian et al., (2018) reported the maximum number of lesions as 30 lesions in their study in Jahrom, southwestern Iran. This finding may be different due to the types and behaviour of insect blood-feeding, the number of bites feeding by sandflies per feeding time, and the frequency of infectious sandflies in that region [29].

In conclusion, according to the results of the present study, the incidence of CL in Neyshabur was higher in north and west regions; and autumn was a more suitable season for the prevalence of CL in the region. Most patients were housewives, children under the age of ten, and people living in the city. Therefore, measures should be taken to improve environmental health, public health education, and making people aware of the positive impact of early diagnosis to achieve successful treatment by public health authorities and control the disease. Due to multiple lesions with different sizes in this disease, the likelihood of scar is higher, and thus the future studies should investigate the status of these scars and their causes. As the incidence of disease increased in the county in 2017, further studies should be conducted to clarify its causes.

Ethical considerations

This study was approved by the Research Ethics Committee Neyshabur University of Medical Sciences under Opinion number IR.NUMS.REC.1397.020.

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Review Article

The Prognostic Significance of Phosphatase and Tensin Homolog Loss in Breast Cancer

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Abstract

AIM: This study aims to determine the prognostic significance of phosphatase and tensin homolog (PTEN) loss in breast cancer.

METHODS: We conducted a meta-analysis study. Sample of this study were research articles that evaluated PTEN loss and prognosis in breast cancer patients. We searched for relevant studies published in PubMed and Proquest from January 2010 to July 2018. We reviewed studies that examined the association between immunohistochemical expression of PTEN and breast cancer prognosis using meta-analysis methods. Pooled risk ratios (RR) were calculated using fixed and random-effect models. Data were processed using Review Manager 5.3 (RevMan 5.3).

RESULTS: There were 7 studies conducted a systematic review then continued to evaluate the association of PTEN loss and breast cancer prognosis by meta-analysis. There was a significant association of PTEN loss with poor prognosis of breast cancer (RR = 0.76 [95% CI 0.59-0.98 p <0.07), and there was not any significant publication bias for studies included.

CONCLUSION: This study confirmed PTEN loss is an important independent factor for breast cancer prognosis.

Introduction

Breast cancer is the most common malignancies in women worldwide. Breast cancer incidents rapidly increased from 641,000 cases in 1980 to 1.6 million cases in 2010 [1]. GLOBOCAN's 2012 International Agency for Research on Cancer (IARC) data notes that there are approximately 1.67 million new cases of BC diagnosed in 2012 or 25% of all cancers [2].

In Asia Pacific region breast cancer was the most common type of cancer among females, accounting for 18% of all cases in 2012, and was the fourth most common cause of cancer-related deaths (9%). Globally, one of three women (33%) diagnosed with breast cancer in a woman under 50 years old during 2008. The proportion of female breast cancer diagnosed among women under 50 years of age ranged from 21% to 58% in the Asia Pacific region [3].

Prognosis of breast cancer influenced by many factors. Prognostic factors such as tumour size, lymph node involvement and metastasis play a big role in prognosis. Standard adjuvant treatment reduces the risk of disease recurrence and improves prognosis [4]. Most relapses of breast cancer

occurred in the second year of treatment. About 62.71% of patients with primary tumour ≥ 5 cm, 79.65% of patients with ≥ 4 involved of axillary lymph nodes, and 72.41% of hormone receptor-negative tumours could relapse. Large tumour size, number axillary lymph nodes metastasis, and hormone receptor status were strongly associated with risk of relapse [5].

One biomarker of breast cancer growth is phosphatase and tensin homolog (PTEN). PTEN dysfunction on tumour cells causes increasing of NF-kB activity resulting in growth, proliferation, survival, and metabolism of tumour cells [6], [7], [8]. Previous studies revealed tumour cells with PTEN loss of function have a poor prognosis [9], [10], [11], [12]. But there are also studies showed different results [13]. Changes of PTEN expression can be accurately identified by Immunohistochemical analysis (IHC). IHC analysis is quicker and more cost-effective than molecular genetic techniques to detect PTEN status. Therefore, this study aims to determine the prognostic significance of PTEN loss in breast cancer by IHC methods using meta-analysis.

Material and Methods

Study design and research sample

This study was quantitative with meta-analysis study design. The meta-analysis followed PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) Statement. Meta-analysis was used to determine the association between PTEN loss expression and breast cancer prognosis. The result of published articles on the internet through databased on PubMed and Proquest that published from January 2010 to August 2018 were analysed. The inclusion criteria of this study were cohort studies. Exclusion criteria of this study were articles which are not available in full-text form.

Operational definitions

The variables of this study are PTEN loss expression as an independent variable, and a dependent variable is a prognosis.

Research procedure

This study was conducted by collecting data through the identification of published research articles on the association between PTEN loss expression and breast cancer prognosis by using the PubMed and Proquest search engines (Figure 1).

