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Cryptosporidium Sp. Findings and Its Symptomatology among Immunocompromised Patients

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Keywords: Cryptosporidium sp.; Diarrhea; Protozoa

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Abstract

BACKGROUND: *Cryptosporidium sp.* is an apicomplexan protozoa, and it is related to an immunocompromised state. As it develops diverse clinical manifestations, mild to life-threatening conditions, administration of anti-parasitic medication and its management remain problematic.

AIM: The study aimed to provide Cryptosporidiosis symptomatology and its prevalence among HIV-infected patients in a tertiary referral hospital, Haji Adam Malik General Hospital, Medan, Indonesia.

MATERIAL AND METHODS: Symptomatology was noted using short-questionnaire, and laboratory findings were obtained from the hospital medical record registry on the same day of admission. We enrolled 24 patients were suffered from HIV infection for a certain period and more than one-week diarrhoea including 18 males and 6 females. Routine faeces examination using wet mount, Kinyoun-gabet, and trichrome staining was performed for all samples in Parasitology Department, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia. Numerical data were evaluated using the Mann-Whitney test while Fisher Exact test was used to determine any association between categorical variables.

RESULTS: Our study found that 8 of 24 patients were positive with *Cryptosporidium sp.* while its symptomatology including abdominal cramp (66.7%), nausea and vomiting (70.8%), and fever (62.5%) is prevalent from our study. We obtained significant association between CD4 cell count (p = 0.006), diarrhea duration (p = 0.007), abdominal pain (p = 0.005), and nausea and vomiting (p = 0.021) with cryptosporidiosis.

CONCLUSION: High consideration of several symptoms related to cryptosporidiosis leads a clinician to initiate prompt management particularly in a high-risk population.

Introduction

Cryptosporidium sp. first appearance was noted in 1976 when the organism infected a three-year-old child with self-limiting enterocolitis while in early 1980s Cryptosporidium sp. had been linked to the immunocompromised population [1]. *Cryptosporidium hominis* and *Cryptosporidium parvum* are commonly associated with human infection. Ingestion of fully and chlorine resistant sporulated oocyst also initiate the infection in human. After the oocyst ingestion, upper small bowel will become the milieu for infection, and it develops into the further stage, as the sporozoite penetrates the enterocyte and transforms into the mature form [2]. The global burden for cryptosporidiosis was noted from several reports in Africa and Asia, 11.3% in Turkey [3], 6% in Iran [4], and the highest prevalence found in Uganda as much as 73.6% [5]. Indonesia

data prevalence of the infection is available based on two studies ranging from 4.9-52.5% [6], [7]. Most studies revealed that the prevalence variation of *Cryptosporidium sp.* among HIV-infected population occurred since the limitation of using proper diagnostic method particularly in developing regions is established [8].

The infection could produce broad clinical manifestations ranging from mild to life-threatening symptoms depends on the presence of comorbidities particularly HIV/AIDS status, malignancy, patients undergoing dialysis, and organ transplantation [9]. *Cryptosporidium sp.* as the most common species found in the HIV population and it is linked to higher mortality among population. Diarrhoea associated with cryptosporidiosis is watery or mucoid diarrhoea that persists ranging from acute to chronic form which caused several clinical implications to the patients including severe dehydration, abdominal pain, nausea and vomiting, fever, malnutrition, and significant weight loss [10]. Medication for cryptosporidiosis still becomes a problematic issue as its effectivity among the certain population is questionable while U.S Food and Drug Administration (FDA) only approved nitazoxanide which does not effectively eradicate the organism in immunocompromised hosts [11].

There has been no study related to symptomatology and cryptosporidiosis in Indonesia recently. Our study aimed to provide the prevalence of cryptosporidiosis among the HIV population and its symptomatology in a single institution, tertiary referral centre. Assessment and evaluation may be used to determine the likelihood that patients with certain symptomatology have *Cryptosporidium sp.* infection.

Methods

Study location and population

We enrolled in a cross-sectional study conducted in one tertiary referral centre, Haji Adam Malik General Hospital, Medan, Indonesia. The study included 18 males and 6 females who had met the inclusion criteria, for instance: HIV positive patients, suffered from more than one-week diarrhoea and admitted to the outpatient clinic or hospitalisation ward. WHO defines diarrhoea as three or more loose stool per day for 72 hours (acute for no more than 13 days; chronic or persistent diarrhoea is defined as diarrhoea for 14 days or more). Medical record registry was assessed to fulfil several variables related to demographic data (gender and age), and laboratory results including CD4+ cell count, haemoglobin, hematocrit, leukocyte, thrombocyte, urea, creatinine, and glucose levels. The laboratory results were obtained from Haji Adam Malik medical record registry on the same day of their admission to the hospital. A short questionnaire was also used to determine symptomatology of all participants consisting of three-panel question about the presence of abdominal pain, nausea and vomiting, and fever; the physical examination was performed to ascertain the symptomatology.

Parasitological examination

All participants had given their approval to include in the study. On the day of admission, a small faecal sample container was given to the patients. On the following day, sample collection was performed by our laboratory staff; no preservation was applied to samples. In the same day of sample collection, the microscopic examination was carried out in the Parasitology Department, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia. A battery of examination was conducted to determine the presence of Cryptosporidium sp., and other pathogenic parasites including routine faeces examination using wet mount, Kinyoun-gabet, and trichrome staining. As a matter of limitation in the study, bacterial culture and viral marker examination were not screened. The study had been evaluated by Health Research Ethical Committee of Faculty of Medicine, Universitas Sumatera Utara and obtained their approval.

Statistics and study approval

The research form and questionnaire were collected then transformed into one table in Microsoft Excel. Statistical Package for Social Sciences 21 (SPSS Inc. version 21) in univariate fashioned using Fisher Exact test was used to determine any significant presence relationship between several variables and Cryptosporidiosis. Furthermore, numeric data were evaluated using the non-parametric Mann-Whitney test to evaluate the mean difference between positive and negative groups of cryptosporidiosis including age, CD4+ cell count, diarrhoea duration and several laboratory results.

Results

We proved that 8 of 24 patients were positive for Cryptosporidium sp. and one patient was positive with double infection, *Cryptosporidium sp.* and *Blastocystis hominis*. The demographic data were depicted in Table 1. One-week diarrhoea was commonly found in our study while there was one patient who had suffered from 24-week-diarrhoea. Symptomatology among patients was also assessed; it revealed that abdominal pain (66.7%), nausea and vomiting (70.8%), and fever (62.5%) were prevalent

among participants. Laboratory results baseline also listed in Table 1.

The symptomatology including abdominal pain, nausea and vomiting, and fever was evaluated using short questionnaire while abdominal pain and nausea and vomiting had a significant association with the infection, p = 0.005 PR 21.00 95% CI 2.372-185.93 and p = 0.021 PR 0.086 95% CI 0.011-0.673 respectively. Abdominal pain was complained by 6 of 8 cryptosporidiosis patients. Nevertheless, we proved descriptively that most of the patients who complained about nausea and vomiting were demonstrated as negative for *Cryptosporidium sp.* Several variables, then, was also statistically evaluated using Mann-Whitney testing since the data was not normally distributed. We obtained significant association between CD4+ cell count (p = 0.006), diarrhea duration (p = 0.007), and leukocyte (p = 0.038) with cryptosporidiosis.

Table 1: Several variables among cryptosporidiosis patients

Characteristics	Cryptosporidiosis (Mean ± SD)		p-value
	Positive (8/33.3%)	Negative (16/66.7%)	
Age (year)	36.50 ± 7.28	42.93 ± 12.10	0.238
Gender (Male/Female)	5/3	13/3	0.621
CD4+ cell count (/µL)	87.37 ± 141.16	319.50 ± 160.39	0.006*
Diarrhea duration (weeks)	7.25 ± 7.34	1.81 ± 1.10	0.007*
Laboratory results			
Hemoglobin (gr/dL)	8.98 ± 1.42	10.64 ± 1.88	0.070
Thrombocyte (cell/ µL)	195,250.00 ± 81,32	229,537.50 ± 117,216.30	0.490
Hematokrit (%)	37.10 ± 11.83	35.37 ± 7.09	0.834
Leukocyte (cell/µL)	6,428.75 ± 2,475.04	9,238.12 ± 3,220.81	0.038*
Glucose levels (mg/dL)	86.12 ± 15.19	117.18 ± 76.95	0.320
Creatinin (mg/dL)	0.88 ± 0.60	2.04 ± 2.33	0.291
Ureum (mg/dL)	32.40 ± 21.51	68.49 ± 73.15	0.320
Symptomatology (Yes/No)			
Abdominal pain	6/2	2/14	0.005*
Nausea and vomiting	3/5	14/2	0.021*
Fever	5/3	10/6	1.000

*, statistically significant.

Discussion

As part of the neglected tropical disease, cryptosporidiosis still becomes problematic since it can produce several symptoms ranging from mild, self-limiting disease to severe dehydration or death [12]. Also, *Cryptosporidium sp.* is categorised as a highly infectious organism, infectious dose until it can initiate infection as low as 10 oocysts [13]. Immunocompromised status particularly HIV/AIDS infection also had been linked to the infection and its presence become a leading causative agent can produce the debilitating condition among the population. Inadequacy of the diagnostic method of Cryptosporidium sp. also challenges the findings of the organism [14].

Immunologic response of *Cryptosporidium sp.* invasion is emphasised from literature, the elevation of certain cytokines such as interferon-gamma (IFN-Y), interleukin-1β (IL-1β), tumour necrosis factor-alpha (TNF-α), interleukin-10 (IL-10) and transforming growth factor-β (TGF- β), is evident [15]. Intestinal epithelial cells (IECs) detect *Cryptosporidium sp.* and initiate the recognition of the organism to CD4+ cell via Major Histocompatibility Complex-II (MHC-II) [16]. As HIV destruct CD4+ cell, cell-mediated immunity arm among HIV-infected individuals will severely impair resulting in ineffective eradication of the organisms [17], [18]. Our study proved a significant difference in CD4+ cell count between two groups of patients, infected- and uninfected-group (p = 0.006). Cryptosporidiosis group had CD4+ cell count which was significantly lower than the negative group. Furthermore, the likelihood of getting the infection increases after CD4+ cell count falls to 50 cell/mm3 [19], [20].

Morphologic changes including loss of microvilli resulting in disruption of several enzymes involved in the digestion process are crucial for developing symptoms among infected-individual [21]. Cryptosporidiosis patients mostly complained of watery diarrhoea, abdominal pain, anorexia, flatulence, nausea and vomiting, and mild fever. Significant association was obtained from our study particularly abdominal pain was related to cryptosporidiosis (p = 0.005) while nausea and vomiting was significantly associated with cryptosporidiosis in inversion manner (p = 0.021) since our study showed most patients complained the symptoms were negative for *Cryptosporidium sp.* (infected-patients complained nausea and vomiting versus uninfected-patients; 37.5% versus 87.5%). There are few published articles linked *Cryptosporidium sp.* with the presence of certain symptoms among the HIV population in Indonesia. Notwithstanding, several studies revealed the prevalence and symptoms produced during the infection, for instance, Wanyiri et al., [22] conducted a study in Kenya found 56 of 164 (34%) of HIV/AIDS patients were positive for *Cryptosporidium sp.* A significant association between abdominal pain and vomiting was also evident in the study. In Iran, Izadi et al., [23] also revealed similar results that two common symptomatology commonly found in cryptosporidiosis patients including abdominal pain and nausea and vomiting had a significant association with the infection.

Association between chronicity and cryptosporidiosis among the HIV population stated from the literature [24], [25]. While our study obtained a significant difference between two groups, the mean of diarrhoea duration among infected group versus uninfected group was 7.25 versus 1.81 (p = 0.007). The condition may be clearly explained since the mainstay of therapy among HIV/AIDS population is the restoration of immune function. Immune reconstitution shortly occurred after effective administration of combination antiretroviral therapy has been associated with parasitic clearance. Duration of symptoms especially diarrhoea is directly linked to the immune response in each [26].

Also, symptomatic management including

replacement of fluids and electrolytes is essential to prevent further complications. Furthermore, administration of any anti-parasitic medication remains debatable [27].

In conclusion, the study has revealed several significant results by using a smaller sample size, yet it becomes the limitation of the study. Also, the evidence also has brought several clinical significances among cryptosporidiosis patients and the symptomatology. Therefore, the combination of HIV/AIDS positive status, diarrhoea, and abdominal pain increase the likelihood of a patient having the infection. The infection remains neglected not only in the general population but also among HIV/AIDS patients. The physician should increase awareness of the infection among HIV/AIDS patients since the infection and diarrhoea shown to be an independent predictor of mortality in the population.

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Low Degree Hyaluronic Acid Crosslinking Inducing the Release of TGF-B1 in Conditioned Medium of Wharton's Jelly-Derived Stem Cells

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Abstract

BACKGROUND: Presently, the application of stem cells and their paracrine effect for anti-ageing therapy has commenced. Wharton's jelly-derived stem cell conditioned medium (WJSCs-CM) is renowned for increasing proliferation, migrate ageing skin fibroblasts and increase consumption of extracellular transforming growth factor-β (TGF-β). With more than 85% of frequently used dermal filler procedures are hyaluronic acid fillers (HA), a mixture of both with optimal HA crosslinking degree has not yet been identified.

AIM: This study aimed to determine the discrepancies in the results of various HA crosslinking degree in WJSCs-CM concerning various levels of growth factors (GF).

METHODS: Conditioned medium was obtained from mesenchymal stem cells Wharton's jelly of the newborn umbilical cord with caesarean section procedure, fabricated with hypoxia method (HCM). HA was obtained from preparations on the market with crosslinking degrees of 3%, 4%, and 10%. GF levels were measured using sandwich ELISA method based on the protocol provided by anti-TGF-β1, platelet-derived growth factor (PDGF), and basic fibroblast growth factor (bFGF) antibody producers (Cloud-Clone Corp®, Texas, USA).

RESULTS: Low degree HA crosslinking (3% and 4%) elevated TGF-β1 release in WJSCs-CM. HA crosslinking did not provoke increased levels of PDGF and bFGF in WJSCs-CM, both at low and higher degrees.

CONCLUSION: Low degree HA crosslinking induced the increase of TGF-β1 release in WJSCs-CM.

Introduction

The intrinsic ageing process of the skin is accelerated by several extrinsic factors, with the most frequent is sun exposure. This process is known as photoaging skin, premature ageing skin, or ageing skin [1], [2]. Fibroblasts are the cells being most responsible for the occurrence of ageing skin manifestations [3]. One method in ageing skin therapy perceptible to be less invasive is filler injection. In filler injection, utilised materials range from autologous materials such as collagen, fat cells, fibroblasts, to synthetic materials [1], [4]. More than 85% of dermal filler procedures often used as ageing tissue fillers are hyaluronic acid (HA) [5].

Today experts are turning their attention away from stem cell transplants to stem cell products because the limitations of treatment for diseases using heterologous stem cells are rejection reactions from recipients [6]. Wirohadidjojo *et al.* found the benefit of Wharton's jelly-derived stem cell conditioned medium (WJSCs-CM) in the recovery of human ageing skin fibroblast activity [7]. It was reported that WJSCs-CM could increase proliferation, fibroblast migration and increase extracellular transforming growth factor-β (TGF-β) consumption.

This dermal filler injection with HA crosslinking would give immediate results in clinically

reducing wrinkles after being injected, but these results possess no durability as its bane, with occurrence possibility of biocompatibility, encapsulation and granuloma formation [8], [9]. While WJSCs-CM is used to provide indirectly visible improvement in relieving wrinkles clinically, after a certain period the results commence to appear. This is due to the time taken for the growth factors (GF) to stimulate autologous cell or fibroblasts regeneration [9], [10], [11].

If HA crosslinking combination with WJSCs-CM is envisioned to be promising to possess better outcomes and benefits compared to a sole HA crosslinking, clinicians can obtain additional biological material in the form of stem cell products, as a combination of skin rejuvenation methods to increase the effectiveness of the method. The risk of rejection due to the use of heterologous stem cells in photoaging skin sufferers can be avoided, and the necessity of using autologous material can be eliminated.

The optimal HA crosslinking degree has not yet been identified. This study aimed to determine the effect of crosslinking HA degrees on GF levels in WJSCs-CM.

Methods

This study was experimental in vitro with post-test control group design. This study was conducted at the Research Laboratory of the Department of Dermatology and Venereology, Faculty of Medicine, Universitas Gadjah Mada, Radiopoetro Building, Yogyakarta, Indonesia.

Inclusion criteria of mesenchymal stem cell donor were the newborn's umbilical cord from normal childbirth or cesarean section, term and healthy. This study had received approval from the Ethics Committee of the Faculty of Medicine, Universitas Gadjah Mada (Ref: KE/FK/0845/EC/2018). Mesenchymal stem cell (MSC) culture samples were subcultured to passage > 4. Conditioned medium (CM) was obtained from the stem cell laboratory, Institute of Tropical Disease (ITD), Universitas Airlangga. CM was taken from mesenchymal stem cells Wharton's jelly of newborn's umbilical cord with caesarean section and fabricated with the hypoxic method (hypoxic conditioned medium = HCM) with 1% nitrogen content and harvested at 72 h.

HA was obtained from preparations in the market with crosslinking degrees of 3%, 4% and 10%, from the fermentation of *Staphylococcus equine* bacteria (NASHA = non-animal stabilised hyaluronic acid). WJSCs-CM was isolated from the embryoid body of Wharton's jelly mesenchymal stem cell culture with the content of 50% dissolved in DMEM ± 1% FBS (Gibco™, Massachusetts, USA). HA and WJSCs-CM were mixed using the three-way connecting syringe method which was mixed repeatedly until homogeneous. The HA used in this combination was of 30% preparation concentration in WJSCs-CM. Comparison of HA and WJSCs-CM was 0.3 ml HA:0.7 ml WJSCs-CM. GF levels were measured in a solution or medium by sandwich ELISA method based on the protocol provided by anti-transforming growth factor-β1 (TGF-β1), platelet-derived growth factor (PDGF), and basic fibroblast growth factor (bFGF) antibody producers (Cloud-Clone Corp®, Texas, USA). Data were presented as mean ± SD.

Results

In HCM without HA crosslinking, the level of growth factor for TGF-β1 was 28.51 ± 9.41 pg/ml, PDGF-BB 144.79 ± 67.57 pg/ml, and bFGF 0.00 pg/ml. The HA group of low degree crosslinking (3% and 4%) resulted in the release of TGF-β1 in WJSCs-CM much higher compared to the group without HA crosslinking and crosslinking of 10%. TGF-β1 level in 3% HA crosslinking was 170.89 ± 128.36 pg/ml and 4% HA crosslinking was 105.26 ± 18.44 pg/ml. Whereas for the 10% HA crosslinking group, TGF-β1 level was only 19.62 ± 15.20 pg/ml, even lower than the group without HA crosslinking.

Table 1: Growth factor levels in cross-linked HA and HCM

Group	TGF-β1 (pg/ml) Mean ± SD	PDGF-BB (pg/ml) Mean ± SD	bFGF (pg/ml) Mean ± SD
HCM	28.51 ± 9.41	144.79 ± 67.57	0.00 ± 0.00
HCM + HA 3%	170.89 ± 128.36	141.89 ± 25.64	0.00 ± 0.00
HCM + HA 4%	105.26 ± 18.44	101.05 ± 19.15	0.00 ± 0.00
HCM + HA 10%	19.62 ± 15.20	102.02 ± 13.10	0.00 ± 0.00

HCM: hypoxic conditioned medium; HCM+HA 3%: conditioned medium + hyaluronic acid crosslinking grade 3%; HCM+HA 4%: conditioned medium + hyaluronic acid crosslinking grade 4%; HCM+HA 10%: conditioned medium + hyaluronic acid crosslinking grade 10%.

As for PDGF-BB levels, GF levels were reduced in all degree HA crosslinking groups. For bFGF, no release of GF was perceptible, with or without HA crosslinking. Contrary to TGF-β1, low degree HA crosslinking (3% and 4%) did not elevate PDGF-BB and bFGF levels in WJSCs-CM.

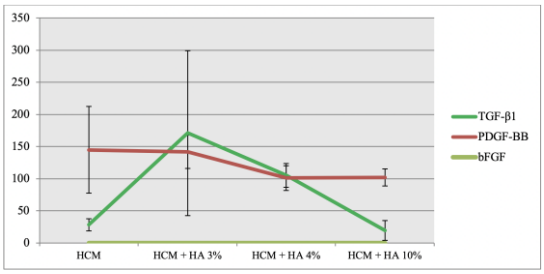

Figure 1: Growth factor levels in crosslinked HA and HCM; HCM: hypoxic conditioned medium; HCM+HA 3%: conditioned medium + hyaluronic acid crosslinking grade 3%; HCM+HA 4%: conditioned medium + hyaluronic acid crosslinking grade 4%; HCM+HA 10%: conditioned medium + hyaluronic acid crosslinking grade 10%

Discussion

In this study TGF-β1, PDGF-BB and bFGF were selected in the analysis due to being the most important GF related to senescent fibroblasts in the ageing skin. Fibroblasts are the cells most responsible for the onset of ageing skin [3]. Fibroblasts are the main cellular elements in the human dermis because these cells are responsible for the synthesis of the extracellular matrix, both collagen, elastin synthesis, and the synthesis of other basal dermis substances.