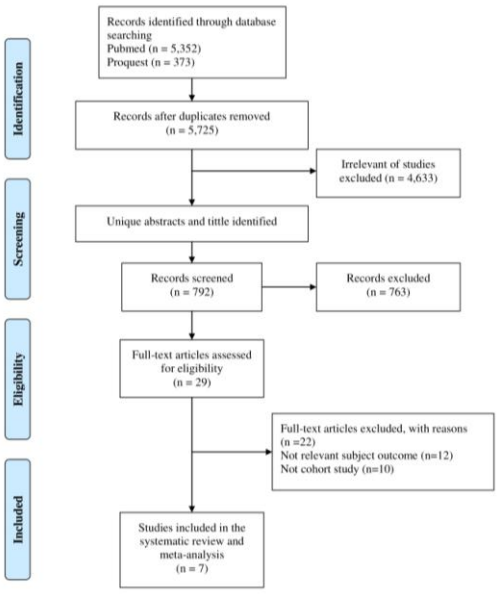

Figure 1: Flow diagram research procedure

Identification of 5,725 articles, reviewed through the title of the articles, continued by reviewing the abstract, and then the full-text form. The article was excluded if: (a) not the relevant subject outcome, (b) the information provided in the results was insufficient for data extraction and (c) duplicate studies.

Data collection technique

The search was limited to English language articles. The article type was limited to research articles. The research subject was limited to a human subject. The time of publication was limited from January 2010 to August 2018 period. The abstract of articles with relevant title continued to review process, and the articles with the irrelevant title were excluded. After that, articles with relevant abstract were continued to be reviewed in full-text, while the others were excluded. The articles that used immunohistochemistry method only and the relevant outcome will be reviewed and analysed.

Data analysis

The analysis was conducted to obtain the value of pooled risk ratio as the combined risk ratio value from the collected researches. Data analysis was performed using the Mantel-Haenszel method with a fixed-effect model and the DerSimonian-Laird random-effect model. Publication bias was visually evaluated by using funnel plots and statistically assessed through Egger's and Begg's tests. Meta-analysis was carried out by using *Review Manager* 5.3 (RevMan 5.3).

Results

The selection of studies was conducted to identify 7 studies related to the PTEN loss expression. The characteristic of eligible studies (Table 1). These publications estimate association between PTEN loss expression and prognosis with various sample size, staining methods, cut off and methods of interpretation.

Table 1. A systematic review of the role of loss of PTEN expression in breast cancer prognosis

First Author	Procedure	Histology typing	Cut off	Staining methods	Number of patients	Scoring	Outcome
Jensen et al., [11]	IHC	mixed	> 40	Cytoplasmic	235	IRS	OS
Wu et al., [14]	IHC	TN	> 5	Cytoplasmic	65	IRS	OS
Perez et al., [15]	IHC	mixed	> 0	Nuclear / cytoplasmic	1286	SI	DFS/OS
Tang et al., [16]	IHC	mixed	> 0	inti	68	SI	OS
Beelen et al., [17]	IHC	mixed	> 3	Cytoplasmic	436	SI	OS
Stern et al., [18]	IHC	mixed	> 5	Cytoplasmic	2354	SI	DFS/OS
Beg et al., [19]	IHC	Mixed	> 90	Nuclear and cytoplasmic	957	H-score	OS

Abbreviation: IHC: Immunohistochemistry; TN: Triple-negative; IRS; immunoreactive score; SI: Staining intensity; OS: overall survival; DFS: disease-free survival.

Forest plots of PTEN loss expression in breast cancer prognosis (Figure 2). Figure 2 revealed the significance association of PTEN loss expression with prognosis of breast cancer (RR = 0.76 [95% CI = 0.59-0.98, p < 0.07]).

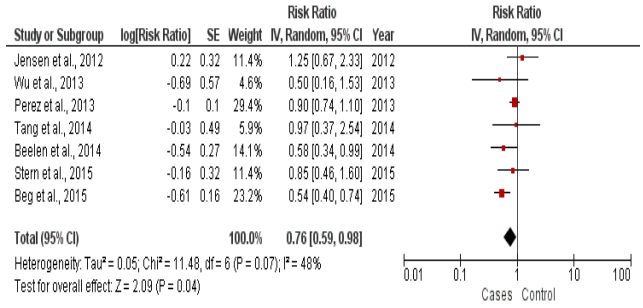

Figure 2: Forest plots the association of PTEN loss and breast cancer prognosis

Funnel plot association of PTEN loss expression with breast cancer prognosis (Figure 3).

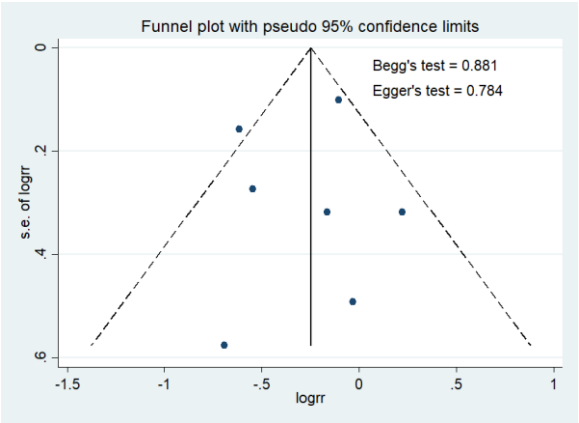

Figure 3: Funnel plots the association of PTEN loss and breast cancer prognosis

Figure 3 showed the association of PTEN loss expression with the prognosis of breast cancer has a variety of homogeneous research on breast cancer. It means that if the analysis is done on the population, time and place and different conditions the results will be consistent. There was not any significant publication bias for the association of PTEN loss expression with breast cancer prognosis, Egger's test (P = 0.784) and Begg's test (P = 0.881).

Discussion

Identification of biomarkers and gene expressions are needed to improve early diagnosis and prognosis, as well as to provide the most effective drug suitable with molecular characteristics of the patients. Studies over the years have indicated that prognostic and predictive biomarkers are molecules involved in the regulation of cellular mechanisms, including proliferation, apoptosis, angiogenesis, metastasis and therapeutic resistance. Evidence has shown that PTEN is an important factor in many processes related to cancer progression [20], [21].

PTEN is one of the tumour suppressor genes that play a role in breast cancer development. PTEN is an antagonist of phosphatidylinositol 3-kinase (PI3K) and plays a role in dephosphorylation of PIP3 into PIP2. Loss of PTEN can cause overgrowth, proliferation, survival, and metabolism of tumour cells. PTEN consists of phosphatase homolog and cytoskeletal tensin protein. PTEN protein contains 403 amino acids. The crystal structure of PTEN consists of two main functional domains (phosphatase domain and C2 domain) and three structural PIP2 binding domains, C-terminal tail [22]. PTEN loss can also cause phosphorylation mediated by Akt and increase of NF-kB activity which promotes P53 degradation. P53 degradation reduced the apoptotic ability and induced cell cycle progression [8]. PTEN gene located on chromosome 10q23. Loss of heterozygosity on chromosome 10q23 often occurs in advanced sporadic tumours, including breast cancer [23]. PTEN dysfunction, especially its inactivity in tumour cells, causes PIP3 accumulation in cells and regulate Akt recruitment. Furthermore, it can activate downstream kinases, increasing NF-kB activity resulting in growth, proliferation, survival, and metabolism of tumour cells [6], [7], [24].

Some studies suggested PTEN loss in tumour cells are associated with drug resistance [6,15]. Other studies showed a significant association between PTEN immunoscoring and mRNA expression. A previous study also revealed that PTEN loss has a significantly worse prognosis than PTEN positive breast cancer cells [17], [18]. PTEN loss is also associated with aggressive behaviour and poor prognosis [14], [19].

Changes of PTEN expression can be identified by IHC analysis, Fluorescent In situ Hybridization (FISH) and PCR. There were previous meta-analyses of PTEN loss, but it did not elaborate on the methods. The previous study found IHC analysis is quicker and more cost-effective than molecular genetic techniques to detect PTEN expression [25], [26]. FISH and PCR is limited to sophisticated laboratories; it cannot be done routinely. In a developing country, not all laboratories have FISH and PCR services. This study answers the need for an analysis of the association of PTEN with prognosis in the use of IHC staining methods.

This study has identified the heterogeneity of published articles ($p = 0,04$, $I^2 = 45\%$), then used the random effect models. The analysis revealed significance association of PTEN loss with prognosis of breast cancer (RR = 0.76 [95% CI = 0.59-0.98, $p < 0.07$]). The pooled finding of these seven articles provides evidence of the significance of PTEN loss of expression with prognosis. This meta-analysis showed that PTEN could provide prognostic information for the clinician during the decision-making process.

Although we try to make our study as good as possible, the limitation of this study should be considered. First, data suitability of several studies included in this meta-analysis showed different sample sizes (65-2354 samples). Second, there are different scoring methods for PTEN status with different numbers of positivity of tumour cells. The study was using immunohistochemical analysis and could be carried out with different evaluation methods using IS (intensity staining), histoscore (H-score) and percentage of positive cells. Different studies with different PTEN classifications and different patient sets are hardly comparable.

Cut off points are varying from > 0% to > 90%. These differences in the method could be responsible for various PTEN expression observed in tumour cells. Although the robustness of PTEN antibodies has been controversy, optimal IHC conditions including concentration of anti-PTEN antibody as well as different antigen retrieval conditions should be considered too. Scoring methods against PTEN's requires a more valid and reliable standard methodology to assess PTEN expression. In conclusion, the result of this meta-analysis showed that loss PTEN expression is associated with poor prognosis of breast cancer.