Ultraviolet A (UVA) exposure in the long term attenuates dermal structures causing premature photoaging. Reactive oxygen species (ROS) yielded from UV radiation leads to oxidation at the cellular level, clinically presented by skin inflammation, erythema, tanning, immunosuppression, photoaging, and skin cancer. Antioxidant molecules (i.e. glutathione, carotenoids, ascorbate, and tocopherol) and proteins (i.e. ferritin, heme oxygenase, glutathione peroxidase, superoxide dismutase, catalase, etc.) ruled as the defences against UVA. UVA nevertheless can transgress to the dermis, altering the dendritic cells, matrix metalloproteinase (MMP), T-lymphocytes, mast cells, endothelial cells, and fibroblasts [12].

Human skin fibroblasts activity is very dependent on the race of various cytokines and GF. Most responsible GF for human skin fibroblast activity is transforming growth factor-β or TGF-β [13]. Exposure to UVA was shown to inhibit the proliferation of fibroblasts, inhibiting the synthesis of collagen by fibroblasts and producing collagen damage due to increased activity of MMP enzyme that yielded collagen fibres breakage [14]. Collagen fibres breakage would lead to a decrease in the mechanical power that yielded wrinkled fibroblasts, and TGF-βII receptors on cell membranes would become sealed against their ligands [14], [15]. Thus, the TGF-β signalling pathway would be disrupted, whereas the TGF-β-Smad signalling pathway is the most important signalling in fibroblast proliferation and collagen synthesis [15].

In this study, low degree HA crosslinking elevated TGF-β1 release in WJSCs-CM. Low degree HA crosslinking provoked the release of GF greater than the higher HA crosslinking degree. HA crosslinking did not provoke increased levels of PDGF-BB and bFGF in WJSCs-CM, both at low and higher degrees. There was almost no difference in other levels of GF, namely PDGF-BB and bFGF between groups of HCM mixtures with various HA crosslinking degrees. This was likely due to HA crosslinking in the HCM mixture causing binding of proteins including GF in HCM so that GF levels would be reduced.

It had been reported that the addition of monomeric exogenous HA to fibroblast culture triggered TGF-β signalling and collagen production. HA which was involved in wound healing and its biological properties depended on its molecular size [16]. Inhibition of HA synthesis in dermal fibroblasts had been shown to cancel the proliferation induction of TGF-β1 [17]. In the study of Quan et al., it was known that HA filler locally injected into the skin would fill the space and push the area around the extracellular matrix (ECM) so that fibroblasts underwent morphological extension [14]. This elongation of fibroblasts was associated with upregulation of the TGF-β signalling pathway.

Quan et al., and Fisher et al., showed that the decrease in collagen content in the dermis would result in a decrease in mechanical power which caused fibroblasts (which were in a statically bound state by collagen fibres) to morphologically became shrinking, so that the TGF-β receptor became closed and did not respond to TGF-β. This was evidenced by a dermal filler of HA injection on ageing skin which increased mechanical power, would cause an increase in TGF-β receptor expression, a change in the morphology of fibroblasts to be longer, and an increase of fibroblast proliferation with the content of procollagen-1. Reduced fibroblast size or decreased mechanical power of the fibroblasts caused the failure of TGF-β/Smad signalling due to decreased expression of TGF-βII receptors [14], [15].

In the processes of wound repair, expression dynamics of growth factor and cytokines, such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), epidermal growth factor (EGF), exhibited characteristics of temporal and spatial regulation. Impaired wound healing was associated with alteration in growth factors expression pattern [18]. WJMSCs-CM had been shown to increase the regulation of re-epithelialization gene expression, TGF-β, neovascularisation (hypoxia-inducible factor-la) and fibro-proliferation (plasminogen activator inhibitor-1), with an increase of normal human skin fibroblasts proliferation and to aid wound healing on injured mice skin [19]. In a study by Wirohadidjojo *et al*., the benefits of WJSCs-CM was discovered in the recovery of human ageing skin fibroblasts activity due to UVA exposure by increasing proliferation, migration of aging skin fibroblasts and increasing consumption of TGF-β [7]. Conditioned medium derived from fat cells was also proven to stimulate the production of TGF-β1, immunoglobulin binding protein-7 (IGBP-7), collagen type 1 and fibronectin, as well as to restore collagen synthesis through increased procollagen-1 gene expression, suppress MMP-1 release, and restore human skin fibroblast proliferation [20], [21].

In conclusion, low degree HA crosslinking induced the increase of TGF-β1 release in WJSCs-CM. HA crosslinking did not provoke increased levels of PDGF and bFGF in WJSCs-CM, both at low and higher degrees.

Authors Contribution

Authors equally contributed to design, data compiling and analysis, and the composing of the manuscript.

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Basic Science

Efficiency of Crystal Violet Stain to Study Mitotic Figures in Oral Epithelial Dysplasia

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Keywords: Crystal violet stain; Mitotic figures; Oral epithelial dysplasia; Oral cancer

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Abstract

AIM: To evaluate mitotic activity in the different grades of oral epithelial dysplasia using 1% crystal violet stain.

MATERIAL AND METHODS: A descriptive study was conducted in the Department of Histopathology of the Post Graduate Medical Institute, Lahore on a total of thirty-three cases of the Oral Epithelial Dysplasia (OED). Fresh, frozen paraffin-embedded archival tissue blocks were collected from Lahore General Hospital, Lahore & Oral & Maxillofacial Surgery Department of Nawaz Sharif Hospital, Yakki Gate, Lahore. The representative sections were taken and, after processing, mounted on glass slides and stained with H&E and crystal violet stains. The stained slides were then examined under an optical microscope. The efficacy of 1% crystal violet stain to identify mitotic figures in the different grades of oral epithelial dysplasia was assessed with the sample t-test. A difference of $p < 0.05$ was considered to be significant.

RESULTS: A comparison of the mitotic figure count in two categories in sections stained with both stains showed a statistically significant difference. An increase in the mean mitotic count was noted in the sections of OED stained with crystal violet in comparison to the sections of OED stained with H&E which was statistically significant ($p = 0.00$).

CONCLUSION: Counting of mitotic cell is the rapid and simplest way of evaluating the proliferative activity of cells. Crystal violet stain can be a rationalised step in the staining of mitotic figures compared to the usual H&E staining and can be employed as a selective stain during routine histopathological procedures.

Introduction

There are many lesions in the oral cavity which are characterised by various morphological and cellular changes. These lesions may proceed to cancer. Dysplastic changes are considered to be the most important of these identifiable changes in the oral cavity [1].

One of the most complex topics of head and neck pathology is the precancerous lesion. The idea of step-by-step development of malignancy in the oral cavity mucosa is well recognised. The presence of dysplastic epithelium may play an even more important role in foretelling the development of cancer compared to the clinical presentation [2].

Dysplasia is a Greek word which means abnormal, atypical proliferation. Reagon used the term “dysplasia” in 1958 to describe cells exfoliated from a lesion of the uterine cervix. It is encountered predominantly in epithelia. In 1977, Pindborg defined epithelial dysplasia as “a lesion in which part of the thickness of the epithelium is replaced by cells

showing varying degrees of cellular atypia." In 1981, Burkhart and Maerker specified that the degree of dysplasia is defined "as a measure of tissue and cellular deviation from the normal," whereas Kumar et al., (1992) stated that dysplasia is a "disturbance in the maturational sequence of the stratified squamous epithelium and disturbance in cell kinetics of the proliferative compartment with cytological changes [3].

Freedmen and Kerpel (1995) explained it as a diagnostic term which is used to describe the histopathological changes seen in premalignant disorders of the oral mucosa. Oral Epithelial Dysplasia (OED) is the diagnostic term which describes the histological and cellular changes that are seen in a chronic, progressive and premalignant disorder of the oral mucosa. OED is not linked with any definite clinical picture. However, lesions like leukoplakia and erythroplakia are typically connected with dysplastic changes. Therefore, white, red, or mixed white and red lesions are those which have most commonly revealed OED. It has also been constantly seen in the mucosa next to the malignancy in patients with invasive SCC [4].

There is more of a risk of the development of oral cancer in oral potentially malignant disorders which are diagnosed as OED. The studies have reported a transformation rate ranging from approximately 6.6-36.4% after the mean follow-up periods of 1.5-8.5 years. The definite mechanism of neoplastic transformation is not clearly understood and it is not unavoidable that a dysplastic lesion will lead to malignancy [5].

Epithelial dysplasia is found in 5-25% cases of leukoplakia. It is suggested that a histological report should always describe the absence or presence of epithelial dysplasia and also account for the determination of the severity of epithelial dysplasia in the case of its presence. On the other hand, erythroplakia, which is a less common lesion than leukoplakia, almost consistently reveals epithelial dysplasia [4].

The diagnosis of dysplasia is described in terms of the existence of particular histological and cytological characteristics [6]. A chain of subtle changes occurs in dysplasia, which suggests the development of anaplasia. Dysplasia is a reversible condition, and is hence not yet cancerous [7].

Dysplasia is characterised as unusual, abnormal, proliferation occurring in the epithelium. In most cases, dysplastic changes are the earliest microscopic evidence that represent the progression of malignancy [3]. Mitotic figures play an important role in the assessment of cellular proliferation and act as a prognostic sign in OED and OSCC [8].

Mitosis involves the division of mother cells into two daughter cells that look alike. These are further subdivided into prophase, metaphase, anaphase, and telophase, which are seen in the histological sections. There are many nuclear abnormalities like pyknotic nuclei, micronuclei, binucleation, an increase in the quantity of mitotic figures, and abnormalities in the number of mitotic figures as a consequence of defects of mitosis. One of the most common findings in OED and OSCC is presence of abnormal mitotic figures or an increase in the number of mitotic figures. Mitotic figures are one of the important criteria that carry importance in the grading of OED [9].

Atypical mitotic figures or an increase in the number of mitotic figures is one of the standards for the grading of OED according to the WHO [8].

Abnormal and increased mitosis reflects genetic injury. Hence, identifying and quantifying mitotic cells is an important aspect of the histological grading system which is used for determining the prognosis of premalignant and malignant lesions [5].

Mitosis serves as the basis for cell proliferation and the study of mitosis plays an important role in determining the aggressiveness and prognosis of lesions. Numerous authors have reported various stains and techniques which can detect or study mitotic activity [10].

Previous studies have shown that there are various selective stains such as toluidine blue, giemsa and crystal violet which can detect chromatin patterns [9]. Among these, crystal violet is the one which is used to study chromosomal pattern in cells based on the hydrolysis of DNA [8]. The objective of the current study was to evaluate mitotic figures in the oral epithelial dysplasia using crystal violet staining over routine H&E staining in order to judge its reliability in the early diagnosis of oral precancerous and cancerous conditions.

Table 1: Histological Grading of Oral Epithelial Dysplasia (Smith and Pindborg, 1969)

	Type of change	Severity of dysplasia		
1	Drop-shaped retepegs	None (0)	Slight (2)	Marked (4)
2	Irregular epithelial stratification	None (0)	Slight (2)	Marked (5)
3	Keratinization of cells below keratinised layer	None (0)	Slight (1)	Marked (3)
4	Basal cell hyperplasia	None (0)	Slight (1)	Marked (4)
5	Loss of intercellular adherence	None (0)	Slight (1)	Marked (5)
6	Loss of polarity	None (0)	Slight (2)	Marked (6)
7	Hyperchromatic nuclei	None (0)	Slight (2)	Marked (5)
8	Increased nucleo-cytoplasmic ratio in basal and prickle cell layers	None (0)	Slight (2)	Marked (6)
9	Anisocytosis and anisonucleosis	None (0)	Slight (2)	Marked (6)
10	Pleomorphic cells and nuclei	None (0)	Slight (2)	Marked (6)
11	Mitotic activity	None (0)	Slight (1)	Marked (5)
12	Level of mitotic activity	None (0)	Slight (3)	Marked (10)
13	Presence of bizarre mitoses	None (0)	Slight (6)	Marked (10)

Material and Methods

The present study was conducted on a total of 33 specimens of OED which were further divided into two groups for staining with H&E and crystal violet, respectively. Here, the 1% crystal violet staining was performed according to the modified Fraser FJ

method (11). The prepared slides were examined under a CX31 Olympus microscope. Histological grading of oral epithelial dysplasia was performed according to the Smith and Pindborg classification system [12] (Table 1 and 2).

TABLE 2: Scoring of dysplasia (Smith and Pindborg, 1969)

Total score (EDI)	Grade
0-10	No dysplasia
11-25	Mild Dysplasia
26-45	Moderate dysplasia
46-75	Severe dysplasia

Criteria for identification of Mitotic Cells

To label a structure as a mitotic figure, criteria given by Van Deist et al. were used in this study [13]:

1. Cells have conceded prophase, indicating the absence of nuclear membrane.

2. The presence of condensed chromosomes (clear, hairy extensions of nuclear material) must exist either in the clotted form (beginning metaphase), in a plane (metaphase or anaphase) or in separate clots (telophase).

3. Two parallel, clearly separate chromosome clots to be counted as if they are separate.

These criteria helped to differentiate between different phases of mitosis from other, commonly seen nuclear changes like apoptosis, pyknotic nuclei, and karyorrhexis.

Counting of Mitotic Figures

Ocular graticule was calibrated using a light microscope (Leica, DM 1000) by the method described by Culling [14].

- A 20 × 20 square (area = 1mm^2) grid eyepiece graticule, engraved on a disc, was placed inside the eyepiece of the microscope.
- Stage micrometre, a 3 × 1 inch slide onto which a 1 mm scale was divided into 100 equal divisions is engraved, was placed under the objective.
- A 10X objective was selected and focused on the stage micrometre scale.
- The number of transverse and vertical segments of ocular graticule squares equal to an exact number of divisions of the stage micrometre scale was determined.
- Every 4 transverse and vertical segments of ocular graticule squares were equal to 10 stage divisions, therefore:

100 stage divisions = 1 mm = 1000 µm.

1 stage division = 1000/100 = 10 µm.

1 stage division = 10 µm.

10 stage division = 10 x 10 = 100 µm.

4 segments of graticule = 10 stage divisions = 100 µm.

1 segment of graticule = 100/4 = 25 µm

- The area of the graticule was calculated by multiplying the calibrated factor 25µm with transverse and vertical segments of 20 small squares of the graticule as follows:

(20 × 25 = 500 µm. or 0.5 mm.) × (20 × 25 = 500 µm. or 0.5 mm.)

or 0.5 × 0.5 = 0.25 mm^2.

- The counting of mitotic figures was performed by superimposing the ocular graticule onto the tissue preparation.
- Each slide was then viewed under a high-power field (400X) for counting of mitotic figures using ocular grid eyepiece. Counting of mitotic figures was done in stepladder fashion in 10 different high-power fields. The area selected for the counting of mitotic figures included the most invasive part and the most cellular part of the tissue. The areas showing necrosis, inflammation, tissue folds and calcifications were not considered for counting.

Mitotic count was described as the mitotic count per grid field and the mitotic count per square millimetre.

The mitotic count per grid field was calculated as:

Mitotic count/grid field = Total number of mitotic figures observed/Number of grid fields counted

The mitotic count per square millimetre was calculated as follows:

i. *Area of 1 grid field = 0.25 mm^2* [14]

ii. Mitotic count per square millimetre = Average number of mitotic figures per grid field/0.25 mm^2

Each slide was observed by two separate obs ervers without any exchange of information regarding study sample details.
Observations made by each observer regarding numb er of MFs were recorded separately and average valu e was calculated for both observations. The data was entered and analyzed using the Statistical Package for Social Sciences (SPSS) [Version 20]. Quantitative data was presented as Mean and Standard deviation. Qualitative data was presented as frequency and percentage. Comparison of staining of mitotic figures between crystal violet and H & E staining in oral epithelial dysplasia was done using T-test. p-value ≥ 0.05 was significant.

Results

A total of 33 patients presenting with different grades of OED was collected from Lahore General Hospital, Lahore & Oral & Maxillofacial Surgery Department of Nawaz Sharif Hospital, Yakki gate, Lahore. Out of the 33 patients of OED, n = 23 (69.7%) were males and n = 10 (30.3%) were females, with a male to female ratio of 2.3:1 (Figure 1).

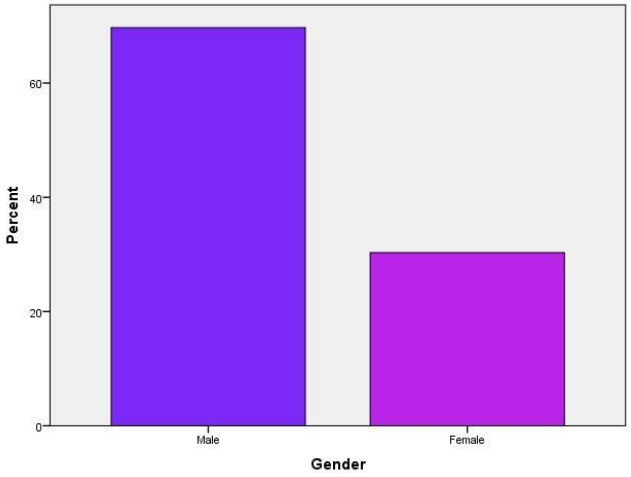

Figure 1: Gender distribution

The overall mean age of the patients reported with OED was 48.81 ± 13.05 years, with most patients presenting between the ages of 40 and 60 years. The age range in females was 35-85 years, with the youngest female patient presenting at the age of 35 years and oldest at the age of 85. A quite similar age range was seen in males, from 33-80 years. The minimum and maximum ages in male patients was 33 years and 80 years, respectively. The data indicate that the age incidences in both genders are quite similar (Figure 2).

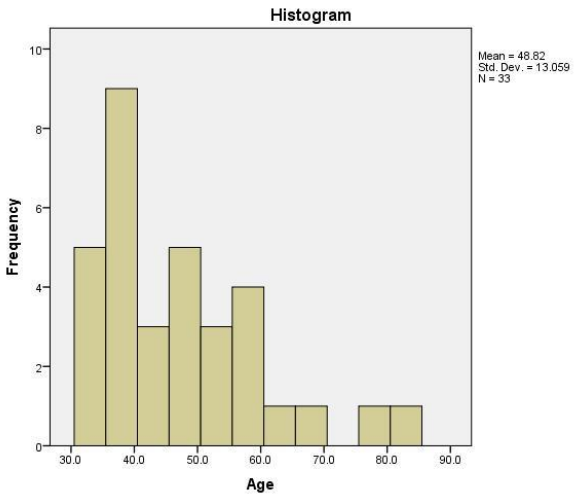

Figure 2: Age incidences in both genders

The predominant site of involvement in OED was buccal mucosa (66.7%), followed by tongue (18.2%), retromolar area (9.1%), floor of mouth (3.0%) and lip each (3.0%) (Figure 3).

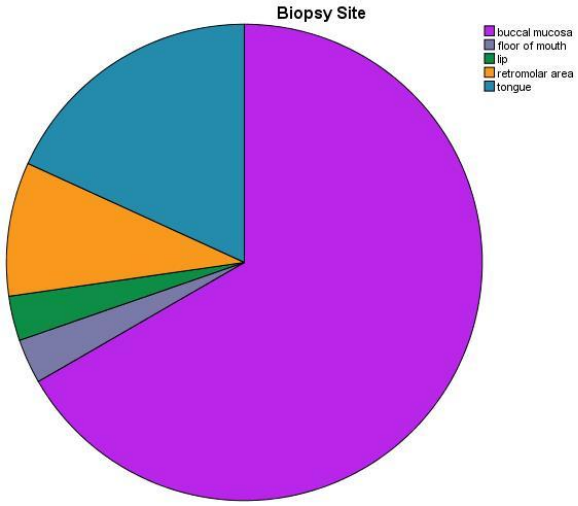

Figure 3: Site of involvement in OED

When the OED were sub-classified on the basis of their histological subtypes, we observed that the most common histological grade among the 33 cases was mild epithelial dysplasia, which was seen in n = 15 (45.5%) cases, followed by moderate epithelial dysplasia in n = 9 (27.3%) and severe epithelial dysplasia in n = 9 (27.3%) cases, respectively (Figure 4).