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Review Article

Efficacy of Minimally Invasive Surgery on Giant Cell Tumour of the Bone: A Systematic Review

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Abbreviations: GCT: Giant Cell Tumor; MIS: Minimally Invasive Surgery; CT: Computed Tomography; MRI: Magnetic Resonance Imaging

Abstract

BACKGROUND: Although major reconstructive surgeries in the form of excision and reconstruction have been the mainstay treatment for Giant Cell Tumour (GCT) of the bone, its recurrence rate remains high and poses various morbidities such as implant failure or skin breakdown. Minimal invasive surgery (MIS) techniques, which has gained popularity mostly in other fields in orthopaedic surgery, are being introduced as an alternative to limit the surgical complications while achieving the best possible outcome. Until now, there has been no literature summarising the evidence of MIS outcome in treating GCT of the bone.

AIM: The purpose of this systematic review was to investigate the efficacy of this relatively new treatment.

METHODS: We comprehensively searched PubMed, EMBASE, and Cochrane Library to search for studies about MIS for GCT of the bone treatment up to March 1, 2019. The selection of appropriate studies was performed by two independent investigators based on PRISMA guideline. Given the limited number of studies, there was no restriction in terms of patient's demographics, the specific minimally invasive surgical method, and publication status.

RESULTS: We found 120 articles from the database. After evaluating full text, 5 articles (16 patients) were found to be eligible. The minimally invasive methods were curettage, cryosurgery, and argon beam coagulator. The visualisation methods include a computer-assisted navigation system, endoscope, otoscope, CT, and MRI. Location of tumours includes axial and long bones. The follow-up period ranges from 7 to 126 months. The functional and oncological outcome was found to be satisfying with no recurrence or complications.

CONCLUSION: In conclusion, MIS is a familiar method in orthopaedic surgery with potential expansion in tumour field. The current evidence shows that this approach for GCT results in good functional outcome, with low risk of recurrence.

Introduction

Giant cell tumour (GCT) is a primary bone tumour with low metastatic potential, yet locally aggressive. Despite that surgery is the mainstay treatment for this entity; its recurrence rate remains high with < 20% for en block excision and 40%-50% for intralesional curettage [1]. Furthermore, radical resection of GCT has been known to result in poor functional outcome, especially in areas such as pelvis [2], [3]. Recently, minimally invasive techniques for treating GCT are being introduced to reduce the morbidity of GCT treatment while achieving treatment goals effectively. This study aims to review recent evidence concerning such treatment options systematically.

The surgical approach towards localised GCT highly relates to significant damage to the surrounding tissue, hence the poor functional outcome. Adjunctive therapies were proposed as an alternative to manage GCT while minimising side effect. The therapies include pharmacologic therapies with RANKL inhibitors or bisphosphonates for patients with bad prognostic factors and radiotherapy (RT) for non-resectable cases that are unresponsive to systemic drugs [4]. However, none of the adjunctive therapies was proven to be highly effective.

Recently, minimally invasive techniques were offered as definitive therapy, which attempts to reduc morbidity to patients, resulting in faster recovery and less hospitalisation period. Minimally invasive surgery is defined as a surgical procedure that is done using

state-of-the-art technology to reduce the damage to human tissue when performing surgery. Minimally invasive surgery uses a small incision to create small "ports" from which the surgeon inserts small instruments. A miniature camera can be placed inside to view the procedure as a magnified image on video monitors in the operating room. Other specialised small surgical instruments can then insert through the trocars based on the type of surgery. Cryoablation method, for example, utilised 4 probes and the patient underwent minimum pain relieved by basic analgesic [5]. It is usually combined with the computer navigation system to navigate the limited visual field [6]. These methods have gained popularity mostly in other fields in orthopaedic surgery, with the possibility of expanding its use in the orthopaedic oncology field.

Despite the potential of MIS, there has never been any literature summarising this latest trend of minimally invasive techniques for treating GCT. To advance it even further, a thorough analysis of previously treated cases, the specific indication, the methods used along with its advantages and disadvantages, and other lessons learned should be performed. This systematic review aims at presenting current evidence about the efficacy of this relatively new treatment for GCT.

Materials and Methods

Search strategy

This systematic review was conducted based on PRISMA guideline. Literature research was primarily performed using the Pubmed, EMBASE, and Cochrane Library to search for studies about MIS for GCT of the bone treatment up to March 1, 2019, with the keywords: 1. “giant cell tumour” AND “minimally invasive”; and 2. “giant cell tumour” AND “endoscopic”.

We filtered the search to include only studies in human, published in the last 10 years, and ones written in English. After that, we combed through all articles cited and citing the articles so as not to miss any relevant articles.

Inclusion criteria

Inclusion and exclusion criteria of this study were described using the method of PICO (Population, Intervention, Comparison, and Outcome) (Table 1). The inclusion criteria were GCT of bone in any location, with the intervention of minimally invasive surgery as compared to conventional open surgery, and recurrence rate and hospitalisation period as an outcome. Case reports and case series were also included. Given the limited number of studies, there was no restriction in terms of patient’s demographics, the specific minimally invasive surgical method, and publication status. We excluded all cases of GCT of soft tissue and ones located in the cranium.

Quality evaluation

First, all authors screened eligible studies through the titles and abstracts based on inclusion criteria. Then, all authors screened the full articles of all the collected studies. The authors had a meeting and agreed on highly relevant publications to be included in this study. All authors performed an appraisal of study quality independently and any disagreement was resolved through discussion.

All inherent aspects of the studies, including study quality, variables for which data were sought, and assessment of the risk of bias, were appraised by all authors independently by filling up forms. The forms were collected by the first author and the contents were scanned for any disagreement. The authors then gathered again for discussing any contradicting points.

Results

The electronic search resulted in 120 records, after the elimination of duplicate results. Based on titles and abstracts screening, a total of 99 records were excluded. The remaining articles were subsequently studied by two independent investigators based on the full text extracted. A list of inclusion and exclusion criteria (Table 1) previously agreed by the three authors were utilised for screening the full text. This selection process yielded 5 final articles to be included in the systematic review and was depicted in Figure 1.

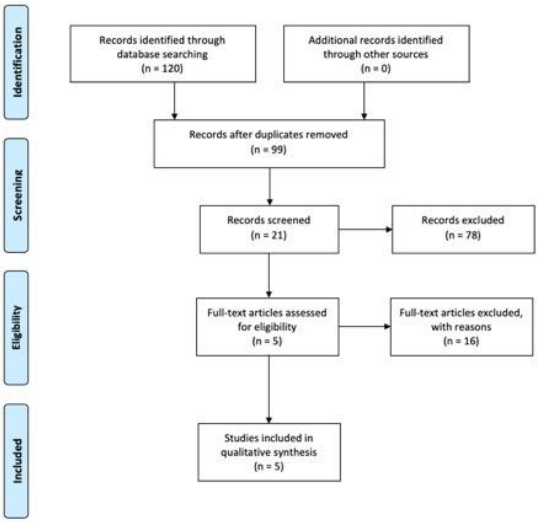

Figure 1: Article selection based on PRISMA Guidelines

In total, 16 patients were eventually included in the analysis. Among these patients, 3 patients had surgeries in the axial bones (vertebral body, sacrum, and pelvis) while the remaining 13 patients were of long bones (femur, tibia, and fibula). The axial bone group had a CT guided procedure to treat the GCT. In the long bones group, image intensifier was utilised for all 13 patients [5], [7].

Table 1: PICO Table Describing Inclusion and Exclusion Criteria

Study Component	Inclusion	Exclusion
Population	Any age Patients with Giant Cell Tumour of the bone in any location	Animal studies Patients with benign neoplasms other than GCT of the bone Infection or deformity
Intervention	Minimally invasive surgery as primary treatment for GCT	Adjunctive therapies for GCT Minimally invasive surgery as a treatment for GCT complication
Outcome	Functional outcome Oncological outcome	Radiological outcome
Publication	Studies published in English in peer-reviewed journals Any publication year	Abstracts, editorials, letters Duplicate publications of the same study that do not report on different outcomes Meeting presentations or proceedings
Study Design	All study design	-

Among them, 8 patients underwent the procedure with endoscope or osteoscope through a small incision, and the other 5 patients did not have additional visualisation inside the lesion [6], [8], [9]. The indication for studies of MIS of GCT in axial bones was tumours with high morbidity and failed conventional treatment [5], [7]. As for studies of long bones GCT, special attention was given to lesions around joint and one that still has cortical wall even though thin [6], [8], [9]. The summary of used methods and their indications were described in Table 2.

Table 2: Summary of minimally invasive methods recorded in the literature

No	Author	Method	Navigation	Indication
Axial bones				
1	Takeda, 2009 [7]	Curettage and argon beam coagulator	CT and MRI	Sacral and spinal GCT of bone
2	Panizza, 2015 [5]	Cryoablation	CT-guided	Extensive pelvic bone tumour. Failure of clinical treatment with denosumab and refusal of hemipelvectomy.
Long bones				
3	Robinson, 2004 [8]	Argon-based cryosurgery	Image intensifier	Lesions close to joint
4	Wong, 2010 [6]	Intralesional curettage and bone cement	Computer-assisted navigation and endoscope	Benign bone tumours without extraosseous extension.
5	Futani, 2018 [9]	Curettage and adjuvants of argon plasma coagulation (APC).	Osteoscopy with 30^{o} endoscope.	GCT was developing in the proximal fibula. Tumour located inside the bone cavity surrounded by a cortical wall, even if this is thin.