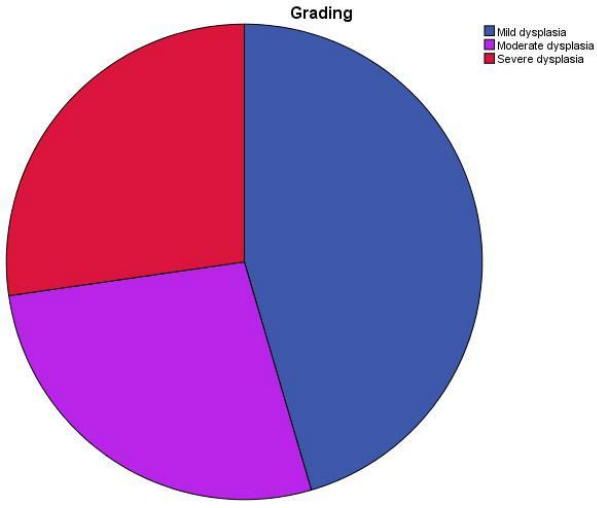

Figure 4: OED sub-classification on the basis of their histological subtypes

The mean (average) mitotic count in 33 cases of epithelial dysplasia was 4.4606/10 grid fields in H&E stained sections compared to 5.3212/^{10} grid fields in the sections stained with crystal violet (p = 0.00) (Tables 3 and 4).

Table 3: Mean mitotic count per square millimetre in OED cases

	N	MEAN
H&E	33	4.4606
CV	33	5.3212

A significantly increased mitotic count was observed in crystal violet-stained sections of the oral epithelial dysplasia when compared with H&E stained counterparts.

Table 3: Mean mitotic count per square millimetre in OED cases

	N	MEAN
H&E	33	4.4606
CV	33	5.3212

Discussion

Divisions of cells are needed to maintain the integrity of tissue [9]. Mitosis indicates genetic damage that is increased and abnormal. This is one of the essential features seen in the precancerous and cancerous condition [15]. The present study was carried out for the localisation of mitotic figures and highlights the importance of mitosis in assessing cell proliferation.

The oldest and simplest way of assessing cell proliferation is the counting of mitotic figures. It is a way to assess cell proliferation and is used as a diagnostic tool in tumour pathology. Cellular proliferation is widely used for primary diagnostic purposes, as well as a guide to prognosis in the assessment of tumours [16].

Thus, the identification and quantitation of mitotic cells plays an important role in histological grading systems which are used for determining the prognosis of premalignant and malignant lesions [9]. Clinical measures of proliferation are often included in the histological grading system as those tumours which exhibit increased proliferation have a tendency to be more aggressive [16].

Over the decades, the quantification of mitotic figures has been on the backseat. Newer prognostic indicators are now on the forefront, for example immunohistochemistry, autoradiography, flow cytometry and DNA ploidy measurements [9]. To study the frequency of mitotic activity, physiological markers for mitotic cells like protein kinases, as well as the accumulation of different molecules such as dyenins and cyclins in the different stages of mitosis can also be used [17]. Special stains like Nissl stain, gallocyanin, Giemsa, toluidine blue and Feulgen have also been used to study mitotic figures [18].

Although time and cost elements make them less feasible, a well standardised histological stain along with accurate use of the morphologic criteria for the identification of mitotic figures can overcome these difficulties [9].

The present study was carried out to overcome such limitations. The aim of this study was to use easy and economical methodology to study mitotic figures. In this study, 1% crystal violet is used as a selective stain and then compared with H&E staining in thirty three cases of OED.

When determining mitotic activity, methodological errors are of great interest [19]. Therefore, well-standardised procedures were used in the counting of mitotic figures, involving the analysis of multiple microscopic fields in representative areas of the tumour.

The counting of mitotic figures was done and the result in each sample was expressed in terms of the number of mitoses per high power field (hpf) and also in 1 square millimetre of the tumour tissue (mitoses per mm^2). As the microscopic objectives used (numeric aperture, field diameter at specimen level) are difficult to control, mitoses per high power field may be quite variable; because of this, results cannot be compared between various laboratories. Therefore, counting per mm^2 reduced the need for additional standardisation and gave significant findings [11] (Table 3).

In this study, the mean (average) mitotic count in 33 cases of epithelial dysplasia was 4.4606/10 grid fields in H&E stained sections compared to 5.3212/10 grid fields in the sections stained with crystal violet. In the present study, we meant to define a simple, economical and prompt technique to identify the mitotic figures and also to evaluate its role in histological grading of OED and OSCC. It was observed that crystal violet delivered superior staining of mitotic figures compared to H&E stains.

A similar study was conducted by Ankle MR on mitotic figures, which was used to determine the 1% crystal violet stain selectivity by comparing it with the H&E staining method. The findings of the study showed a statistically increased mean mitotic count in the sections of OED stained with 1% crystal violet and OSCC in comparison to sections stained with H&E [9]. From the present study, it can be easily observed that crystal violet provides efficient staining of mitotic figures and assists in its identification.

In another study which was carried out by Jadhav, it was seen that crystal violet provided a clear-cut advantage over H&E-stained sections in the selective staining of mitotic figures. A significant increase in the number of mitotic figures was noticed in both OED and OSCC [15]. The present study is in accordance with the previously conducted studies.

The higher sensitivity of crystal violet could be described due to its basic nature and its high affinity for highly acidic chromatin [9]. The overall higher

diagnostic efficiency of crystal violet could also be explained due to the modification of a staining method by using 1N HCL at 60°C, therefore causing an increase in contrast due to the light staining of the cytoplasm, which is likely to be a result of the decreased RNA content following hydrolysis [11].

From the results of the present study, it is concluded that mitotic cell counting is the quickest and simplest way of assessing the cellular proliferation. Crystal violet provides the efficient staining of mitotic figures and assists in its identification. It can be used for the localisation of mitotic figures and to assess proliferation, even in small scale laboratories, as crystal violet staining is easier, cheaper and more feasible. Crystal violet staining can be a rationalised step in the staining of mitotic figures compared to the usual H&E staining and can be employed as a selective stain during routine histopathological procedures.

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Basic Science

Allele-Specific Loop-Mediated Isothermal Amplification for the Detection of IVSII-I G>A Mutation On β-Globin Gene

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Abstract

BACKGROUND: Thalassemia is one of the most common genetic health problems in the world. More than 200 different mutations have been identified in the beta-globin gene and among the 24 β-globin gene mutations in β-thalassemia carriers in the north of Iran IVSII-I G>A mutation has the highest frequency. Using fast, inexpensive, simple and reliable methods for the detection of the mutations in β-thal carriers is very important in prenatal diagnosis, and introduction of alternative methods to the existing ones can help to simplify the detection of mutations. Since its introduction, different methods derived from LAMP have been widely used for SNPs detection.

AIM: This study was aimed to design a new method for the detection of IVSII-I G>A mutation on β-globin gene based on AS - LAMP technique.

METHODS: Primer explorer V5 software was used for the design of LAMP primers. Three sets of primers were designed. In the first set, the BIP primers were exactly complementary to the normal and mutant alleles. In the second set, 1 nucleotide (T) was inserted at the 5' end of BIP primers, and in the last set, one nucleotide at the 5' end of BIP primer was changed. The other required primers for the LAMP reaction (FIP, B3, and F3) were the same for all 3 sets of primers. The LAMP reaction was applied on three DNA samples (Wild type, Heterozygote and Homozygote for IVSII-I G>A mutation) and synthetic DNA.

RESULTS: The results of the present study showed that LAMP reaction using three sets of primers could not successfully detect the IVSII-I G > A mutation among subjects DNA sample and synthetic DNA.

CONCLUSION: Although several studies have successfully used ARMS-LAMP method to detect the SNPs, and other studies use a variety of methods to identify IVSII-I G>A mutation, the present study was unable to differentiate between a normal allele and IVSII-I G>A mutation. Hence further studies are recommended to consider redesigning of primer set, DNA concentration and using commercial LAMP Master Mix to detect the mutation.

Introduction

Thalassemia is one of the most common genetic health problems in the world resulting from the reduced or absent synthesis of the haemoglobin chains synthesis. Thalassemias are classified into two main types of α- or β based on the absence production of α- or β-globin chains. β-thalassemia is caused by inherited mutations on the β-globin gene that leads to reduced synthesis of the β-globin chain of the haemoglobin. The highest frequency of β-thalassemia mutations is reported from Mediterranean countries, the Middle East, and East Asia. More than 200 different mutations have been identified in the beta-globin gene, causing wide genotypic and phenotypic variability of this blood disorder [1], [2].

Alpha and beta thalassemia mutations are very common in Iran [3], and the highest prevalence of β-Thalassemia (around 10%) has been reported from north of Iran, in Southern coastlines of Caspian Sea, and South of Iran close to the Persian Gulf. The prevalence of β-thalassemia mutations in most parts of the country has been estimated to be 4-8% [4], [5],

[6]. Overall, there are 20,000 affected β-thalassemia patients and 3,750,000 carriers of the diseases in Iran [7]. Among the 24 β-globin gene mutations in β-thalassemia carriers in the north of Iran IVSII-I G>A mutation has the highest frequency (61%) [8].

The national program for the prevention of thalassemia in Iran has been started since 1997. This premarital screening program is compulsory in all provinces, and all couples should be screened before they get married. The aim of this program is screening β-thal carriers and preventing the child-birth with β-thal major [9], [10], [11]. Since in lots of the cases in screening program the mutations of the carriers must be identified, using fast, inexpensive, simple and reliable methods for the detection of the mutations in β-thal carriers is very important in prenatal diagnosis, and introduction of alternative methods to the existing ones can help to simplify the detection of mutations.

For the identification of the mutations among β-thal carriers, PCR-based methods are usually used. Although PCR-based methods are cost-effective techniques that are very robust for nucleic acid amplification, they cannot be performed in every lab. Also, the methods require a thermocycling system, which limits their application in the field. There are few genetic laboratories that collaborate with the screening programs to detect the mutations among carriers and patients.

In the past years, numerous nucleic amplification approaches have been introduced that do not need the thermocycling system. Isothermal techniques to amplify nucleic acids include loop-mediated isothermal amplification (LAMP), nucleic acid sequence-based amplification (NASBA), helicase-dependent amplification (HDA), rolling circle amplification (RCA), multiple displacement amplification (MDA), and recombinase polymerase amplification (RPA); these new approaches can easily be used at a constant temperature [12], [13], [14], [15], [16], [17], [18], [19].

The LAMP method was first introduced by Notomi et al., in 2000 [20]. Since then, different methods derived from LAMP have been widely used for pathogen detection [21], [22], [23], [24].

The LAMP method uses four sets of primers that are specially designed to identify six distinct regions on the objective gene. These sets of primers are the outer and inner primers (F3 and B3). The forward inner primer (FIP) consisting of the F2 zone at the 3' end with the sequence similar to the F1c region at the 5' end, is complementary to the F2c zone, while the backward inner primer (BIP) consisting of the B2 zone at the 3' end with the sequence similar to the B1c region at the 5' end, is complementary to the B2c zone (Figure 1). The two primers are complementary to the downstream zone of the opposite strand in the objective region (F1 and B1) [20].

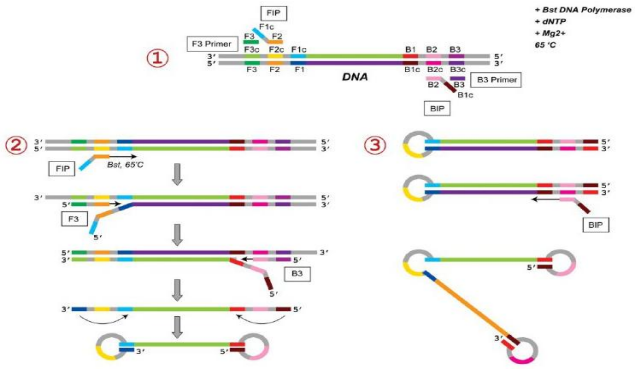

Figure 1: Schematic view of LAMP Primers Using 4 sets of Primers. These sets of primers are the outer and inner primers (F3 and B3). The forward inner primer (FIP) consisting of the F2 zone at the 3' end with the sequence similar to the F1c region at the 5' end, is complementary to the F2c zone, while the backward inner primer (BIP) consisting of the B2 zone at the 3' end with the sequence similar to the B1c region at the 5' end, is complementary to the B2c zone

The LAMP method yields a substantial amount of amplicons in a short time (around 60 min), which is 103 times higher than the amount produced with PCR [25].

Because the LAMP method uses four primers sets that are specific to the objective regions, it has rapid amplification, a higher amount of amplification products, and smaller detection limits compared with PCR-based methods [23]. Hence, LAMP is more beneficial for point-of-care testing (POCT) than PCR [24].

The LAMP-based methods have rarely been used for genotyping and detecting point mutations. Thus, designing new LAMP methods capable of genotyping could be an alternative method to other genotyping techniques. Moreover, the LAMP technique is a very suitable, easy to use, and cost-effective approach for POCT or on-site testing. This study was aimed to design a new method for the detection of IVSII-I G>A mutation on β-globin gene based on AS (Allele-Specific)-LAMP technique.

Material and Methods

Among the registered patients at the thalassemia research centre, Mazandaran University of medical sciences, the cases that were heterozygote and homozygote for IVSII-I G>A mutation were selected. The mutation in patients was detected using standard ARMS-PCR method and confirmed with PCR-sequencing. The genomic DNA was extracted from peripheral blood applying QIAamp DNA Micro Kit (Qiagen, USA).

Some healthy subjects with no mutations on the β-globin gene were also selected with the help of

PCR-sequencing method. Synthetic DNAs complementary to the wild type and mutated region were also designed.

Primer designing for AS-LAMP

For primer designing Primer explorer V5 software, which is a specific tool for the design of LAMP, primers were used (Table 1), and NM_000518.4 reference sequence was considered as a template. To select the primers that could successfully differentiate the IVSII-I G>A mutation (dB SNP rs#:33945777) from normal allele, three sets of primers were designed. In the first set, the BIP primers were exactly complementary to the normal and mutant alleles. In the second set, 1 nucleotide (T) was inserted at the 5' end of BIP primers, and in the last set, one nucleotide at the 5' end of BIP primer was changed. The other required primers for the LAMP reaction (FIP, B3, and F3) were the same for all 3 sets of primers (Table 1).

Table 1: The sequences of primers used for AS-LAMP method for the detection of IVSII-I G>A method

Set1: BIP-Wild = B1C (5-3) + B2 (5-3)	GTGAGTCTATGGGACGCTTGATTATCCCCTTCCTATGACATGA
Set1: BIP-Mutated = B1C M (5-3) + B2 (5-3)	ATGAGTCTATGGGACGCTTGATTATCCCCTTCCTATGACATGA
Set 2: BIP-Wild = B1C (5-3) + B2 (5-3)	GTTGAGTCTATGGGACGCTTGATTATCCCCTTCCTATGACATGA
Set2: BIP-Mutated = B1C M (5-3) + B2 (5-3)	ATTGAGTCTATGGGACGCTTGATTATCCCCTTCCTATGACATGA
Set3: BIP-Wild = B1C (5-3) + B2 (5-3)	GTCAGTCTATGGGACGCTTGATTATCCCCTTCCTATGACATGA
Set3: BIP-Mutated = B1C M (5-3) + B2 (5-3)	ATCAGTCTATGGGACGCTTGATTATCCCCTTCCTATGACATGA
FIP= F1C (5-3) + F2 (5-3)	CCTGAAGTTCTCAGGATCCACGCCTTTGCCACACTGAGTG
F3	TCACCTGGACAACCTCAA
B3	5'CCATTCTAAACTGTACCCTG3'

Set1: primer designed by http://primerexplorer.jp/lampv5e/index.html, Set2: In this sets of primers 1 nucleotide was inserted at the 5' end of BIP primers, Set3: The sequence of primers in which one nucleotide at the 5' end of BIP primer was changed

PCR-Sequencing reaction with B3 and F3 primers

To find that the primers could amplify the target region PCR reaction with the help of F3 and B3 primers were applied, and the PCR product was sequenced using ABI Capillary system.

AS-LAMP reaction

Conventional LAMP mix in total volume of 20µL that contained 2 µL of 10 X Bst DNA polymerase reaction buffer (New England Bio-labs), 8 mM MgSo4, 0.8 M betaine, 1.4 mM of each dNTPs, 1.6 mM FIP, 1.6 mM BIP (wild or Mutated), 0.2 mM B3, 0.2 mM F3 and 8U Bst DNA polymerase (New England Bio-labs). The master mix was dispensed in each microtube, and 50 ng from the DNA template was added to the mixtures. Then the reaction mixes were incubated at 60°C for 60 min. It should be mentioned that for each sample, two LAMP reactions were done, one with wild primers and another with Mutated primer. The LAMP products were run on 1% agarose gel.

Results

PCR sequencing

The results of PCR sequencing with the help of F3 and B3 primer showed that the primers could successfully amplify the target region on the beta-globin gene (Figure 2).

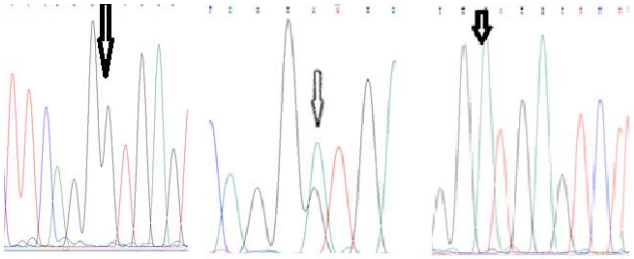

Figure 2: The results of PCR-Sequencing using F3 and B3 primers for the identification of IVSII-I G > A mutation: from left to right: Homozygote wild type, Heterozygote, Homozygote mutated

LAMP reaction with the first primer set

The LAMP reaction using first sets of primers (without any modification) showed that the primers could amplify the target DNA, but this set of primer was unable to differentiate the normal allele from mutated one (Figure 3).

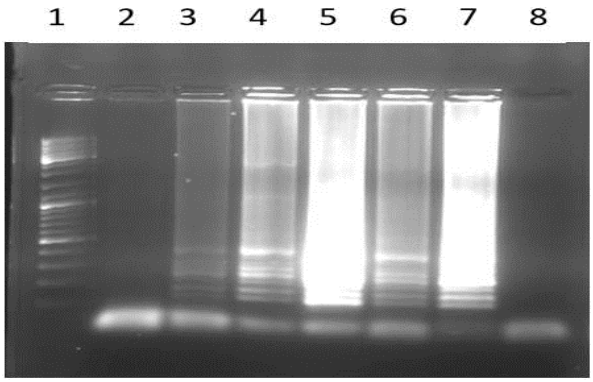

Figure 3: The results of AS LAMP reaction using primers without any modification; 1) Ladder 100bP; 2) Normal DNA with wild type primer; 3) Normal DNA with mutant type primer; 4) Mutant DNA (Homozygote) with wild type primer; 5) Mutant DNA (Homozygote) with mutant type primer; 6) Heterozygote DNA with wild type Primer; 7) Heterozygote DNA with mutant type primer; 8) blank

LAMP reaction with second primer set (with a nucleotide insertion)

Like the LAMP reaction with the modified primers that a nucleotide (T) was inserted one nucleotide before mutation site at the 5 ends of BIP primers indicated that these primers could not differentiate the normal DNA from the mutant one (Figure 4).

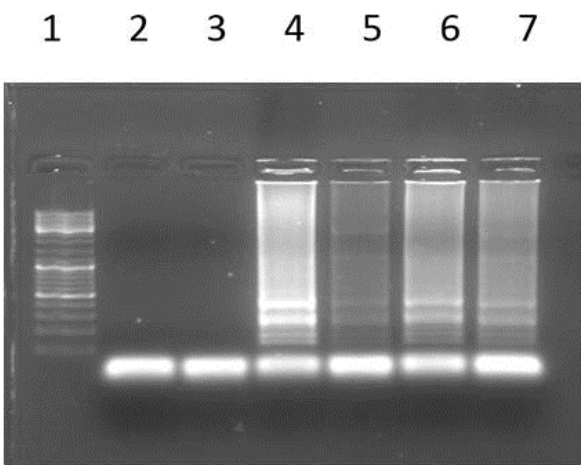

Figure 4: The results of AS LAMP reaction using primers with a T insertion; 1) Ladder 100bP; 2) Normal DNA with wild type primer; 3) Normal DNA with mutant type primer; 4) Mutant DNA (Homozygote) with wild type primer; 5) Mutant DNA (Homozygote) with mutant type primer; 6) Heterozygote DNA with wild type Primer; 7) Heterozygote DNA with mutant type primer; 8) blank

LAMP reaction with third primer set (with a nucleotide substitution at the third nucleotide before mutation site)

The results of LAMP reaction using the third set of primers with a nucleotide substitution at the third nucleotide before mutation site indicated that the LAMP reaction could not successfully distinguish the mutant and normal alleles (Figure 5).