The baseline characteristic of the study of Robinson (2004) was not described in details. As for the other studies, the patients were aged between 17 until 59 years old, and most were male (Table 3). The characteristic of the tumour was similar for the axial bones, a large lytic lesion in the sacrum, vertebral body, and pelvis. In the study by Panizza (2015), the pelvic tumour was described to have well-defined and sclerotic borders. While for the long bones, the tumours were of Campanacci grade 2-3 and located in femur, tibia, and fibula.

Table 3: Baseline Characteristics of Patients

No	Author	Number of patients	Age	Gender	Tumour characteristics	Location
Axial bones						
1	Takeda, 2009 [7]	2	46	F	Large purely lytic process.	Right hemisacrum
			25	M	Large osteolytic lesion	L5 vertebral body, expanding to the spinal canal
2	Panizza, 2015 [5]	1	45	M	Large lytic, expansible, a locally aggressive lesion with well-defined and sclerotic borders.	Pelvis
Long bones						
3	Robinson, 2004 [8]	5	NA	NA	NA	Proximal fibula [1], NA [4]
4	Wong, 2010 [6]	3	47	M	NA	Right medial distal femur
			34	F	NA	Left proximal lateral tibia
			26	F	NA	Right lateral distal femur
5	Futani, 2018 [9]	5	17-59	All male	Campanacci grade 2-3	Proximal fibula

Abbreviations: NA, not available.

Patients of GCT in axial bones had follow-up between 60-74 months, and long bones GCT between 7-126 months. The outcome for axial bones GCT was overall satisfying, with the preserved function of lower limbs, bladder and bowel, and no recurrence or metastasis. There was however a movement impairment of hallux for pelvic tumour reconstruction case. MIS for long bones yielded an acceptable result with mean early postoperative VAS of 2.2 (range, 1-3), full ROM and unaided walking, no knee instability or decreased Tegner scores, and MSTS of 100%. The oncological outcome was good without any metastasis or recurrence except on one patient with tumour recurrence at proximal fibula (Table 4).

Table 4: Functional outcome of each study

No	Author	Follow up (months)	Functional Outcome	Oncological Outcome
Axial bones				
1	Takeda, 2009 [7]	60-74	The function of lower limbs, bladder, and bowel preserved.	No recurrence.
2	Panizza, 2015 [5]	NA	Able to walk a 10 km distance. Movement impairment of hallux.	No local or metastatic progression of tumor.
Long bones				
3	Robinson, 2004 [8]	24	NA	Tumour recurrence at the proximal fibula.
4	Wong, 2010 [6]	7-9	Mean early postoperative VAS 2.2 (range, 1-3). All patients had full ROM and walked unaided at 4 weeks post-surgery.	No local recurrence. Good cementation of the skeletal defect.
5	Futani, 2018 [9]	24-126	Knee instability negative in all cases. Mean MSTS rating was 100%. Tegner scores the same as before surgery.	No local recurrence. No pulmonary metastasis.

Abbreviations: NA, not available; VAS, Visual Analog Scale.

Quality of evidence was assessed with all studies categorised as level IV.

Table 5: Characteristics of journals used in the study

No	Reference	Journal	Study Design	Level of Evidence
1	Takeda, 2009 [7]	Journal of Orthopaedic Science	Case Series	Level IV
2	Panizza, 2015 [5]	Cardiovascular and Interventional Radiological Society of Europe	Case Report	Level IV
3	Robinson, 2004 [8]	Technology in Cancer Research & Treatment	Case Series	Level IV
4	Wong, 2010 [6]	Computer-Aided Surgery	Case Series	Level IV
5	Futani, 2018 [9]	Anticancer Research	Case Series	Level IV

Each one had the different instrument to assess the functional outcome, while the oncological outcome was assessed almost similarly according to recurrence and metastasis rate (Table 5).

Discussion

The traditional method of managing locally aggressive bone tumour such as GCT includes intra-lesional curettage followed by local adjuvants. The bone is decorticated to access the tumour, which is then curetted. The osseous surface of the tumour cavity is then cleared using high-speed burr. This conventional invasive method inflicts a lot of damage to the living tissue, something that could be improved by introducing a less invasive approach. The advantage of the minimally invasive approach is particularly the decrease of morbidity in GCT of axial bones and the function preservation of periarticular lesions of long bones.

One of the problems commonly found regarding GCT treatment is a local recurrence and increased risk of pulmonary metastases. In surgically inaccessible lesions such as spine and pelvis, sometimes the lesion cannot be completely resected, further contributing to this problem. Conventional treatment using curettage with or without adjunct, burring, bone cement, H_2O_2, and/or sponge-bone have not proven to be satisfactory in terms of outcome, as well as wide resection procedure. Furthermore, the recurrence rate was still high (± 30.8%), and even more in long bones (45.5-88.9%). Pulmonary metastases rate was ± 3.3%, not to mention the possible metastases in other organs, such as soft tissue, brain, and small intestine, resulting in increased mortality and hospitalisation cost [10].

In a study by van der Heiden *et al*., (2014), en bloc resection was known to result in the lowest recurrence rate (0-16%). However, this procedure has higher complication risk, increased possibility of the subsequent need for revision surgery, and worse functional outcome compared to MIS. For GCT of axial bones, invasive surgery may result in higher morbidity, as it may increase the possibility of bleeding, infection, neurological deficits, bladder, rectal, and sexual dysfunction [2].

MIS for GCT of axial bones

Sacral GCT is a rare difficult case, and there are still controversies on the optimal treatments for the lesion. Conventional methods still result in a low rate of tumour control, high rate of complications and functional outcome. It is even more challenging when the defect is significantly large that excessive bleeding and pelvic instability might ensue after resection [11], [12], [13]. Extensive surgery might also damage neighbouring nerves and may result in cauda equina syndrome. Bladder and rectal dysfunction also lead to increased morbidity [14], [15]. The restricted use of radiotherapy for sacral GCT (as it may result in radiation-induced sarcoma) makes the conventional treatment more difficult [16].

In this study, we interpret “less invasive” as an approach which introduces less damage to tissue than mainstay treatment. This is especially true for GCT of axial bones whose conventional treatment is complete excision, including complete removal of sacral nerve roots [5], [7]. Aiming at minimal damage to surrounding tissues, the surgeons devised minimal invasive procedures using curettage and argon beam coagulator [7] or cryoablation [5] while protecting relevant nerve roots throughout procedure under direct visualisation. In one case where nerve injury still occurred despite the precautions, Panizza et al., (2015) recommended the electrophysiological monitoring in addition to displacement techniques using gas or fluid to protect the nerve roots.

MIS for GCT near joint of long bones

Some serious postoperative complications associated with resection of GCT of the proximal fibula are peroneal nerve palsy and local recurrence. Local recurrence rate differs depending on tumour histology and resection type, while the incidence of postoperative peroneal nerve palsy ranges from 3% to 57% [17] and closely relates to the patient’s functional outcome. The fate of the peroneal nerve here also depends on preoperative chemotherapy response, which if the tumour responds well, then the amount of tissue resected can be minimalised and the peroneal nerve can be more well preserved. In patients with peroneal nerve palsy, the functional outcome is lower and peroneal braces might be needed [18]. For selected patients, MIS techniques in treating GCT of the bone in certain locations of the body have advantages such as preservation of anatomically important tissue and also the possibility of intra-lesional resection. Futani *et al.* (2018) used MIS to treat GCT at the proximal fibula since the area is highly important for knee stability. MIS allowed thorough tumour removal without compromising ligaments attached around that area.

The other highlight of this technique is the use of magnification with equipment which provides excellent visualisation of the bone cavity. Direct visualisation can be provided by osteoscopy or endoscopy which magnifies the inside of lesion onto large monitors, enabling surgeons to identify small residual tumours [6], [9]. Additionally, the computer navigation system can also be used to confirm whether the curettage is already as thorough as a preoperative plan [6]. A combination of these visualisation methods permits assurance of complete

curettage without compromising the tissue too much.

Limitation of MIS: Despite the success of the studies presented, there are still limitations. Cryoablation in the study of Panizza, 2016, was performed in 2 stages after ensuring that the first stage did not yield unwanted necrosis of adjuvant tissues, a reported complication of the surgery [19]. Cryoablation technique might also be challenging when it comes to treating a large complex bone lesion, where multiple probes and imaging might be needed to plan serial repositioning sessions [5]. Whereas in argon beam coagulator technique for treating GCT of axial bones, there is a risk of damaging neural tissue, when the tip of the nozzle is initiated before reaching at least 1 cm of the tissue surface [7]. For extraskeletal lesions, osteoscopic surgery method should also be used cautiously, where if the lesion fails to create a sclerotic wall using denosumab, then this method is no longer suitable to apply [9]. In general, MIS requires surgeons who are well-adapted to endoscopic and navigation surgeries.

Furthermore, the novelty of the system results in higher cost and the need for sophisticated supporting facilities. Wong *et al.,* (2010) also hoped that in the future, there would be an established navigation software system along with more developed special tools for endoscopic tumour surgery, to facilitate this advanced method of treatment [6]. There is a need for future studies with better study design to assess the added value of developing and utilising sophisticated equipment for treating GCT of the bone.