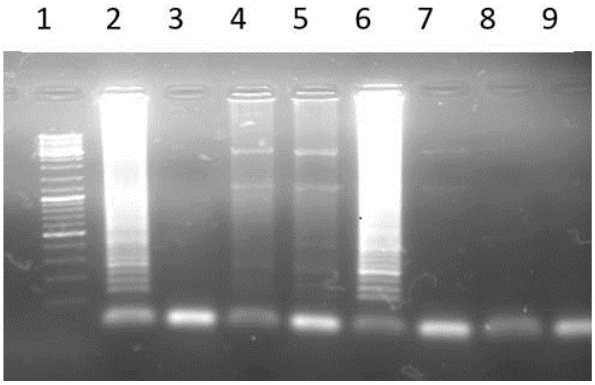

Figure 5: The results of AS LAMP reaction using primers with a nucleotide substitution at the third nucleotide before mutation site; 1) Ladder 100bP; 2) Normal DNA with wild type primer; 3) Normal DNA with mutant type primer; 4) Mutant DNA (Homozygote) with wild type primer; 5) Mutant DNA (Homozygote) with mutant type primer; 6) Heterozygote DNA with wild type Primer; 7) Heterozygote DNA with mutant type primer; 8) blank with wild primer; 9) blank with mutant type primer

The gradient of mgso4 and betain for LAMP reaction

The gradient of mgso4 (8-10-12-16 mM)and betain (0.8-1.6 -2.4-4.8 M) was separately applied to Optimize LAMP reaction, and the results showed that different concentration of these materials was unable to distinguish the mutant and normal alleles.

Discussion

Since the introduction of the LAMP approach, most of the studies have been applying the conventional type of this technique to detect the desired segment of DNA of the pathogens. However, some studies have used this method for genotyping and detection of the SNPs. These studies argued that the AS-LAMP method is a valid method for the detection of the SNPs. the present study aimed to detect the IVSII-I G→A mutation which is the most frequent mutation among β- thalassemia patients in the north of Iran.

In the ARMS-LAMP method, two sets of LAMP primers are designed to differentiate two different nucleotides at the same position in the target DNA: BIP or FIP primers which are specifically designed complementary to the mutation point at the 3' end of the B2 primer (5' end of the BIP primer). The remaining primers, F3, B3, and FIP or BIP, are common for each primer set. Two LAMP reactions should be prepared with each set of primers for each sample.

Several studies have used the AS-LAMP method for SNP detection. For instance, this method was used for the identification of the West African kdr (kdr-w; L1014F) [26]. And G119S ace-1R mutations [27] in field-collected Anopheles gambiae. Yongkiettrakul et al., (2017) designed an SNP-LAMP assay to detect the N51I mutation on the *dhfr* gene that is associated with pyrimethamine resistance in Plasmodium falciparum with 100% specificity [28].

Ikeda et al. in 2007, designed an ARMS-LAMP approach for the identification of L858R mutation on the epidermal growth factor receptor gene (*EGFR*) [29]. They used an in situ LAMP in which The FIP and BIP primers were labelled with fluorescein isothiocyanate (FITC) for mutation detection on paraffin-embedded tissues.

In 2017, Tamura et al., Used the ARMS-LAMP method to detect the N526K mutation in the *ftsI* gene that was involved in H. pylori-resistant Haemophilus influenza with a negative effect on ampicillin beta-lactamase. Using this method, they succeeded in identifying this mutation in the mentioned species [30].

Using the AS-LAMP method, Duan et al., in 2016, identified spot mutations (TTC → TAC, F200Y) in the β2-tubulin gene, which is involved in resistance to benzimidazole in Fusarium asiaticum fungus. The comparison between the results of this method with the PCR test showed that this method could successfully detect this mutation in resistant strains of agricultural products [31].

β-thalassemia is the most common haemoglobin disorder in Iran and IVSII-I G→A mutation is the most frequent mutation in the north of

Iran. Several studies have introduced a variety of methods for the identification of this mutation. Zafari et al., in 2010 developed the real time HRM (High-Resolution Melting) method in which they identified the mutation IVSII-I G→A on serum samples of pregnant mothers. The purpose of the research was to detect the fetus's IVSII-I G→A mutation in the serum of pregnant mothers. The sensitivity and specificity of the introduced method were 92.6% and 82.6%, respectively [32].

Heidari Sharafkhalkali et al., in 2017, using a Ligation Assay method combined with Nanoparticles could successfully identify the IVSII-I G→A mutation. They used probes that were labelled with magnetic nanoparticles and quantum dots. In the mentioned study, two types of the probe were designed, one complementary to the normal region and another complementary to the mutant area. Fifteen homozygote and 21 heterozygote patients were successfully identified using this method [33].

Hamid et al., in 2012 have also used the HRM (High Resolution Melting) method to identify 4 common mutations in Iran including IVSI-5 (G>C), CD36/37 (-T), IVSII-I (G>A), IVSI-110 (G>A). However, in the IVSII-I G→A mutation, 2 peaks were observed due to the presence of a polymorphism close to mutation site [34].

Multiplex HRM method to identify common beta-globin gene mutations in Greece was also developed by Chassanidis et al., in 2016. They designed several primers for mutation sites. Several mutations, including IVSII-I, in multiplex conditions, were simultaneously amplified [35].

Haji Hosseini et al., in 2015, using Tetra-Primer ARMS PCR method could detect IVSII-I G→A and FSC 8/9 InsG mutations in 30 affected beta-thalassemia patients in Isfahan province. This method has been able to differentiate normal, heterozygous, and homozygous samples in patients with 100% specificity and reliability. The frequency of IVSII-I G→A and FSC 8/9 InsG mutations in Isfahan province were 86.6% and 16.6 % respectively [36].

Honardoost et al., in 2014, compared the results of Tetra Primer ARMS PCR method with conventional ARMS PCR technique to detect IVSII-I mutation in β-thalassemia patients. The PCR-sequencing approach was considered as a standard gold test. Fifty-seven subjects, including 2 homozygous, 49 heterozygotes, and 6 normal samples were analysed using both techniques. They reported that Tetra Primer ARMS-PCR was able to detect IVSII-I mutation with 100% sensitivity and specificity while the conventional ARMS test had a specificity and sensitivity of 100% and 92.55% [37].

In 2015, Alfdali et al., identified common β-globin gene mutations in Egypt using Reverse Dot-Blot method among 40 β-thalassemia patients. Using PCR sequencing as a standard gold method, the results showed that this method had a sensitivity of 92.5% [38].

Although the mentioned studies have successfully used ARMS-LAMP method to detect the SNPs, and other studies use a variety of methods to identify IVSII-I G→A, the present study was unable to differentiate between a normal allele and IVSII-I G→A mutation. At the present study, three different primer sets could not detect the mutation in the patient's DNA and synthetic DNA. Hence further studies are recommended to consider redesigning of primer set, DNA concentration and using commercial LAMP Master Mix to detect the mutation.

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Basic Science

Transplantation of Bone Marrow-Derived Mesenchymal Stem Cells Preserve the Salivary Glands Structure after Head and Neck Radiation in Rats

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Abstract

BACKGROUND: The salivary glands are one of the radiation sensitive tissues during radiotherapy in the treatment of head and neck cancer. Within the first weeks of radiotherapy, the radiation causes progressive loss of gland function, then continue throughout the later of the patient's life.

AIM: The present work was designed to discover the potential effect of bone marrow-derived mesenchymal stem cells (MSCs) injected locally and in decreasing the unwanted effects of radiation on rats salivary gland.

MATERIAL AND METHODS: 6 rats used as the control group (N) and 12 rats had a single radiation dose of 13Gy in the head and neck then, they were equally allocated into two groups: Irradiated only as a group (C), Irradiated then treated with MSCs as a group (S). The animals were euthanised 7 days post radiation. Then, submandibular salivary glands were cut up; the histological examination was done.

RESULTS: Histological examination of the treated group(S) shown an apparent improvement in the SG structure and function compared to the irradiated group (C), this improvement represented mainly as preserving acini diameter (mean diameter in µm group (C) 183.1 ± 4.5, in group (S) 356.3 ± 33.5 while, in (N) group 408.9 ± 5.9) and decrease in fibrotic areas in the gland (mean fibrosis parentage in group (C) 26.5 ± 5.9 in (C) group , in group (S) 11.7 ± 4.13 while in (N) group 0.2 ± 0.31).

CONCLUSION: BM-MSCs has revealed to be promising in mitigating the side effects of radiotherapy on salivary glands structure.

Introduction

Xerostomia, which is hypofunction of the salivary glands, is the greatest noteworthy undesirable sequelae of radiotherapy in the management of head and neck neoplasms [1]. Approximately, more than 500000 patients experience radiotherapy every year all over the world, and as an outcome, the greater part of these patients will encounter serious hypofunction of the salivary glands (SGs) [2].

This could prompt an expansive series of medicinal complications and symptoms, for example, dry mouth, improper function of speaking, deglutition and mastication, serious dental caries, dysgeusia and oral mucositis. These long-lasting complications extremely lessen the post-treatment personal satisfaction and increment the pain of a substantial number of patients undergoing radiotherapy in the head and neck area [3].

The obvious destruction of salivary glands as a result to radiation treatment radiation-actuated SG harm shows the necessity for progressively dynamic measures through as well as directly after the prior stage post-radiation, as opposed to managing hypofunction till the point when permanent harm has happened. Available approaches avoiding or limiting damage to salivary glands as a result to radiation incorporate the utilisation of defensive substances, for example, amfostin or tempol, submandibular salivary gland relocation surgically and reduction of the radiation dosage conveyed to non-target typical tissues of the salivary glands [4], [5]. However, a reasonable protective treatment has not been

developed to defend the salivary glands from damage produced by radiotherapy.

Attention in the treatment approaches intended to heal and additionally reestablish harmed SGs is expanding, and with regards to tissue engineering and regenerative medicine, these approaches involve the re-grafting of autologous SG cells [6], the embedding of engineered salivary glands synthetically prepared [7], stem cell treatment [8], and gene therapy [9]. Bone-marrow-derived mesenchymal stem cells (BM-MSCs) were in recent times suggested as probable applicants for the treatment of hypofunction of salivary glands [10].

The present work was designed to discover the potential effect of bone marrow-derived mesenchymal stem cells injected locally and in decreasing the unwanted effects of radiation on rats salivary gland.

Material and Methods

Experimental animals and ethical statement

The current study was done on 24 healthy male albino rats, weight about 100 to 150 grams and age ranged between 3 to 4 months. The sample size was divided into 6 rats in each group. The animals were obtained and housed under standardised conditions with controlled temperature and humidity (30-35%) and a 12-12 h light-dark cycle.

The rats had free access to standard rat chow and tap water at the research animal house of National Cancer Institute – Cairo, Egypt.

All aspects of the animal's care and experimental protocols were reviewed and approved by the ethical committee in the faculty of Oral and Dental Medicine– Cairo University. Furthermore, the protocol was by the recommendation of the National Institutes of Health guide for the care and use of Laboratory Animals (NIH Publications No. 8023, revised 1978).

BM-MSCs Preparation

Six 6 weeks old male white albino rats (100-120 g) were euthanised by cervical dislocation, isolation of bone marrow was done in the Research animal house of National Cancer Institute – Cairo, Egypt. Bone marrow was harvested by flushing the tibiae and femurs of rats with DMEM (GIBCO/BRL) supplemented with 10% fetal bovine medium (GIBCO/BRL). Nucleated cells were isolated with a density gradient [Ficoll/Paque (Pharmacia)]. The cells were then incubated in a CO_2 incubator at 37°C in 5% humidified CO_2 for 12-14 days as a primary culture or upon the formation of large colonies. When large colonies developed (80-90% confluence), the cultures were washed twice with phosphate buffer saline (PBS), and the cells were trypsinised with 0.25% trypsin in 1 mM EDTA (GIBCO/BRL) for 5 minutes at 37°C. After centrifugation, cells were resuspended in PBS. MSCs in culture were characterised by their adhesiveness, fusiform shape and by detection of CD29, one of the surface markers for MSCs by RT-PCR [11].

Experimental Design

The selected rats were randomised into 3 groups, one normal control group (N) and two experimental groups. In experimental groups, rats were anaesthetised by i.p. Injection of sodium pentobarbital (Nembutal®, 40 mg/kg body weight) and ketamine chloride (ketalar®, 40 mg/kg body weight) then received a single radiation dose of 13 Gray (Gy) to the head and neck region at the National Cancer Institute, Cairo, Egypt, using linear accelerator (Electa-precise T System) using 4 MeV electron beam with 1 cm bolus(the source of radiation, the dose and the time for evaluation were determined based on a pilot study to generate salivary gland injury in rats in response to regional radiation), then they were equally subdivided to Irradiated only group (C) + (PBS), Irradiated + mesenchymal stem cells (MSCs) group (S). Group (S), received an injection with (1×10^5 cells in 0.2 ml) in (PBS). The injection in both groups was immediately after irradiation within the first hour using a 1 ml insulin syringe with a needle size 27 gauge x 1/2 inch (BD Nokor™). It was at submandibular gland through a horizontal incision in the neck to expose the gland. Euthanasia by concussion occurred in day 7 after radiation. Then, submandibular salivary glands collected and were fixed in 10% formalin for specimen preparation and histological evaluation. Acini diameters and area were assessed histologically in the H & E section in addition to an assessment of areas of fibrosis in Masson's trichrome MTC stained sections.

Histological evaluation

The assessor was completely blind about the sample groups during evaluation. The diameter of the acini was assessed using Leica Quin 500 analyser computer system (Leica Microsystems, Switzerland). The cursor was utilised to draw a straight line representing the length of the acinus in H & E stained sections. The image analyser is calibrated automatically to transform the measurement units (pixels) produced by the image analyser program into actual micrometre units. The length was estimated in 2 different acini in 5 different fields, in each specimen using magnification (x 100). The area percentage of MTC stain in the sections were analysed in 3 sections for each slide. At least 3 fields per section were

accounted for using CellSens dimensions software (Olympus).

Statistical analysis

Statistical Package for Social Sciences (SPSS) was used, Version 18.0 for Windows. As the data following a normal distribution, ANOVA was utilised to relate the values between the experimental and control groups, followed by post hoc Tukey, if results of ANOVA were significant. A p-value of ≤ 0.05 was considered statistically significant.

Results

Microscopic examination of the normal group showed the normal histological structure of submandibular salivary gland that composed of the mixed serous-mucous acinus, the serous acinus forms serous demilune around mucous acinus and intercalated ducts, the later were lined by simple cuboidal epithelium and surrounded by myoepithelial cells with limited periductal and perivascular fibrous connective tissue. Meanwhile, examination of the stem cells treated group (S) showed relatively well preserved histological structure compared to irradiated only group (C), this presented in the detected changes in acini diameter in the MSCs group (mean 356.3 ± 33.5 µm) is significantly higher than in irradiated only group (mean 183.1 ± 4.5 µm) but did not reach the normal group (mean 408.9 ± 5.9 µm, P value ≤ 0.05). The irradiated only group showed necrosis of the ductal lining epithelium with apoptosis that was less commonly observed in the stem cells treated group.

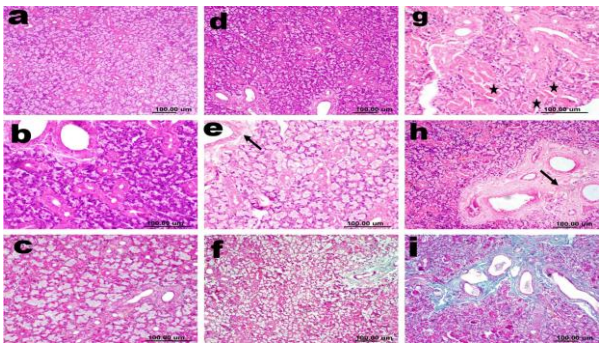

Figure 1: Photomicrographs of rat submandibular salivary gland a) and b) control group showing normal gland composed of mixed secretory units with intercalated ducts (H & E); c) Control group showing normal fine connective tissue stroma (MTC); d) Stem cells treated group showing well preserved histological structure of the gland (H & E); e) Stem cells treated group showing less periductal fibrosis (arrow) (H & E); f) Stem cells treated group showing less periductal and perivascular fibrosis by (MTC); g) Irradiated group showing ductal necrosis (stars) (H & E); h) Irradiated group showing diffuse fibrosis periductal, perivascular and around acini (arrow) (H & E); i) Irradiated group showing diffuse fibrosis periductal, perivascular and around acini (MTC)

Also, fibrosis was evaluated in tissue sections stained with Masson's trichrome (MTC) stain; irradiated group showed significant diffuse periductal and perivascular fibrosis that was extending into the lobules in-between the acini (area of fibrosis 26.5 ± 5.9) compared to the treated group (area of fibrosis 11.71 ± 1.8, P value ≤ 0.05) Figure 1, and 2.

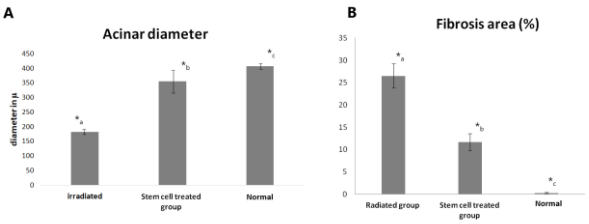

*Figure 2: Bar charts representing A) changes in mean acini diameter among the groups, while B) changes in the percentage of fibrosis among the groups; Values are the Means ± standard deviation *(P-value ≤ 0.05), a, b and c indicate significant differences between values*

Discussion

Our results demonstrate that intraglandular transplantation of BM-MSCs during the early post-radiation phase mitigates SG damage following head and neck radiation at a single dose of 13 Gy. Many former studies utilised a dosage of 15 Gy to examine the outcome of stem cell in the management of radiation brought SG destruction [1], [10], [12], [13]. In the current study, we designated dose of 13 Gy based on a pilot study to detect the lowest dose that induces detectable histopathological changes after single dose during different time points in the first week after regional irradiation. The same dose was used in the model created by Fang et al., [14]. We choose to use intraglandular route this was by [10], [15] to ensure homing and detect the early changes and the ability of the treatment to preserve the structure. We found that radiation caused a remarkable decrease in the acinar field area as well as acinar diameter, a remarkable increase in fibrosis and a significant decrease in proliferation activity. However, in the stem cells treated animals, these changes were significantly decreased with obvious preservation of the normal structure — the definite underlying mechanisms explaining the role of BM-MSCs in the protection of radiation-induced SG damage controversial. Paracrine effects, trans-differentiation and/or cell fusion and angiogenesis may be involved in the protection of SG structure [4], [5], [16]. On the one hand, Lombaert et al. found that transplanted BM-MSCs could engraft in the damaged SGs inducing repair by paracrine stimulation preserving glands function and structure [17].

Similarly, the findings of Tran et al., that BM Soup was as effective as whole live BM cells in

repairing irradiated-SG, suggesting a paracrine effect of BM cells on SGs [12]. Recently, a study by Fang et al. reported that injections of BM soup within 3 weeks after irradiation could preserve 90–100% of salivary flow in irradiated mice [14]. The paracrine actions of MSCs, via secreting anti-inflammatory, anti-apoptotic agents and increase expression of proliferation-promoting factors, have been suggested to have a role in tissue repair in many diseases [18], [19], [20], [21], 22], [23]. On the other hand, few studies showed that intravenous transplantation of BM-MSCs [24], or intraglandular injection with BM-MSCs co-cultured with acinar cells [25] could preserve the function of SGs post radiation via transdifferentiation into glandular cells. These studies used mixed cells so that the type of cells that improves the SG function is not identified.

In conclusion, immediate transplantation of BM-MSCs after regional radiotherapy has shown to be effective in preserving the salivary gland morphology and function, minimizing the side effects of radiation on normal salivary glands.

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Basic Science

Correlation of CD8+ Expression, Foxp3+ Expression, and CD8+/Foxp3+ Ratio with Triple Negative Breast Cancer Stage in Sanglah General Hospital

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Keywords: TNBC; CD8+; Foxp3+; CD8+/Foxp3+ ratio; Advanced stage

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Abstract

BACKGROUND: Triple negative breast cancer (TNBC) is a breast cancer sub-type that lacks ER, PR and HER-2 expression. This type tends to be more aggressive than other types of breast cancer, with poor prognosis, distant metastases, higher recurrence rate, and lower overall survival. The TNBC is resistant to hormonal therapy, but generally very susceptible to chemotherapy. Expression of CD8+ and Foxp3+ were parts of the TIL, which often found in TNBC as an immune response to tumour antigens following antigens presenting cell (APC) stimulation.

AIM: This study was conducted to find out whether the expression of CD8+, Foxp3+ and CD8+/Foxp3+ ratio was associated with the stage of TNBC.

METHODS: This cross-sectional study was conducted from January 2014 until December 2016 at Sanglah Hospital with 46 research subjects. Two paraffin blocks were prepared for each sample to examine the CD8+ expression and Foxp3+ expression. Data were analysed using the Chi-Square test or Fisher's Exact tests as an alternative for bivariate analysis and logistic regression for multivariate analysis.