In conclusion, despite the limited number of studies, MIS yields satisfying functional and oncological outcome for GCT around axial bones and long bones. In axial bones, MIS has a particular role in treating GCT of spine and pelvis with high morbidity by negating the need of en bloc resection. There are also certain benefits of treating GCT of long bones with periarticular lesions. The most prominent advantage of MIS is its excellent intralesional visualisation with equipment which has been widely utilised in orthopaedics fields, thus reducing the cost of developing sophisticated new equipment. However, further cost-benefit analysis is needed to justify the addition of these sophisticated equipment.

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Letter to the Editor

Successful Treatment of Isotretinoin Induced Musculoskeletal Pain by Vitamin B12 and Folic Acid

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Abstract

BACKGROUND: Daily supplementation with vitamin B12 and folate, which are the cofactors of the enzymatic reactions involved in Hcy metabolism, can lower plasma levels of Hcy and prevent osteoporosis induced by retinoid

CASE PRESENTATION: We reported six patients with nodulocystic acne who reported musculoskeletal pain after taking isotretinoin which successfully treated with daily supplementation of folic acid and B12.

CONCLUSION: We suggest our colleagues consider these supplements when a patient suffers from musculoskeletal pain following starting isotretinoin and continue the treatment as well. In the end, the authors concluded that robust trials with more patients are needed to establish the efficacy of Vit B12 and folic acid in the treatment of isotretinoin induced musculoskeletal pain.

Introduction

Acne vulgaris is a chronic inflammatory disease affecting the pilosebaceous unit in the skin. Isotretinoin is a vitamin A derivative is the most effective agent in the treatment of acne. A substantial amount of studies reports of adverse effects of isotretinoin on the skeletal system [1], [2], [3], [4], [5]. Namazi and Feily in 2010 suggested that retinoid-induced hyperhomocysteinemia may account for osteoclast overactivity, osteoporosis, and increased risk of bone fracture associated with retinoid use. As a hypothesis, they suggested Daily supplementation with vitamin B12 and folate, which are the cofactors of the enzymatic reactions involved in Hcy metabolism, can lower plasma levels of Hcy and prevent osteoporosis induced by retinoid [4], [5].

Accordingly, we reported six patients with nodulocystic acne who reported musculoskeletal pain after taking isotretinoin which successfully treated with daily supplementation of folic acid and B12.

Six patients (Table 1) had previously been treated with isotretinoin reported musculoskeletal pain after starting isotretinoin for their nodulocystic acne. Three of them around 20 days, one of them one mouth and the other two patients after 45 days of taking isotretinoin reported the musculoskeletal pain. Four of them reported back pain and two reported leg pain. Two of the patients had the history of taking isotretinoin and musculoskeletal pain which caused them to discontinue the treatment. There was not any other rheumatologic or other past history for all of them. Routine labdates were normal too.

Table 1: Patients Treatment and Evolution

Patient	Sex / Age (yrs)	Follow up	Area of musculoskeletal pain	Time of the pain after starting isotretinoin	History of pain after taking isotretinoin	Response to vitb12 and Folic acid
1	F/18	8 months	Back	Around 20 days after starting isotretinoin	Negative	Positive
2	F/21	6 months	Leg	Around 20 days after starting isotretinoin	Negative	Positive
3	F/24	8 months	Back	Around 45 days after starting isotretinoin	Positive	Positive
4	M/20	6 months	Back	Around 30 days after starting isotretinoin	Negative	Positive
5	F/29	8 months	Leg	Around 20 days after starting isotretinoin	Positive	Positive
6	F/22	6 months	Back	Around 20days after starting isotretinoin	Negative	Positive

In all patients, we started Folic Acid 1 mg daily and vitamin B12 injection every two weeks until more than 6 months follow up. After two weeks of treatment, the partial improvement was noted, and over the next week, further reduction in musculoskeletal pain was observed and After 6 weeks the symptoms completely disappeared, and all patients continued their treatment until 6 months.

It has been shown that the amino acid homocysteine level is elevated in patients on isotretinoin treatment for acne, which may be due to the inhibition of cystathionine-beta-synthase by the drug and/or the drug-induced liver dysfunction [4], [5], [6].

Higher levels of Hcy have been linked to higher fracture rate in the elderly and have been noted as a new risk factor for osteoporosis. Also, hyperhomocysteinemia has some adverse effects on the extracellular bone matrix by damaging collagen crosslinking [4], [5]. Accordingly, we think Daily supplementation with vitamin B12 and folate, maybe by affecting on enzymatic reactions involved in Hcy metabolism, could lower their plasma levels of Hcy and musculoskeletal pain. We suggest our colleagues consider these supplements when a patient suffers from musculoskeletal pain following starting isotretinoin and continue the treatment as well. In the end, the authors concluded that robust trials with more patients are needed to establish the efficacy of Vit B12 and folic acid in the treatment of isotretinoin induced musculoskeletal pain.

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