RESULTS: On bivariate analysis, we found a low of CD8+ expression in advanced stage ($p < 0.001$ with OR 3.5; CI 1.611-7.727). Expression Foxp3+ in advanced stage ($p = 0.482$; OR 0.8; CI 0.497-1.374), while the ratio of CD8+/Foxp3+ ($p = 0.213$; OR 2.2; CI 0.650-7.132). On multivariate analysis, a low of CD8+ expression (adjusted OR 16.5; CI 3.735-7.370; $p < 0.001$) was obtained.

CONCLUSION: Low expression of CD8+ was associated with the advanced stage of TNBC. The risk of becoming an advanced stage in TNBC patients with low CD8+ expression was 16.5 times higher than those with high of CD8+ expression. High expression of Foxp3+ was not associated with an advanced stage of TNBC. The low CD8+/Foxp3+ ratio was not associated with the advanced stage of TNBC.

Introduction

Triple-negative breast cancer (TNBC) is one of the subtypes of breast cancer that has a poor prognosis for free survival disease and shorter survival overall. The highest prevalence is in African-American women. Until now, there is no specific targeting therapy for TNBC. Significant overlap found in BRCA 1 associated breast cancer with TNBC phenotype [1].

TNBC is aggressive and has higher mortality due to metastases and has a high character of cell proliferation, poor cell differentiation, and in many cases, mutations in the TP53 tumour suppressor gene. TNBC often occurs at a younger age (< 40 years) [2]. TNBC recurrence increases in the first three years after diagnosis and decreases after eight years [3], and the mortality rate will increase within 5 years after diagnosed [4].

The poor prognosis of TNBC is related to tumour grading, lymph node status, tumour size and management [1]. TNBC sufferers also tend to have lower life expectancies but have a better chemotherapy response [5].

The potential mechanism that causes higher lymphocyte infiltration in TNBC because TNBC is a subtype of breast cancer that is immunogenic

because of genetic instability and increased mutation and expressing certain proteins [6]. An increase in tumour infiltrating lymphocytes (TIL) in TNBC is associated with increased disease-free and overall survival and complete pathologic response (pCR) on neoadjuvant therapy [6], [7].

High pathological complete response (pCR) values after neoadjuvant chemotherapy have been reported in patients with high levels of expression of TIL components such as CD3+, CD4+ and CD3+, CD8+, and Forkhead box protein 3 (Foxp3+). High pCR values in TNBC were also reported with a high CD8+/Foxp3+ (CFR) ratio [8].

Tumour infiltration by CD8+ can control tumour growth with cytotoxic effects on tumour cells. CD8+ expression is associated with a better prognosis if found in large quantities. Almost 20% of TNBC expresses strong TIL and if the amount of TIL is more in the tumour stroma is associated with a higher likelihood of healing in the early stages of TNBC. This presents that high CD8+ is associated with the early stages of TNBC sufferers [9].

There is a strong relationship between Foxp3+ with the development and progression of cancer. Some research evidence shows Foxp3+ effectively protects tumours from our body's immune response. Foxp3+ in tumours, ascites and peripheral blood of cancer patients was stated to be associated with a poor prognosis.[10] This presented that high Foxp3+ was associated with advanced stages of TNBC sufferers. The high ratio of CD8+/Foxp3+ T cells can predict that the clinical outcome is better in TNBC patients [11]. This can illustrate that the high ratio of CD8+/Foxp3+ T cells is associated with early stages in TNBC patients.

Many studies linking CD8+, Foxp3+ expression and CD8+/Foxp3+ ratio with complete pathological responses (pCR) to neoadjuvant therapy on TNBC patients made us want to know how the relationship of CD8+, Foxp3+, and CD8+/Foxp3+ expression to TNBC patients at Sanglah General Hospital Denpasar.

Methods

We conducted a cross-sectional study of 46 samples of TNBC patients (fulfilling inclusion and exclusion criteria) recorded from January 2014 to December 2016 at Sanglah General Hospital. Furthermore, the data is taken from the medical record to find out the age and clinical stage of TNBC sufferers as well as from the Anatomical Pathology report obtained from all TNBC patients who underwent a diagnostic procedure with biopsy or surgery. All operating specimens are then processed in paraffin blocks. The subsequent examination was performed by immunohistochemistry to determine CD8+ and Foxp3+ expressions.

The collected data were analysed using the Chi-Square test or Fisher's Exact test as an alternative for bivariate analysis and logistic regression for multivariate analysis.

Results

Characteristics of the 46 research subjects that we obtained can be seen in Table 1. The data on the subject of this study showed an age range between 31-75 years, with an average age of 47.6 years. The age grouping in this study was the early age group (< 40 years) and the advanced age group (> 40 years). From the table, it can be seen that the early age group is 13 (28.3%), and the elderly group is 33 (71.7%). Grading in this study was high grading groups as much as 28 (60.9%), low grading groups as many as 14 (30.4%) and unknown groups grading as many as 4 (8.7%). The clinical stage, which is the dependent variable in this study, is divided into 2, namely the advanced stage group (stage III and IV) and the early stage group (stage I and II). From the table, it was found that the advanced stage group was 26 (56.5%), and the early stage group was 20 (43.5%). For the low CD8+ expression group, there were 25 (54.3%), and the number of high CD8+ expressions was 21 (45.7%).

Whereas for the high Foxp3+ expression group, 32 (69.6%) and low Foxp3+ expressions were 14 (30.4%). The low CFR group (CD8+/Foxp3+) ratio was 39 (84.8%) while the high CFR group was 7 (15.2%). Based on the results of the bivariate analysis (Table 2), the low CD8+ expression group found at an advanced stage was 84.0% while in the high CD8+ expression group at an advanced stage as much as 23.8%. Based on statistical tests using the Chi-Square test showed that the relationship was significant ($p < 0.001$) with a 95% confidence interval of 1.611-7.727.

Table 1: An Overview of the characteristic of the research subjects

Variable	n = 46
Age (year), average ± SD	47.6 ± 11.5
≤ 40 years	13 (28.3)
> 40 years	33 (71.7)
Grading	
High	28 (60.9)
Low	14 (30.4)
Unknown	4 (8.7)
Stage	
Advanced (III-IV)	26 (56.5)
Early (I-II)	20 (43.5)
CD8+	
Low	25 (54.3)
High	21 (45.7)
Foxp3+	
High	32 (69.6)
Low	14 (30.4)
CFR (Rasio CD8+/Foxp3+)	
Low	39 (84.8)
High	7 (15.2)

The high Foxp3+ expression group was found at an advanced stage of 53.1% and 64.3% in the Foxp3+ low expression group. Based on statistical tests using the Fisher Exact test, the relationship was not significant (p = 0.482) with a 95% confidence interval of 0.497-1.374.

Table 2: Bivariate analysis of CD8+, Foxp3+, and CFR expressions (CD8+/Foxp3+ ratio) with clinical stage

Variable	Clinical Stage		OR	95%	P Value
	Advanced	Early			
CD8+					
Low	21 (84.0)	4 (16.0)	3.5	1.611-7.727	< 0.001[a]
High	5 (23.8)	16 (76.2)			
Foxp3+					
High	17 (53.1)	15 (46.9)	0.8	0.497-1.374	0.482[b]
Low	9 (64.3)	5 (35.7)			
CFR					
Low	24 (61.5)	14 (38.5)	2.2	0.650-7.132	0.213[b]
High	2 (28.6)	5 (71.4)			

[a]Uji Chi-Square; [b]Uji Fisher's Exact.

In the low CFR expression group at an advanced stage as much as 61.5% while in the high CFR expression group as much as 28.6%. But based on statistical tests using the Fisher Exact test, the relationship was not significant (p = 0.213) with a 95% confidence interval of 0.650-7.132.

Based on the bivariate analysis, it is necessary to continue the multivariate analysis to assess the pure effect of each variable. The variables included in the multivariate test are age variables and CD8+ expressions. Grading variables are not included because the data is missing so that it does not represent in its entirety (Table 3). Based on the table, there is a pure relationship between low CD8+ expression and advanced stage in TNBC patients with adjusted OR 16.5 after calculating age variables.

Table 3: Multivariable Analysis of CD8+ Interaction with Clinical Stages after controlling the age

Variable	*Adjusted* OR	95% CI	p-value
Low CD8+	16.5	3.735 – 7.370	< 0.001
Age	0.9	0.182 – 4.753	0.930

Discussion

The TNBC breast cancer subtype compared to other breast cancer subtypes is associated with younger age (< 40 years) when diagnosed [2], [12], [13]. The earlier the age of cancer patients the greater the influence of internal factors on the occurrence of malignancy compared to external factors, especially there is a delay in diagnosis and has been found in an advanced stage, the prognosis is getting worse.[14] In this study, the whole study subjects with TNBC were found to be older (> 40 years), namely 71.7% compared to young age (28.3%). Besides that, there were more advanced groups from the early stage group (56.5% vs 43.5%). This may be due to the delay of the patient in examining the health service centre.

In breast cancer, grading is a prognostic factor where high grading has more aggressive behaviour and poor prognosis; the recurrence rates are four times more than low grading [4]. The poor prognosis of TNBC is related to tumour grading, lymph node status, size tumour, and management [1]. In this study, grading was included as a confounding variable. The above is by this study where high grading was 60.9%; low grading was 30.4% while unknown grading was 8.7%.

From the characteristics of the research subjects above, the stadium obtained more groups than the early group stadiums. This means that TIL, in this case, is CD8+ with a lower expression more than high CD8+ expression. This is consistent with the results of a study that cites CD8+ low (54.3%) more than high CD8+ expression (45.7%). Meanwhile, for Foxp3+, according to the characteristics of the subject of this study, Foxp3+ groups (69.6%) were higher than Foxp3+ low expressions (30.4%). Likewise, from the CFR group (CD8+/Foxp3+ ratio), there was a lower CFR group (84.8%) more than the high CFR group (15.2%).

In the advanced stage, it was found that the low CD8+ expression group was 84.0% while the high CD8+ expression group was 23.8%. This shows that low CD8+ expression at an advanced stage is not capable of carrying out its function as surveillance by recognising and killing malignant cells that express peptides produced by mutant cell proteins or oncogenic viral proteins presented through MHC class I [15].

There is a pure relationship between low CD8+ expression and advanced stage (p < 0.001) in TNBC patients with adjusted OR 16.5 which means that the risk of TNBC patients with low CD8+ expression is 16.5 times higher than TNBC patients with high CD8+ expression. This is consistent with research that says that nearly 20% of TNBC expresses strong TIL and if the amount of TIL is more in the tumour stroma is associated with a higher likelihood of healing in the early stages of TNBC [9].

The distribution of Foxp3+ expression at an advanced stage showed that the high Foxp3+ expression (53.1%) was lower than Foxp3+ low expression (64.3%) and statistical analysis of Foxp3+ high expression with an advanced stage was not proven to have a significant relationship (p = 0.482). This is different from previous studies which stated that there was an increase in the appearance of CD4+, CD25+, Foxp3+ T Cell in malignancies such as the lungs, head and neck, ovary, gastrointestinal, and skin [10]. This shows that at an advanced stage, Foxp3 + expressions should be higher than low Foxp3+ expressions.

The causes of the absence of such

relationships include two hypotheses. First, as it is known that tumour progression is not only influenced by Foxp3 +, but there are also other influential factors such as CTLA4 and PD-1. T cell activity requires antigen recognition by TCR and the introduction of costimulatory, mainly B7 by CD8+. CTLA-4 resembles CD8+ receptor activity, is bound to B7 molecules. CTLA-4 has a greater affinity ability than CD8+ to B7 family receptors thus preventing B7 costimulatory in APC from CD8+ bond, then produces a signal that inhibits T cells which cause the non-working interaction of APC-complex T cells. CTLA-4 will cause no T cell sensitisation, T cells become anergic, and even T cell apoptosis can occur. Ultimately energy from T cells will correlate to the degree of differentiation and progression of cancer cells [15]. Other receptor blockers on CD8+ family are PD-1 (Programmed Cell Death 1). The use of PD-1 by other ligands leads to T cell inactivity. It is said that CTLA-4 main function is to control initial T cells that are active in lymphoid organs while PD-1 plays an important role in limiting the response to effector cell differentiation in peripheral tissues [15]. Second, in the early stages, higher Foxp3+ expressions were more found than Foxp3+ low expressions. This is probably because Foxp3+ circulates a lot in the peripheral circulation but is mostly in FoxP3+ naive T cell conditions, so it has not carried out its activities to inhibit CD8. This is by the results of the study that in the circulation of the Treg cell periphery, it is 5-10% naive CD4+ T cell [16].

In the advanced stage, the CFR group was low (61.5%) while in the CFR group it was high (28.6%), but the statistical test showed that the relationship was not significant (p = 0.213). This is because in the calculation of the CFR there are 2 independent variables, namely CD8+ and Foxp3+. The Foxp3 variable statistically did not show a significant relationship causing the results of the CFR to be meaningless.

In conclusion, low CD8+ expression is associated with advanced stages of TNBC sufferers. High Foxp3+ expression is not associated with advanced stages of TNBC sufferers. Low CD8+/Foxp3+ ratio is not associated with advanced stages of TNBC sufferers.

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Basic Science

Comparison of Honey versus Polylactide Anti-Adhesion Barrier on Peritoneal Adhesion and Healing of Colon Anastomosis in Rabbits

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Abstract

BACKGROUND: Postoperative adhesion is still a consequence of intra-abdominal surgeries, which results in bowel obstruction and abdominopelvic pain. Bowel anastomosis as a common abdominal surgery has the incidence of leakage in up to 30% of patients that increase morbidity and mortality. Due to similar pathways of adhesion formation and wound healing, it is important to find a way to reduce adhesions and anastomosis leakage.

AIM: This study was designed to compare antiadhesive as well as anastomosis healing improvement effect of honey and polylactide anti-adhesive barrier film.

METHODS: Forty-five rabbits divided into three groups of honey, adhesion barrier film, and control group in an animal study. Under a similar condition, rabbits underwent resection and anastomosis of cecum under general anaesthesia. In the first group, honey was used at the anastomosis site, in the second one polylactide adhesion barrier film utilised, and the third one was the control group. Adhesion, as well as anastomosis leakage, was assessed after 21 days. Data were analysed using the Statistical Package for Social Scientists (SPSS) for Windows version 25.

RESULTS: Three groups of 15 rabbits were studied. The results showed that mean peritoneal adhesion score (PAS) was lower in the honey group (1.67) in comparison to the adhesion barrier film group (3.40) and the control group (6.33).

CONCLUSION: Bio-absorbable polylactide barrier has an anti-adhesion effect but is not suitable for intestinal anastomosis in rabbits. Further studies needed to evaluate these effects on human beings.

Introduction

Postoperative peritoneal adhesion is still an obvious consequence of intra-abdominal surgeries in spite of modern surgical techniques and precautions [1], [2]. Peritoneal surfaces trauma and tissue ischemia cause adhesion resulting in bowel obstruction, abdominopelvic pain, and female infertility [3], [4], [5]. Adhesion will lead to prolonged hospitalisation and increased health care cost [6], [7].

Bowel anastomosis is a common abdominal operation, and the incidence of anastomosis leakage has been reported in different studies between 2.6% to 30% depending on the type of procedure, surgical technique and patient demographics [8], [9], [10], [11]. Anastomosis leakage leads to increase hospitalisation, morbidity and mortality ranging from 6.2% to 37% [12], [13]. Due to similar pathways of adhesion formation and wound healing, finding a way to reduce adhesions will probably lead todiscovering measures to reduce anastomosis leakage.

Although various methods have been used to reduce tissue adhesion and improve anastomosis

healing, there is no significant effect yet [14], [15], [16]. Honey has a long history in medicine as an anti-inflammatory, anti-bacterial, and wound healing agent [17], [18]. Recent animal studies have reported anti-adhesion effect and anastomosis healing properties of honey [19], [20]. Moreover, some commercially available barriers have reported having both anti-adhesion and healing improvement effects [21], [22], [23]. Research has shown that honey can reduce the inflammation, growth and angiogenesis, improve intra-abdominal adhesion and increase antioxidant factors [24], as well as in a review study, bactericide effects, reduction the pH of the wound, chronic inflammation and increment of fibroblasts infiltration has been reported for honey [25].

Considering the beneficial effects of honey are approved, and there is no problem in its accessibility, as well as given that it is affordable, this study was designed to evaluate the effect of honey and polylactide barrier on adhesion formation as well as leakage of anastomosis site in rabbits.

Methods

Animal study

In this study, 45 healthy male rabbits with the mean weight of 3000-3500 grams were divided into three groups (15 rabbits in each group, including honey, adhesion barrier film, and control group). Rabbits were obtained from the Pasteur Institute of Iran (IPI).

They were kept and fed based on standard laboratory diet and water for one week before surgery in a similar condition. Animals were fasted, except for water, 12 hours before surgical intervention. Neither mechanical bowel preparation nor intraoperative bowel irrigation was performed. All experimental procedures were approved by the ethics committee of Iran University of Medical Sciences for the use of laboratory animals .

Rabbits were shaved and anaesthetised in the supine position with intramuscular ketamine (30-35 mg/kg) and intramuscular xylazine (10-15 mg/kg) by a specialised team from the department of veterinary medicine of Tehran University of Medical Sciences.

The shaved area was cleaned and isolated with a sterile dressing. Approximately 6 cm midline incision starting from 10 cm below the xiphoid along the linea alba was made. After entering the peritoneal cavity, the cecum was identified and transected 5 cm distal to ileocecal junction and anastomosis of the transected area was performed using a single running layer Vicryl 3-0 sutures. In the first group, 3 ml of sterile honey was applied at the anastomosis site. In the second group, one adhesion barrier film was used over the anastomosis site. The third was a control group in which no material was used. Finally, abdominal fascia and skin were closed using continuous Nylon 3-0 and interrupted Nylon 4-0 sutures, respectively. The postoperative dressing was applied to prevent self-harm.

In the first group, sterile antibacterial medical honey produced by DERMESIENCES Company was used in our study. A bio-absorbable polylactide adhesion barrier film with a copolymer of 70:30 polys (L-lactide-co-D, L-lactide) produced by MAST company was used in the second group.

Adhesion Barrier Film (SurgiWrap®) is a temporary physical barrier to:

1. Separate opposing tissues and prevent the ingrowth of scar tissues and the formation or reformation of adhesions immediately adjacent to the barrier film.

2. Aid in the reoperation procedures by promoting the formation of a surgical dissection plane immediately adjacent to the barrier film.

3. Promote the formation of a surgical dissection plane to include the following anatomic regions:

1) Peritoneum, peritoneal cavity, bowels, cecum, organs

2) Ob/Gyn (e.g. Female pelvic, reproductive organs, ovaries, uterus, uterine tube, etc.)

Adhesion Barrier Film is a transparent polymer film that is designed to separate opposing tissue during the critical period of peritoneal healing. Made of polylactide (PLA), it comes in 8 different sizes. A copolymer of 70:30 Polys (L-lactide-co-D, L-lactide), it is composed of lactic acid similar to that which occurs naturally in the human body, the material maintains its strength during the healing process, and is slowly hydrolysed into lactic acid. The molecules are then metabolised into carbon dioxide and water and are released from the body through the lungs. SurgiWrap® received FDA clearance to be used in urological, gynaecological, and gastroenterological procedures, either by laparotomy or laparoscopy. Seprafilm Adhesion Barrier is contraindicated for use wrapped directly around a fresh anastomotic suture or staple line; as such use increases the risk of anastomotic leak and related events (fistula, abscess, leak, sepsis, peritonitis) (https://www.seprafilm.us).

The postoperative diet was initiated after complete regain of consciousness with standard laboratory diet and water. Rabbits were returned to the operating room 21 days after the first operation and anaesthetised using the previous method. The previous surgical site was shaved, cleaned, and isolated with sterile dressing in the supine position.

Then laparotomy was done at a previous site by another surgeon and anastomosis leakage, mesenteric ischemia, and peritoneal adhesion index (PAI) were evaluated . The abdomen was divided into nine areas, and an adhesion severity score was related to each area. The sum of the scores was calculated as the PAI. Adhesion severity score was presented as the following order: zero for no adhesions, 1 for filmy adhesions which could remove by blunt dissection, 2 for strong adhesions which were removed by sharp dissection, and 3 for very strong vascularized adhesions which were removed by sharp dissection and tissue damage was hardly preventable. Anastomosis leakage was also evaluated by the second surgeon.

Statistical analysis

The values were expressed as mean, standard errors of deviation. The mean values of the groups were compared by one-way analysis of variance (ANOVA) as well as the least significant difference (LSD) as a post hoc test. $P < 0.05$ was considered significant. The statistical tests were run on a compatible personal computer using the Statistical Package for Social Scientists (SPSS) for Windows version 25.

Results

Four of 15 rabbits in the polylactide anti-adhesive-barrier – film (SurgiWrap®) group died in the second week which immediately underwent laparotomy, and the adhesion severity and leakage status were investigated.

Table 1: The mean and standard deviation of Peritoneal Adhesion Index for the three groups

Variable		N	Mean (SD)	95% Confidence Interval
Peritoneal	Honey	15	1.67 (0.724)	1.27, 2.07
Adhesion	SurgiWrap®	15	3.40 (1.805)	2.40, 4.40
Index	Control	15	6.33 (1.447)	5.53, 7.13
	Total	45	3.80 (2.380)	3.09, 4.51

Descriptive data for 45 Rabbits are presented in Table 1. The results showed that the least adhesion occurred in the honey group, and the most adhesion occurred in the control group.

Table 2: Adhesion Score for the three study groups

Adhesion score	Honey group (n = 15)	SurgiWrap® group (n = 15)	Control group (n = 15)
0	0	2	0
1	7	0	0
2	6	2	0
3	2	3	0
4	0	3	2
5	0	4	2
6	0	1	4
7	0	0	4
8	0	0	2
9	0	0	1
10	0	0	0

Adhesion numbers with different scores for each group are presented in Table 2. Also, the box plot for the comparison of the three groups is presented in Figure 1.

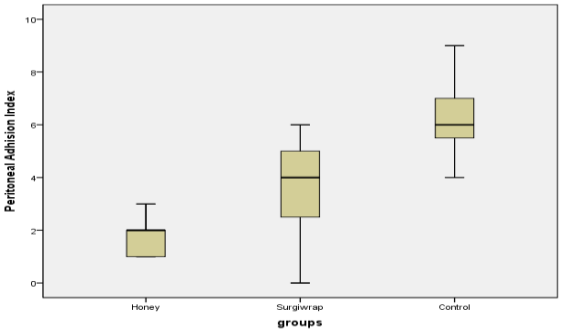

Figure 1: Box Plot of adhesion score for the three study groups

One-way ANOVA for the mean adhesion score of the three groups showed that there was a significant difference between the control group and the two other interventional groups (honey and SurgiWrap®) ($P < 0.001$, $F = 45.2$), so that the honey group had significantly less adhesion score compared with the control and polylactide groups. Post-hoc test (LSD) was used to assess the difference between adhesion scores of all three groups, which are presented in Table 3.

Table 3: Comparison of Peritoneal Adhesion Index in the three groups

Groups		Mean Diff.	Std. Error	P value	95% CI
Honey	SurgiWrap®	-1.733*	0.511	0.002	-2.76 to -0.70
Honey	Control	-4.667*	0.511	0.000	-5.70 to -3.64
SurgiWrap®	Control	-2.933*	0.511	0.000	-3.96 to -1.90

*The mean difference is significant at the 0.05 level.

Discussion

Anastomosis healing without adhesion formation after bowel injury repair or elective bowel resection and anastomosis is still an unmet necessity. In addition to increasing postoperative morbidity and mortality, anastomosis leakage and adhesion formation lead to escalating health costs [3], [4], [12], [13]. Although, several studies have been done on barrier films or chemical agents; there is no effective method yet [15], [16].

Use of barriers has been reported in previous studies as an effective anti-adhesion agent, but adverse effects on bowel anastomosis have limited their widespread application. Bio-absorbable polylactide adhesion barrier films were used in our study, which had significantly less adhesion compared with the control group. The main effect of barriers is to separate injured serosal surfaces so that the mesothelial regeneration can be completed smoothly

[19]. Beneficial anti-adhesion effects of barriers after abdominal surgeries [23], [26], [27] and gynecologic operations [28], [29] have been reported with acceptable results.

In a study conducted by Aly Saber in 2009, which compared the anti-adhesion effect of honey versus integral as an anti-adhesive agent, none of the rats was immune from adhesion formation [19]. Barriers had local anti-adhesion effects and did not decrease adhesion at other abdominal sites. Probably this was due to the good local anti-adhesion effect of polylactide barrier and lower surgical manipulation of other sites in these two rabbits.

In the study of sabre, although all rabbits in the honey group had postoperative adhesion, adhesion score in the honey group was significantly lower than polylactide barrier. Adhesion score in the honey group was between 1 and 3, and there were not strong or very strong adhesions (grade II and III) in none of the honey group rabbits. It may be due to the wider diffusion of honey compared to barrier resulting in less adhesion effect on other abdominal areas leading to lower adhesion score. Saber also reported that 80% of rats receiving intraperitoneal honey had adhesions [19] firmly. Honey' barrier action may be effective in separating traumatised surfaces and reducing extent as well as adhesion severity [19], [30)]. Aysan et al. concluded that the high density of honey would result in its late peritoneal absorption, and this may inhibit adhesion formation up to mesothelial regeneration in their study on rats [31].

Gollu et al. revealed that honey had a protective role against intraperitoneal adhesion [30]. Honey has a long history as an effective subject for wound healing [32]. Moreover, its anti-bacterial and anti-fungal effects may reduce adhesion, since infection is a known trigger of adhesion formation [33], [34]. Furthermore, honey can prevent adhesion formation as a result of anti-inflammatory and anti-oxidant effects [35].

Current anti-adhesion agents improve anastomosis healing and inhibit adhesion formation [30], [36]. But their use is controversial as an increase in anastomosis leakage has been reported in some studies [37]. In our study, both rabbits, there was no anastomosis leakage in the honey and the control groups, but five rabbits in polylactide anti-adhesion group died because of anastomosis leakage. Van Oosterom, in his study on the effect of hyaluronic acid-carboxymethylcellulose membrane on small bowel anastomosis, reported no anastomosis healing impairment [38]. Erturk on his study on the hyaluronic acid-carboxymethylcellulose membrane's effect on colonic anastomosis also reported no adverse effects [39]. Probably this is due to the local anti-adhesion effect of polylactide barrier, which has adverse effects on anastomosis healing as both of the processes have a similar molecular mechanism. Furthermore, honey might be effective on the molecular mechanism of wound healing, but the definite mechanism is unknown [40].

In conclusion, honey has anti-adhesion and wound healing effects on bowel anastomosis in rabbits. Bio-absorbable polylactide barrier has an anti-adhesion effect but is not suitable for intestinal anastomosis in rabbits. Further studies needed to evaluate these effects on human beings.

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Upregulation of SCUBE1 in Dengue Virus Infection

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Abstract

BACKGROUND: Dengue is a major communicable disease in tropical areas, with an increasing prevalence every year. Thrombocytopenia is one of the commonly used laboratory parameters for predicting the severity of the disease. It is detected on day 6 or day 7 after the febrile stage, and its presence indicates that the disease has become potentially fatal. Therefore, it is necessary to identify a marker for the early recognition of dengue virus infection during the febrile stage before the detection of thrombocytopenia on day 6 to prevent severe disease outcomes. Signal peptide-CUB- (complement C1r/C1s)-EGF (epidermal growth factor)-like domain-containing protein 1 (SCUBE1) is secreted in activated platelets under inflammatory conditions and enhances platelet-platelet adhesion and agglutination. This gene was first identified in human vascular endothelium, but its biological role in platelets remains unknown.

AIM: This study aims to identify SCUBE1 expression during the febrile stage of dengue virus infection and examine the correlation of its expression with thrombocytopenia occurrence on day 6.

MATERIAL AND METHODS: Blood samples were collected from 17 patients infected with dengue virus on day-3 fever and from 16 healthy controls who met the inclusion and exclusion criteria for dengue virus infection according to the World Health Organization (WHO) classification for dengue virus infection. All samples were subjected to SCUBE1 gene analysis using real-time reverse transcription quantitative PCR (RT-PCR).

RESULTS: The results showed that upregulation of SCUBE1 gene in infected patients (8.9 ± 3.1-fold) compared to that in healthy controls, indicating SCUBE1 involvement in dengue virus infection. Furthermore, we analysed the laboratory parameters of infected patients on day 3 and day 6, when thrombocytopenia is usually detected. Platelet count was found to be significantly decreased from day 3 until day 6 in the infected patients. Unfortunately, our results showed no correlation between SCUBE1 expression in the febrile stage and the occurrence of thrombocytopenia on day 6.

CONCLUSION: The conclusion of this study is SCUBE1 might play a role in dengue virus infection but does not correlate with thrombocytopenia on day-6 fever.

Introduction

Dengue virus infection is an acute mosquito-borne viral infection and is still one of the major health problems in Indonesia, where its incidence has been rapidly increasing in recent years [1]. Due to the broad spectrum of clinical manifestations of this infection, ranging from asymptomatic, mild-to-severe symptoms, there is a need for new biomarkers for diagnosis, proper treatment, and prognosis [2], [3]. The clinical onset of dengue virus infection is observed as an initial febrile phase on days 1-3, a critical phase where plasma leak is apparent on days 4-7, and a spontaneous recovery phase on days 8-10. Laboratory parameters may vary from mild to moderate thrombocytopenia and leukopenia. In a small proportion of infected patients, systemic vascular leak progresses into impending deterioration symptoms that require clinician awareness [3], [4], [5]. Thrombocytopenia is one of the warning signs for the severity of dengue virus infection. A low platelet count detected in dengue infection might indicate an association with platelet dysfunction. Further decrease in platelet count suggests additional complications. Therefore, monitoring the warning signs of the critical phase of dengue infection is essential to prevent the severity of this infection [6], [7].

Signal peptide-CUB-(complement C1r/C1s)-EGF (epidermal growth factor)-like domain-containing

protein 1 (SCUBE1) is a newly identified secreted and membrane-anchored protein consisting of nine copies of EGF-like repeats and one CUB domain. The expression of SCUBE1 has been reported in the vascular endothelium and activated platelets under inflammatory conditions [6], [7], [8]. A recent report showed that SCUBE1 expression was elevated in patients with Crimean–Congo hemorrhagic fever (CCHF), another viral disease that exhibits mild-to-severe clinical features similar to those of dengue fever. Upregulation of SCUBE1 levels in patients with CCHF may predict a fatal case where thrombocytopenia can cause massive hemorrhage [9]. Considering that SCUBE1 is detected in endothelial dysfunction and activated platelets, we assumed that this gene might play a role in dengue virus infection, which could impact the vascular endothelium and platelets. This study was conducted to assess whether SCUBE1 could be a promising predictive marker for dengue virus infection.

Material and Methods

Collection of blood samples

Blood samples were collected from 17 patients infected with dengue virus with a sudden onset of fever for at least 3 days. The inclusion criteria were fulfilling the World Health Organization (WHO) criteria for dengue virus infection, being anti-NS1-positive, and agreeing to sign the informed consent form. The exclusion criteria were patients confirmed as having no other infectious diseases such as influenza A, influenza B, and HCV infections, and not undergoing any drug treatment. Blood samples were collected from patients confirmed as infected with the dengue virus on day 3 and day 6 after the onset of illness. SCUBE1 expression was measured on day 3 of the febrile stage. Healthy volunteers as controls were randomly recruited from individuals who had no clinical symptoms of infectious diseases. Basic laboratory data included the results of white blood cells, platelet counts, and hematocrit.

RNA extraction and real-time quantitative PCR (qRT-PCR)

Total RNA was isolated using Trizol reagent according to the manufacturer's instruction (Invitrogen, USA). The total RNA was dissolved in 20 µL of RNase-free water and stored at −80°C until further use. Reverse transcription was performed to obtain cDNA using iSCRIPT cDNA synthesis kit (Bio-Rad, USA). SCUBE1 expression was confirmed using SsoFast Evagreen Supermix (Bio-Rad, USA) with Bio-Rad CFX96TM Real-Time PCR System. The primer for SCUBE1 was forward: GTGCCCTATGTCACCTACGAT and reverse: GAACATCTCCTTGGATTCCTGG and that for the reference gene GAPDH was forward: ATG GGT GTG AAC CAT GAG AAG TA and reverse: GGC AGT GAT GGC ATG GAC.

Research Ethics

This study was approved by the Faculty of Medicine, Andalas University Ethics Committee for Health Research.

Data analysis

The relative expression of SCUBE1 was calculated by the 2-ΔΔCt formula. Data were expressed as mean ± SEM, with statistical significance being evaluated using Student's t-test for normally distributed data or Mann – Whitney test for non-normally distributed data. Pearson correlation and significance analysis were conducted using SPSS software. $P < 0.05$ was considered as statistically significant.

Results

In this study, we analysed SCUBE1 expression in 17 patients with dengue virus infection in the febrile phase and healthy donors. We observed an increased SCUBE1 expression (8.89 ± 3.1-fold) compared to that in controls (Figure 1).

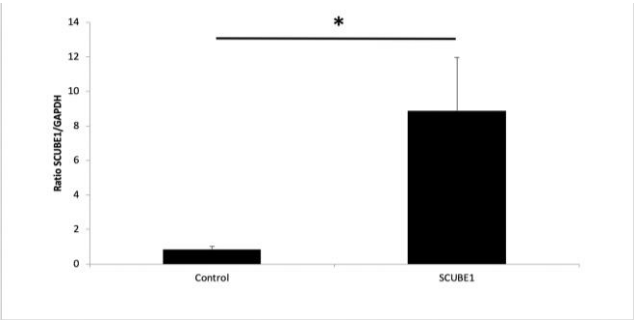

Figure 1: mRNA expression of SCUBE1. An upregulation of SCUBE1 expression was detected in 17 patients infected with dengue virus on day 3 compared with healthy controls

Laboratory parameters of the infected patients revealed a lower platelet count on day 6 than that on day 3 (Table 1).

Table 1: Laboratory parameters of dengue virus-infected patients on day 3 and day 6

Parameters	Day 3	Day 6	p*
Leukocytes	4769 ± 454.18	4605 ±402.02	0.79
PLT (×10^3/mm^3)	168 ± 10.8	137 ± 9.2	0.04*
HB (mg/dL)	14.3 ± 0.4	14.5 ± 0.4	0.61
HT (%)	41.5 ± 0.9	41.3 ±0.9	0.90

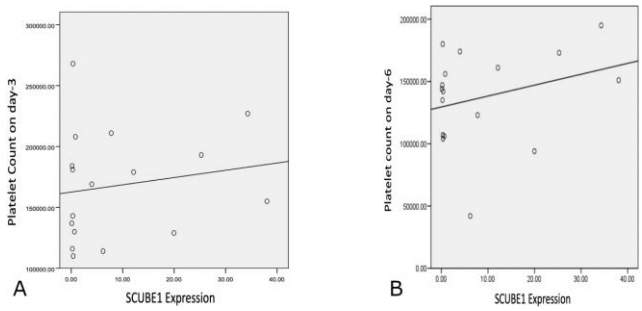

Figure 2: A) SCUBE1 expression and platelet count on day-3 fever showed no statistically significant correlation; B) Extended observation of the platelet count on day 6 showed a decreased count, but no significant correlation was observed with increased SCUBE1 expression

Furthermore, we determined whether there was any correlation between increased SCUBE1 expression on day 3 and platelet counts on day 3 and day 6 among the infected patients. However, we did not observe any significant correlation between SCUBE1 expression and decreased platelet count on day 3 ($r = 0.170$, $p = 0.51$). Similarly, no significant correlation was observed between SCUBE1 expression and platelet count on day 6 ($r = 0.292$, $p = 0.26$) (Figure 2).

Discussion

The mechanism of dengue virus infection and its complexity remain ambiguous. Dengue virus infection exhibits a wide variety of clinical manifestations ranging from asymptomatic to mild-to-severe illness [4], [5]. Awareness during the critical phase is essential to prevent the development of vascular leakage that could lead to dengue shock syndrome [5]. Numerous identification or assay methods have been developed for the detection of dengue virus infection, but they remain suboptimal [3], [4]. Therefore, it is necessary to develop a predictive marker and identify warning signs for close observation in dengue virus infection to reduce mortality due to the severity of illness [5], [6], [7].

SCUBE1 has been documented to be expressed in the vascular endothelium and platelets both at the mRNA and protein levels. Therefore, SCUBE1 may also be involved in the pathogenesis or the progression of atherosclerotic thrombus by promoting platelet adhesion and agglutination [8], [9]. Increased SCUBE1 levels have also been detected in acute coronary syndrome and ischemic stroke, which impact the vascular endothelium [10]. Moreover, a recent study reported increased SCUBE1 levels in patients with CCHF, which predicted severe cases of CCHF [11].

In this study, SCUBE1 expression was observed on day 3 of the febrile stage for early recognition. The expression of SCUBE1 was significantly higher in infected patients than that in the healthy controls ($p < 0.05$). We followed up these infected patients until day 6 of fever and checked their basic laboratory parameters. We observed that the infected patients showed significantly lower platelet counts from day 3 until day 6, implicating a role for SCUBE1 expression in thrombocytopenia occurrence. Furthermore, we analysed whether increased SCUBE1 expression in the febrile stage correlates with platelet count on both day 3 and day 6. However, no significant correlation was observed between increased SCUBE1 expression in dengue virus-infected patients and lower platelet count on either day 3 or day 6. Among the infected patients, some platelet count may decrease from day 3 until day 6, but the count was still in the normal range.

This study demonstrated an increase in the novel secreted protein SCUBE1 in the acute febrile phase of dengue virus infection. Moreover, the platelet count was found to be decreased from day 3 to day 6, but no significant correlation was observed between SCUBE1 expression and platelet count. The major limitation of our study is the small number of patients and controls. Nevertheless, the novel finding of increased SCUBE1 expression in the acute febrile stage of dengue virus infection can be considered as pioneering research for identifying a predictive marker for dengue virus infection. This result can also encourage further comprehensive studies for elucidating the pathogenic mechanisms of this mosquito-borne viral infection.

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Basic Science

Immunohistochemical Expression of MMR Proteins with Clinicopathological Correlation in Colorectal Cancer in Egypt

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Keywords: Microsatellite instability; Colorectal carcinoma; Immunohistochemistry; Mismatch repair; Tumour-infiltrating lymphocyte

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Abstract

BACKGROUND: Microsatellite instability (MSI) is the genetic pathway underlying 15% of sporadic colorectal carcinoma (CRC) and hereditary non-polyposis CRC. MSI-H CRC has a distinct clinicopathological characteristic including excess mucin and signet ring component, proximal colon, Crohn's like reaction, lymphocytic infiltration, and better survival.

AIM: This research aims to screen Egyptian CRC patients for MSI status by IHC testing of expression of the MMR proteins in correlation to its clinicopathological features.

MATERIAL AND METHODS: Immunohistochemistry study for mismatch repair proteins (MMR) was done on 115 cases of CRC. Their expressions were assessed and correlated to clinicopathological parameters in an attempt to obtain the most significant predictors of MSI.

RESULTS: MSI (low and high) represents 67% of the study cases. The most frequent expression pattern was combined loss of MLH, and PMS2 (38% of MSI) followed by a combined loss of MSH2, and MSH6 (29% of MSI). There was significant correlation of expression pattern of MMR proteins with the laterality, lymphovascular emboli, perineural invasion, grade, T stage, N stage, signet ring component, tumor infiltrating lymphocyte, and peritumoral lesion (0.014, 0.035, 0.012, 0.033, 0.013, 0.000, 0.041, 0.012, and 0.009 respectively). Proximal location (right sided) and lower grade, higher nodal stage, and marked TIL were selected as predictors of MS-H CRC (0.005, 0.031, 0.025, and 0.000 respectively).

CONCLUSION: All clinicopathological and histological parameters should be assessed in CRC for the sake of predicting MSI. The optimal approach to MSI evaluation is (IHC) assessment of MMR proteins.

Introduction

Colorectal cancer (CRC) is the most commonly observed cancer worldwide [1]. It is one of the most common cause of cancer-related death worldwide [2]. Cancer colon, as any cancer is caused by environmental and genetic factors [3]. 75% of cancer colon is sporadic and the remaining 30% has a hereditary contribution [4]. Hereditary CRC is appeared in two forms. The first is preceded by familial adenomatosis polyposis (FAP). The second is Lynch syndrome (LS) that has a defect in mismatch repair (MMR) gene and often referred to as hereditary nonpolyposis colorectal cancer. However, there was another category that exhibit gathering of CRC and/or adenomas in families with an identifiable hereditary syndrome, and are known as familial CRC. The genetic basis of familial CRC remains unknown [5], [6].

There are two molecular genetic pathways that underlie colorectal carcinogenesis. The first pathway is chromosomal instability that involves the activation of proto-oncogenes such as K-ras, and

inactivation of tumour-suppressor genes, such as APC, TP53, DCC, SMAD2, and SMAD4 [7], [8]. Chromosomal instability occurs in 85% of sporadic CRC and FAP [9]. The second pathway is the microsatellite instability (MSI) mutational pathway. MSI results from inactivation, mutational and/or epigenetic silencing of mismatch repair (MMR) [8], [10], [11]. MSI is not limited to hereditary non-polyposis cancer colon (HNPCC) but also present in sporadic CRC [9], [11].

The genetic basis for instability in MSI tumours is an inherited germline alteration in any one of the five human MMR genes: MLH, MSH2, MSH6, PMS2, and PMS1 [8], [12]. More specifically, germline mutations in MSH2 and MLH1 are responsible for most HNPCC families, while MSH6 is less common and PMS2 and PMS1 are rare [8]. MSI can be present in 10-15% of sporadic colorectal carcinoma. Acquired hypermethylation of MLH1 promotor and subsequent transcriptional silencing is the cause of high MSI in sporadic CRC [8], [9], [13].

Abnormal expression of MMR in CRC may be due to complete loss of expression, expression of an only truncated protein that does not bind to the antibody or weak /patchy cytoplasmic reaction if the mutation forms premature truncated but stable protein [14].

Hashmi et al., 2017 reported that MSI-H cancers often stimulate a host response that leads to migration of activated T cells into tumour cells. T cell cytotoxicity is activated. The T cells are CD8+, TCR+ cells. Therefore improved prognosis of MSI-H colonic cancers is related to the upregulated immune system that prevents the emergence of metastatic deposit [9].

There were distinct clinicopathological characteristics of CRC with MSI. These include poor differentiation, excess mucin and signet ring component, proximal colon, medullary feature, Crohn's like reaction and lymphocytic infiltration [9]. It is noted that the survival rate of CRC with high MSI is better when it is compared with MSS tumour evidenced by in tumoral lymphocytosis of MSI tumours [9], [15]. However, it sometimes associated with metachronous cancer and resistant to traditional chemotherapeutic agent [16], [17].

Investigation for the presence of MSI in CRC is really important due to many factors. It decides the extent of surgical treatment, the prophylactic surgery of hysterectomy and oophorectomy, and screening of the family member for the presence of the same mutation [9], [18]. Recognition of MSI phenotype can be done by histopathology and IHC because of CRC with MSI shares morphological features such as young patient age, right-sided location, mucinous and signet ring histology and intratumoral lymphocytosis. This fact allows the pathologist to dispense on PCR which remains the gold standard for recognition of MSI phenotype as it is not practicable and expensive in routine pathology lab [9], [15], [19].

Our study aims to screen CRC patients for MSI status in a big histopathology lab in Egypt (Referral lab for a big sector of Egyptian population) by immunohistochemical testing of expression of the MMR proteins and its relation to the clinicopathological features in CRC patients.

Material and Methods

A group of 115 cases of CRC were conducted in the present study. They were retrieved consecutively from the archives of Professor Elia, Anis Ishak Laboratory Pathology Centre (Cairo, Egypt), from 2015 to 2018. One representative slide of all cases were retrieved and reviewed. Then, the allocated paraffin-fixed tissue blocks were selected that showed both tumour and adjacent non-tumor colonic epithelium. The clinical and pathological information as patient's age, gender, tumour laterality (the right side from cecum to splenic flexure), lymphovascular invasion, perineural invasion, T stage, and N stage were obtained from the records. The cases were grouped according to the age of 2 groups (< 50, ≥ 50).

Histopathology

The histopathological features of each slide were reviewed by 3 pathologists as regard the variants (mucinous, cribriform, signet ring, medullary, and poorly differentiated,) according to WHO 2010 [20]. The presence or absence of necrosis (focal and diffuse) was recorded. As regards, intratumoral lymphocytic infiltration, the presence of small round lymphocytes within neoplastic epithelial cells was divided into mild to moderate (up to three intraepithelial lymphocytes (IEL)/HPF) and marked (> 3 IEL/HPF) according to the CAP guidelines [9]. The presence of peritumoral lymphocytic reaction requires 2 or more large lymphoid aggregate. The lab rules include written informed consents of all patients and approval of using their specimen for research purposes.

Immunohistochemistry

IHC examination was performed using a Ventana Benchmark Ultra machine automated staining system. A four-antibody panel of MMR proteins, including MLH1, MSH2, MSH6, and PMS2, are conducted. The primary antibodies used were: anti MLH-1 (M1) Mouse Monoclonal Primary Antibody 1.4 µg/ml) (Ventana, Tucson, AZ, USA), MSH2 (G219-1129), Mouse Monoclonal Primary Antibody 3.04 µg/ml) (Ventana, Tucson, AZ, USA), anti MSH6 (44) Mouse Monoclonal Primary Antibody 0.101 µg/ml) (Ventana, Tucson, AZ, USA), and PMS2

(EPR3947, Rabbit Monoclonal Primary Antibody 11.84 µg/ml) (Ventana, Tucson, AZ, USA). Adjacent normal colonic epithelium, lymphocytes, and stromal cells served as positive internal controls; we use it to check for accuracy. Positive external controls from CRC positive for MLH-1, MSH-2, MSH-6, and PMS2 are used. Negative controls were done by replacing the primary antibody with PBS.

According to the CAP protocol for immunohistochemistry interpretation, any nuclear staining even patchy is taken as “no loss of expression” (Figure 1: C, D, E, and F; Figure 2: C and F). Only absolute absence of nuclear staining was considered “loss of expression” (Figure 2: D and E) provided that internal controls are positive. Hence, carcinoma was considered MSI when nuclear staining was absent for at least one protein [21]. MSI-positive markers were reexamined to confirm the results.

In the present study, expression of proteins was then grouped into 10 categories (A-J) Table (1 and 2): A) no loss of expression; B) loss of expression of all four proteins; C) combined loss of MLH1/PMS2; D) combined loss of MSH2/MSH6; E) combined loss of MSH6/PMS2; F) combined loss of MLH/MSH2 and isolated loss of any of the four proteins G: MLH1; H) MSH2; I) MSH6 and J) PMS2.

MSI-H is considered when two or more markers are demonstrated to be unstable (lost expression). MSI- low is considered when only one marker is unstable. MSS is the case when no markers are unstable. We follow Fujiyoshi et al., 2017 study definition of MSI that was approved for Bethesda [2], [22]. In Fujiyoshi et al., 2017 study, they include MSI-L as MSS. However, in our study, we constellate the results of expression of the 4 markers into 3 categories (MSS, MSI-L, and MSI-H).

Statistical Analysis

All statistical analysis is done with the SPSS version 20 software program. Categorical data obtained are statistically evaluated using the X^2 test. Whereas the only continuous data in the study (age) is evaluated using Mean ± stander deviation. The tests are considered statistically significant when the *P* value less than 0 .05. All clinicopathological parameters of the studied cases are tested for association with the results of expression of the four markers and the status of MSI (Table 1, 2, 3, and 4). Then all candidate predictors with a P < 0.05 in univariate analysis are included in a multivariate logistic regression model in an attempt to discover the factors predicting CRC with MSI. In this study, we use the MSI-H model as a reference model (Table 5).

Results

A group of 115 cases of CRC are included in this study: 72 male and 43 females. The mean age of the patients was 50.88 ± 14.14 years, with an age range of 15-82 years. 72.2% of the cases (n = 83) are adenocarcinoma (NOS) Figure 1A, 24.3% of the cases (n = 28) are mucinous carcinoma Figure 2A, and 3.5% of the cases (n= 4) are signet ring carcinoma. All the assessed clinicopathological parameters categories are illustrated and correlated with the pattern of expression of the 4 markers of MSI (A-J) (Table 1, and 2) and the status of MSI (MSS, MSI-L, and MSI-H) (Table 3, and 4). The significance of the clinicopathological parameters as predictors of MSI-H is illustrated in Table 5.

Table 1: Expression pattern of MMR protein and clinicopathological characteristics of CRC

			MSS (38)					MSI (77)					
			A	B	C	D	E	F	G	H	I	J	P
		n	38	1	29	22	1	1	4	7	9	3	
Gender	M	n	25	0	18	14	0	1	2	5	5	2	0.84
		%	66	0	62	64	0	100	50	71	56	67	
	F	n	13	1	11	8	1	0	2	2	4	1	
		%	34	100	38	36	100	.0	50	29	44	33	
Age	< 50	n	17	1	13	13	1	1	2	2	2	1	0.51
		%	45	100	45	59	100	100	50	29	22	33	
	< 50	n	21	0	16	9	0	0	2	5	7	2	
		%	55	0	55	41	0	0	50	71	78	67	
L	R	n	16	1	22	18	1	0	3	3	8	1	0.014*
		%	42	100	76	82	100	0	75	43	89	33	
	L	n	22	0	7	4	0	1	1	4	1	2	
		%	57.9	0	24	18	0	100	25	57.1	11.1	66.7	
LVE	A	n	30	1	19	10	1	0	1	4	9	2	0.035*
		%	79	100	66	46	100	0	25	57	100	67	
	P	n	8	0	10	12	0	1	3	3	0	1	
		%	21	0	34	54	0	100	75	43	0	33	
PNI	A	n	34	1	29	17	1	0	4	7	9	3	0.012*
		%	89.5	100	100	77	100	0	100	100	100	100	
	P	n	4	0	0	5	0	1	0	0	0	0	
		%	10.5	0	0	23	0	100	0	0	0	0	
G	G2	n	30	0	20	9	1	0	4	5	7	3	0.033*
		%	79	0	69	41	100	0	100	71	78	100	
	G3	n	8	1	9	13	0	1	0	2	2	0	
		%	21	100	31	59	0	100	0	29	22	0	
T	T2	n	6	0	7	3	1	0	0	3	0	0	0.013*
		%	16	0	24	14	100	0	0	43	0	0	
	T3	n	31	1	20	17	0	0	4	4	9	3	
		%	82	100	69	77	0	0	100	57	100	100	
	T4	n	1	0	2	2	0	1	0	0	0	0	
		%	3	0	7	9	0	100	0	0	0	0	
N	N0	n	20	0	23	11	1	0	1	5	9	2	0.000*
		%	53	0	79	50	100	0	25	71	100	67	
	N1	n	5	1	4	10	0	0	3	2	0	1	
		%	13	100	14	45	0	0	75	29	0	33	
	N2	n	13	0	2	1	0	1	0	0	0	0	
		%	34	0	7	4	0	100	0	0	0	0	
V	NOS	n	31	1	21	10	1	0	4	5	7	3	0.3
		%	81	100	72	45	100	0	100	72	78	100	
	MUC	n	6	0	8	10	0	1	0	1	2	0	
		%	16	0	28	46	0	100	0	14	22	0	
	Sign.	n	1	0	0	2	0	0	0	1	0	0	
		%	3	0	0	9	0	0	0	14	0	0	

G: Gender; L: Laterality; LVE: Lymphovascular Emboli; PNI: perineural Invasion; T: T stage; N: Nodal stage; V: Histopathologic variant; NOS: Non otherwise specified; and MUC: Mucinous; sign: signet ring; A: no loss of expression of any marker; B: loss of expression of all markers; C: loss of expression of both MLH, and PMS2; D: loss of expression of both MSH2, and MSH6; E: loss of expression of both of MSH6, and PMS2; F: loss of expression of both of MLH, and MSH2; G: isolated loss of expression of MLH; H: isolated loss of expression of MSH2; I: isolated loss of expression of MSH6; J: isolated loss of expression of PMS2.

Expression pattern of MMR protein, clinicopathological and histopathological characteristics of CRC (Table 1, and 2)

A total of 72 males and 43 females were enlisted in the study. Age of the cases was

subgrouped into < 50 (53 cases) and ≥ 50 years (62 cases). Eighty-three cases were of adenocarcinoma NOS, 28 cases were of the mucinous type, and 4 cases were of signet ring type. The clinicopathological characteristics of each expression pattern of MMR proteins are included in Table 1.

Table 2: Expression pattern of MMR protein and histopathological characteristics of CRC

			A	B	C	D	E	F	G	H	I	J	P
		n.	38	1	29	22	1	1	4	7	9	3	
Mucinous component	Absent	n	26	1	13	7	0	0	2	5	4	2	0.353
		%	68	100	45	32	0	0	50.	72	44	67	
	<10%	n	3	0	3	1	0	0	1	0	2	0	
		%	8	0	10	5	0	0	25.	0	22	0	
	10-50%	n	3	0	6	4	1	0	1	1	1	1	
		%	8	0	21	18	100	0	25	14	11.	33	
	≥50%	n	6	0	7	10	0	1	0	1	2	0	
		%	16	0	24	50	0	100	0	14	22	0	
Signet ring component	A	n	36	1	28	19	1	0	4	6	9	3	0.041*
		%	95	100	97	86	100	0	100	86	100	100	
	P	n	2	0	1	3	0	1	0	1	0	0	
		%	5	0	3	14	0	100	0	14	0	0	
TIL	Absent	n	27	0	15	8	1	1	0	3	2	1	0.012*
		%	71	0	52	36	100	100	0	43	22	33	
	Mild	n	11	1	14	14	0	0	4	3	7	2	
		%	29	100	48	64	0	0	100	43	78	67	
	Marked	n	0	0	0	0	0	0	0	1	0	0	
		%	0	0	0	0	0	0	0	14	0	0	
PTL	Absent	n	38	0	26	18	1	1	4	5	9	2	0.009*
		%	100	0	90	82	100	100	100	71	100	67	
	Present	n	0	1	3	4	0	0	0	2	0	1	
		%	0	100	10	18	0	0	0	29	0	33	
Necrosis	Absent	n	10	0	11	2	0	0	1	2	4	0	0.172
		%	26	0	38	9	0	0	25	29	44	0	
	Focal	n	23	0	15	13	1	1	3	5	5	3	
		%	61	0	52	59	100	100	75	71	56	100	
	Diffuse	n	5	1	3	7	0	0	0	0	0	0	
		%	13	100	10	32	0	0	0	0	0	0	

TIL: tumour infiltrating lymphocyte; PTL: peritumoral lesion; A: no loss of expression of any marker; B: loss of expression of all markers; C: loss of expression of both MLH, and PMS2; D: loss of expression of both MSH2, and MSH6; E: loss of expression of both of MSH6, and PMS2; F: loss of expression of both of MLH, and MSH2; G: isolated loss of expression of MLH; H: isolated loss of expression of MSH2; I: isolated loss of expression of MSH6; J: isolated loss of expression of PMS2.

A (No loss of expression of any MMR proteins): (38 cases) (Figure 1: C, D, E, F)

Around 66% of the cases of this pattern are made with 55% of them ≥ 50 years old, and 58% left-sided. Most of the cases of this pattern reveal no lymphovascular emboli (LVE) (79%), no perineural invasion (PNI) (90%), grade 2 (79%), stage T3 (82%), stage N0 (53%), and of adenocarcinoma NOS (81%). Most of the cases of this pattern do not have a mucinous component (68%), or signet ring component (95%), or TIL (71%), or PTL (100%) and show focal necrosis in 61% of the cases.

B (loss of expression of all MMR proteins)

Only one female case, 30 years old, has right-sided cancer colon. It is of adenocarcinoma NOS G3, stage T3N1, and doesn't have LVE, or PNI, mucinous or signet ring component. It shows mild TIL, PTL, and diffuse necrosis.

C (combined loss of expression of MLH and PMS2 proteins): (29 cases)

Around 62% of the cases of this pattern are male. 55% of them are ≥ 50 years old. 76% is the right side. Most of the cases of this pattern do not have LVE (66%), or PNI (100%). They are of grade 2 (69%), stage T3 (69%), and stage N0 (97%). Most of them are either of adenocarcinoma NOS or mucinous carcinoma (72%, 28% respectively). Some of the cases in this pattern are associated with absence of the mucinous component, or mucinous component ≥ 50% (45%, 21% respectively), absence of signet ring component (95%), TIL (absent, mild) (52%, 48% respectively), and absence of PTL (90%) and show focal necrosis in 52% of the cases.

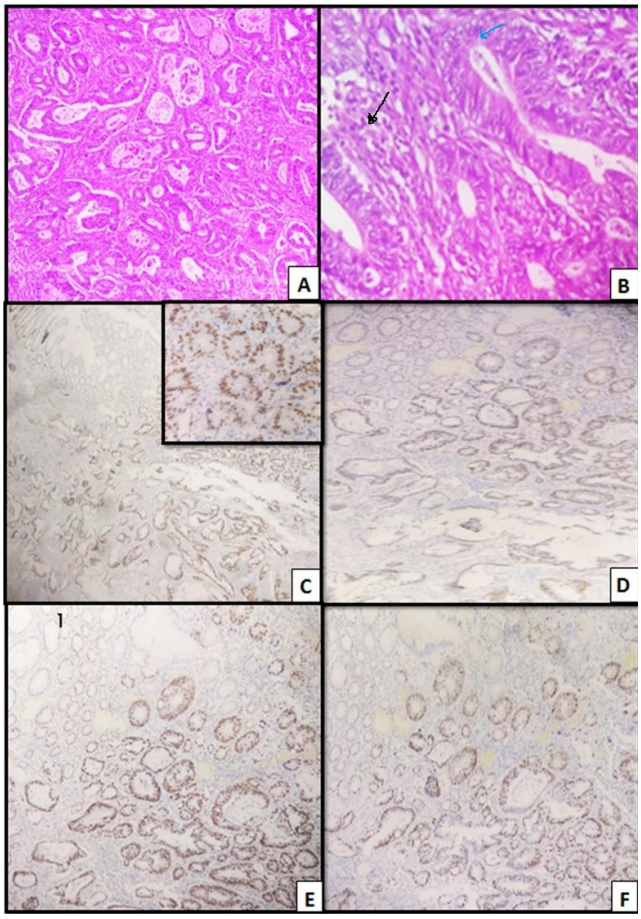

Figure 1: Colonic adenocarcinoma, moderately differentiated, grade II; A) H&E (x 40), Distorted acini of moderately differentiated malignant cells; B) H&E (x 400), showing TIL (blue arrow). PTL (black arrow); C) MLH1, moderately positive nuclear staining of tumor cells (x 40), Inset (x400); D) MSH2, moderately positive nuclear staining of tumor cells (x 100); E) MSH6 (x 100), moderately positive nuclear staining of tumor cells (x 100); F) PMS 2(x100), moderately positive nuclear staining of tumor cells (x 100)

D (combined loss of expression of MSH2 and MSH6 proteins): (22 cases) (Figure 2: D, and E)

Around 64% of the cases of this pattern are male. 59% of them are < 50 years old. 82% is the right side. Most of the cases of this pattern reveal the presence of LVE (54%) and absence of PNI (77%). Most of them are grade 3 (59%), stage T3 (77%), and stage N0 (50%). They are of adenocarcinoma NOS, mucinous carcinoma, and signet ring carcinoma (45%, 46%, and 9% respectively). This pattern in most of the cases is associated with the presence of mucinous component ≥ 50% in 50% of the cases in this pattern. There is no signet ring component in 86% of the

cases of this pattern. TIL (absent, mild) is present in 36%, 64% of this pattern, respectively (Figure 1B, and 2B). Absence of PTL is evidenced in 82% of the cases in this pattern. It shows focal necrosis in 59% of the cases.

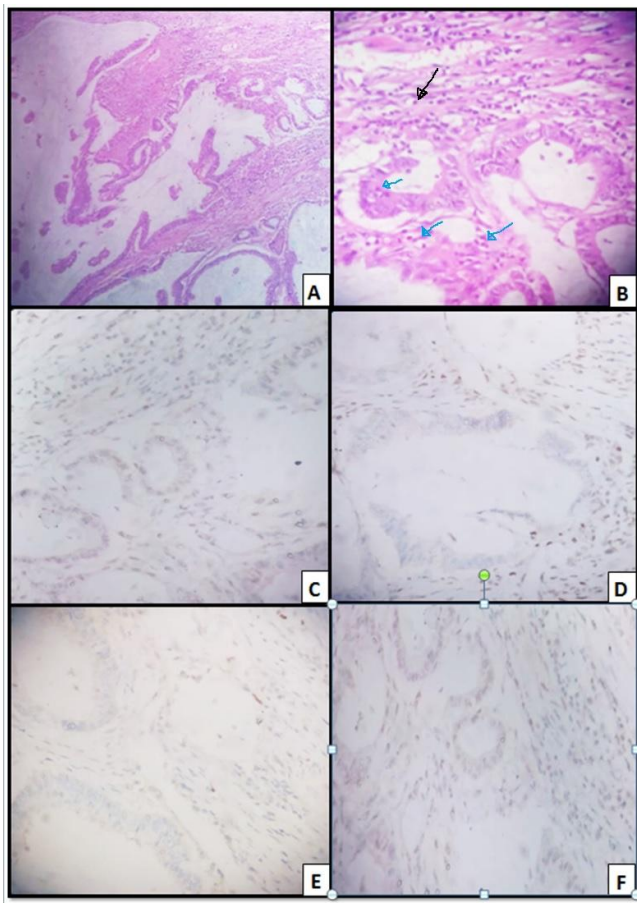

Figure 2: Colonic mucinous carcinoma; A) H&E (x 100), Pools of mucin entangling malignant glands; B) H&E (x 400), TIL (blue arrows); PTL (black arrow); C) MLH 1; Weakly positive nuclear staining of tumor cells (x 400), with positive lymphocytes, internal control; D) MSH2, Negative nuclear staining of tumor cells (x 400). with positive lymphocytes, internal control; E) MSH6, Negative nuclear staining of tumour cells (x 40); F) PMS 2, Weakly positive staining of tumour cells (x 400)

E (combined loss of expression of MSH6and PMS2 proteins)

Only one female case, 35-years-old, has right-sided CRC. It is of adenocarcinoma G2, stage T2N0, and has LVE, PNI, mucous component 20%, signet ring component, and focal necrosis. It doesn't show TIL or PTL.

F (combined loss of expression of MLH and MSH2 proteins)

Only one male case, 24years old, has left-sided CRC. It is of mucinous carcinoma G3, stage T4N2b, and has LVE, PNI, mucous component 60%, signet ring component, and focal necrosis. It doesn't show TIL or PTL.

G (Isolated loss of expression of MLH1): (4 cases)

In 4 cases, 2 of them are male, 2 of them are < 50 years old. 3/4 cases are left sided. Most of the cases of this pattern reveal LVE (75%), no PNI (100%), grade 2 (100%), stage T3 (100%), stage N1 (75%), and of adenocarcinoma NOS (100%). This pattern in most of the cases have mucinous component <50%, no mucinous component, (50%, 50% respectively), no signet ring component (100%), or TIL (mild) (100%), or no PTL (100%) and show focal necrosis in 75% of the cases.

H (Isolated loss of expression of MSH2): (7 cases)

In 7 cases, 5 of them are male, 5 of them are ≥ 50 years old. 4 cases are left sided. Most of the cases of this pattern reveal no LVE (57%), no PNI (100%). They are of grade 2 (71%), stage T3 (57%), and stage N0 (71%), and adenocarcinoma NOS (72%). Most of the cases in this pattern are associated with the absence of mucinous component (72%), absence of signet ring component (86%), TIL (absent, mild) (43% each), absence of PTL (71%) and show focal necrosis in 71% of the cases.

I (Isolated loss of expression of MSH6): (9 cases)

In 9 cases, 5 of them are male, 7 of them are ≥ 50 years old, 8 cases are right sided. Most of the cases of this pattern reveal no LVE (100%), no PNI (100%), grade 2 (78%), stage T3 (100%), stage N0 (100%), and of adenocarcinoma NOS (78%). This pattern in most of the cases is associated with no mucinous component, mucinous component ≥ 50%, mucinous component < 10%), (44%, 22%, 22% respectively), no signet ring component (100%), TIL (mild) (78%), and no PTL (100%). It shows focal necrosis in 56% of the cases.

J (Isolated loss of expression of PMS2): (3 cases)

In 3 cases, 2 of them are male. 2 of them are ≥ 50 years old. 2cases are left sided. Most of the cases of this pattern reveal no LVE (67%), no PNI (100%). They are of G2 adenocarcinoma (NOS) stage T3 (100%), and stage N0 (67%). This pattern in most of the cases is associated with the absence of mucinous component (67%), absence of signet ring component (100%), mild TIL (67%), and absence of PTL (67%). It shows focal necrosis in 100% of the cases. In the present study, there is significant association of expression pattern of MMR proteins with the laterality, the presence of lymphovascular emboli, the presence of perineural invasion, grade, T stage, N stage, the presence of signet ring

component, and the extent of tumour-infiltrating lymphocyte, the presence of peritumoral lesion (0.014, 0.035, 0.012, 0.033, 0.013, 0.000, 0.041, 0.012, and 0.009 respectively). No significant association of expression pattern of MMR proteins with gender, age category, histopathological type, the presence and amount of mucinous component, or necrosis (0.84, 0.51, 0.3, 0.35, 0.17 respectively).

Table 3: Clinicopathological characteristics about the MSI status of CRC

			MSS	MSI-L	MSI-H	P
		n	38 (33%)	23 (20%)	54 (47%)	115
Gender	M	n	25	14	33	088
		%	66	61	61	
	F	n	13	9	21	
		%	34	39	39	
Age	< 50	n	17	7	29	0.169
		%	45	30	54	
	< 50	n	21	16	25	
		%	55	70	46	
L	R	n	16	15	42	0.002*
		%	42	65	78	
	L	n	22	8	12	
		%	58	35	22	
LVE	A	n	30	16	31	0.092
		%	79	70	57	
	P	n	8	7	23	
		%	21	30	43	
PNI	A	n	34	23	48	0.253
		%	90	100	99	
	P	n	4	0	6	
		%	10	0	11	
G	G2	n	30	19	30	0.016*
		%	79	83	56	
	G3	n	8	4	24	
		%	21	17	44	
T	T2	n	6	3	11	0.324
		%	16	13	21	
	T3	n	31	20	38	
		%	81	87	70	
	T4	n	1	0	5	
		%	3	0	9	
N	N0	n	20	17	35	0.001*
		%	53	74	65	
	N1	n	5	6	15	
		%	13	26	28	
	N2	n	13	0	4	
		%	34	0	7	
V	NOS	n	31	19	33	0.146
		%	81	83	61	
	MUC	n	6	3	19	
		%	16	13	35	
	Signet	n	1	1	2	
		%	3	4	4	

G: Gender; L: Laterality; LVE: Lymphovascular Emboli; PNI: perineural Invasion; T: T stage; N: Nodal stage; V: Histopathologic variant; NOS: Non otherwise specified; and MUC: Mucinous.

Clinicopathological and histopathologic characteristics about the MSI status of CRC (Table 3, and 4)

MSI status of the CRC in our study is subdivided into 3 groups (MSS (38 cases), MSI-L (23 cases), and MSI-H (54 cases). Table 3 and 4 illustrate the clinicopathological and histopathological characteristics of each category of MSI.

MSS (microsatellite stable CRC)

About 66% of the cases of this group are male. 55% of them are ≥ 50 years old, 58% are left sided. Most of the cases of this status are characterised by the absence of LVE (79%), and absence of PNI (90%). They are of grade 2 (79%), stage T3 (82%), and stage N0 (53%). 81% of the cases in this status are of adenocarcinoma NOS. Most of the cases in this status do not have either mucinous component (68%), or signet ring component (95%), nor TIL (71%), nor PTL (100%). It shows focal necrosis in 61% of the cases.

Table 4: Histopathological characteristics of the MSI status of CRC

			MSS	L MSI	H MSI	P
		n.	38	23	54	115
Mucinous component	Absent	n	26	13	21	0.089
		%	68	57	40	
	<10%	n	3	3	4	
		%	8	13	7	
	10-50%	n	3	4	11	
		%	8	17	20	
	≥50%	n	6	3	18	
		%	16	13	33	
Signet ring component	A	n	36	22	49	0.653
		%	95	96	91	
	P	n	2	1	5	
		%	5	4	9	
TIL	Absent	n	27	6	25	0.004*
		%	71	26	46	
	Mild	n	11	16	29	
		%	29	70	54	
	Marked	n	0	1	0	
		%	0	4	0	
PTL	Absent	n	38	20	46	0.048*
		%	100	87	85	
	Present	n	0	3	8	
		%	0	13	15	
Necrosis	Absent	n	10	7	13	0.23
		%	26	30	24	
	Focal	n	23	16	30	
		%	61	70	56	
	Diffuse	n	5	0	11	
		%	13	0	20	

TIL: tumour infiltrating lymphocyte; PTL: peritumoral lesion.

MSI-L (low microsatellite instability CRC)

About 61% of the cases of this group are male, 70% of them are ≥ 50 years old, 65% are right sided. Most of the cases of this status are characterised by the absence of LVE (70%), and absence of PNI (100%). They are of grade 2 (83%), stage T3 (87%), and stage N0 (74%). 83% of the cases in this status are of adenocarcinoma NOS. the mucinous component in this status ranges from 0, < 10%, 10-50%, and ≥ 50% in 57%, 13%, 17%, 13% of the cases respectively. It is characterised by the absence of signet ring component (96%), mild TIL (70%), and absence of PTL (87%). It shows focal necrosis in 70% of the cases.

MSI-H (high microsatellite instability CRC)

Around 61% of the cases of this group are male, 54% of them are < 50 years old, 78% are right sided. Most of the cases of this status are characterised by the absence of LVE (57%), and absence of PNI (89%). They are of grade 2, 3(56%, 44% respectively), stage T3 (70%), and stage N0 (65%). They are of adenocarcinoma NOS, mucinous, and signet ring carcinoma (61%, 35%, and 4% respectively). The mucinous component in this status ranges from 0, < 10%, 10-50%, and ≥ 50% in 40%, 7%, 33%, and 20% of the cases respectively). It is characterised by the absence of signet ring component (91%), mild TIL (54%), and absence of PTL (85%). It shows focal necrosis in 56% of the cases.

In the present study, there is a significant association of MSI status with the laterality, grade, N stage, the presence and grade of tumour-infiltrating lymphocyte, the presence of a peritumoral lesion (0.002, 0.016, 0.001, 0.004, and 0.048 respectively).

No significant association of MSI status with gender, age category, the presence of lymphovascular emboli, the presence of perineural invasion, T stage, histopathological type, extent of mucinous component, the presence of signet ring component or necrosis (0.88, 0.16, 0.09, 0.25, 0.32, 0.14, 0.08, 0.65, and 0.23 respectively).

Multivariate analysis of factors predicting CRC with MSI-H (Table 5)

Multivariate logistic regression analysis was conducted including all of the above candidate predictors (laterality, grade, N stage, the extent of tumour-infiltrating lymphocyte, the presence of peritumoral lesion that is significant at univariate analysis). Proximal location (right sided) and lower grade, higher nodal stage, and marked TIL are selected as predictors of MS-H CRC when compared to the model of MSS based on a $P < 0.05$ (0.005, 0.031, 0.025, and 0.000 respectively). However, lower grade only is subsequently selected as predictors of MSI-H when compared to MSI-L based on a $P < 0.05$ (0.027). The final model of MSI-H predictors is shown in Table 5.

Table 5: Multivariate analysis of factors predicting CRC with MSI-H Parameter Estimates

MSI [a]		B	Std. Error	Sig.	95% Confidence Interval for Exp (B)	
					Lower Bound	Upper Bound
MSS	Intercept	1.541	390.22	0.997		
	laterality	-1.466-	0.526	0.005	0.082	0.646
	grade	1.279	0.594	0.031	1.121	11.516
	N stage	-1.892-	0.847	0.025	0.029	0.793
	TIL	-13.860-	0.527	0.000	3.404E-007	2.686E-006
MSI-L	Intercept	3.910	2550.3	0.999		
	grade	1.551	0.701	0.027	1.194	18.625

a: the reference category is MSI-H; TIL: tumour infiltrating lymphocyte; N: nodal stage.

Discussion

The annual incidence of CRC worldwide raises a significant public health impact. Familial CRC represents 20% of all CRC. Lynch syndrome represents 3.5% of all CRC [23]. These figures, besides awareness of the role of genomic instability in initiation and progression in CRC, raise the need for molecular screening of all CRC for Lynch syndrome [14]. Hashmi et al., 2017 reported that PCR amplification of microsatellite repeats remain the gold standard for recognition of MSI phenotype, this approach is not feasible in routine pathology lab [9], [24]. Therefore Hampel 2018 study recommends that IHC is preferred a method to screen for LS as IHC is equally sensitive to PCR, inexpensive, more readily available, and predicts the nonworking gene, so it has a big role in limiting the number of the gene to be sequenced [24]. Hampel 2018 study recommendation confirms the previous conclusion of the national cancer institute workshop on microsatellite instability for cancer detection and Familial Predisposition [22], [24].

The presence of MSI defines a subset of colorectal carcinomas with special molecular aetiology and characteristic clinicopathological features inclusive of increased survival [9], [15]. Since MSI-H CRCs share some morphological features such as young patient age, right-sided location, mucinous and signet ring histology, and intratumoral lymphocytosis, careful observation of tumour histology help identifying these tumours [19]. Thomas, in his study, concluded that quantification of TILs might provide a simple, single criterion for choosing CRC patients as candidates for MSI testing [25].

Frequency of expression pattern of MMR proteins and MSI status of CRC (Table 1, 2, 3, and 4)

In our study, the frequency of MSI (low and high) is 67% of CRC cases of the study (Table 3). MSI-L account for 20% of CRC cases of the study. MSI-H account for 47% of CRC cases of the study. The frequency of loss of expression is found to be quite variable in different studies. Tumours with d MMR status accounted for 34% of the total study cases that was done on the Pakistani population [9]. 21% in Singapore population [26], 6.9% in Chinese population [27] and approximately 15% in western studies [28]. The higher frequency of MSI in cases of CRC of our study compared to the previous studies in the same issue is attributed to that the pathology lab that we retrieve the cases from it is the unique lab if not the only lab in investigation of the expression of MMR protein by immunohistochemistry at this time, so the cases, expected clinically to have MSI, are transferred to this lab for immunohistochemical investigation for MMR protein.

In our study, the expression pattern of the d MMR proteins has variable patterns. No loss of expression of MMR proteins (MSS) accounts for 33% of the study cases. However, MSI includes all other patterns of lost expression. Loss of expression of all MMR proteins (MLH, MSH2, MSH6, and PMS2) is present in one case of the study (0.12% of MSI). The combined loss of 2 MMR proteins includes 4 categories the most frequent in our study is combined loss of MLH, and PMS2 29/115 (38% of MSI) then combined loss of MSH2, and MSH6 22/115 (29% of MSI). The other 2 categories of the combined loss of 2 MMR proteins include only 2 cases (one for each category (MLH + MSH2); (MSH6 + PMS2)) each represents 0.12% of MSI cases of the study. These frequencies as regard combined loss of MMR proteins are in agreement to studies done on Pakistani and Shanghai population [9], [11]. Isolated loss of one MMR proteins (MSI-L) accounts for 23/115 (30% of MSI). Lost MLH expression is present in 4/115 (5% of MSI). Lost MSH2 expression is present in 7/115 (9% of MSI). Lost MSH6 expression is present in 9/115

(12% of MSI). Lost PMS2 expression is present in 3/115 (4% of MSI). This means that the most frequent isolated loss of one MMR proteins in our study is lost MSH6, followed by MSH2 then MLH1, and lastly PMS2.

These results are in agreement with Yuan et al., 2015 as regard MSH6 and PMS2 [11]. Yuan et al., 2015 reported and confirmed that MMR gene products existing in cells always stay as heterodimers complex. And MLH1 and MSH2 are obligatory partners, combined with their secondary partners PMS2 and MSH6 respectively. If the degradation of the former partners occurs, caused by the mutation of the respective MMR gene, the later partners will not exist anymore. But the reverse is not true [11]. This fact was applied by Hall et al., in his study and screened CRC using MSH6, PMS2 proteins only instead of 4 [29]. This means that it is unlogic to find the isolated loss of PMS2 alone without loss of MLH1. However, 4% of MSI in our study, and 9.6% of Kumar et al., 2018 study showed isolated loss of PMS2 without loss of the MLH1 [30]. Hashmi et al., 2017 study found an isolated loss of MLH1 in 5% of study cases which agree with our result as regard isolated loss of MLH1 (5% of MSI) [9]. This controversy between our results and Yuan et al., 2015 can be explained on the basis that some pathogenic MLH1 missense mutations or MLH1 promotor hypermethylation functionally inactivates MLH1 protein and preserve its antigenicity leading to the occurrence of isolated loss of PMS2 [31], [32]. Hashmi et al., 2017 study, like our study, showed isolated loss of MLH1 (without its secondary partner) in 5% of the studied cases of CRC [9]. The frequent failure of antigenic retrieval of MLH1 protein is the logic cause for the lower frequency of isolated loss of MLH1 compared to other studies the problem that similarly faces Yuan *et al.*, 2015 study [11].

Correlation of Expression pattern of MMR protein/MSI status with clinicopathological and histopathological characteristics of CRC

The biological explanations for the characteristic morphology of MSI-H colorectal cancer are not known [33]. However, the increased lymphocytic reaction in MSI-H colorectal cancer may be explained by stimulated immunogenicity associated with the generation of abnormal proteins transcribed from mutant genes [15], [33]. In our study, there is significant correlation of expression pattern of MMR proteins with the laterality, lymphovascular emboli, perineural invasion, grade, T stage, N stage, signet ring component, tumor infiltrating lymphocyte, and peritumoral lesion (0.014, 0.035, 0.012, 0.033, 0.013, 0.000, 0.041, 0.012, and 0.009 respectively) Table (1, 2). These mean that lost expression of MMR proteins (MSI-L, MSI-H) in our study commonly occurs in the proximal colon up to the splenic flexure, and significantly characterized by the presence of lymphovascular emboli, absence of perineural invasion, lower grade, higher T stage, lower N stage, the presence of signet ring component, mild to marked tumor infiltrating lymphocyte, and the presence of peritumoral lesion. While no loss of expression of any MMR proteins (MSS) commonly occurs in the distal colon and rectum, associated with the absence of lymphovascular emboli, presence of perineural invasion, lower T stage, higher N stage, absence of signet ring component, absence of tumour infiltrating lymphocyte, and peritumoral lesion.

These results are approving nearly all studies dealt with this issue [9], [11], [19], [27], [34], [35], [36]. But we are different from most of the studies as to regard the extent of mucinous component and the presence of signet ring component that show no significant correlation with MSI status of the tumour in our study (Table 4) [27], [34], [36]. However Hashmi *et al.*, 2017 and Yuan et al., 2015 studies agree with our result in failure of detection of significant correlation of MSI status of CRC and the extent of mucinous and signet ring component of the tumour [9], [11]. This limited detection of the mucinous and signet ring component can be explained by examination of one slide per case so the exact amount of both components cannot be detected at the research level but if assessed in routine examination of the slides during the initial diagnosis of the mother, the father will be more accurate in prediction of MSI status of CRC. But it puts a big burden on the pathologists.

Multivariate analysis of factors predicting CRC with MSI-H (Table 5)

The current study succeeds in confirmation of that proximal colon CRC, and the presence of marked TIL, lower grade and higher nodal stage have a significant predictive value of MSI-H as compared to MSS CRC. While only lower grade has a significant predictive value of MSI-H when compared to MSI-L CRC. This result when MSI-H model is taken as a reference model.

There is a failure of confirmation of some clinicopathological and histopathological parameters as significant predictors of MSI-H that previously reported in the previous models of MSI-H predictors such as age, mucinous component, and signet ring component [33], [37], [38]. The reasons behind these controversies with our study are in different cut off value used for any of the clinicopathological parameters investigated or inclusion of additional parameters in these studies. For instance, Greenspan et al., 2009 considered any mucinous differentiation, no matter what the overall percentage rather than having at least 50% mucinous differentiation. While in our study, we consider the percentage of the mucinous component. Also, their models included histologic heterogeneity and the type of growth pattern that they are not included in our study [37]. Jenkins et al., 2007 had much younger patients, which probably

enriched the number of HNPCC cases in their study [33]. Revised Bethesda Guidelines for Microsatellite Instability included the Presence of synchronous, metachronous colorectal or other HNPCC-associated tumours regardless of age [38]. The big obstacle of our study is the inability to investigate germline mutations of MMR genes for this large number of cases. However, our study cases are not selected according to their ages, so the effectiveness of the clinicopathological and histological parameters can be noticed accurately.

In conclusion, the prevalence of MSI in our study is relatively high in comparison to international literature. Although they add a big burden on the pathologists, all clinicopathological and histological parameters should be assessed in all CRC for the sake of predicting MSI. Since it is not applicable to test all cases of CRC for MSI, selected cases only (according to clinicopathological predictors) will be proceeded to IHC for MLH1, MSH2 only. Consequently, the optimal approach to MSI evaluation is the immunohistochemical (IHC) analysis of tumours, followed by germline MSH2/MLH1 testing. IHC is easily available and inexpensive as part of the routine services inthe department of pathology.

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Clinical Science

The Impact of Physical Exercise on Brain-Derived Neurotrophic Factor (BDNF) Level in Elderly Population

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Abstract

BACKGROUND: Memory function disorder is a major health problem in geriatric patients. Physical exercise has the potency to decrease the incidence of many degenerative and chronic health problem, related to cognitive deterioration (dementia).

AIM: This research aimed to observe the effect of physical exercise in various doses and duration on memory function by analysing the role of Brain-Derived Neurotrophic Factor (BDNF) as a regulatory protein affected by exercise.

METHODS: This was an analytical observational study with a cohort design. Thirty participants were included in each group, classified as exercise and non-exercise group. The exercise was in the form of jogging for at least fifteen minutes every day. The observation was done for sixty days. Cognitive function assessment was done by using the Mini-Mental State Examination (MMSE) questionnaire. Meanwhile, the BDNF level was assessed by ELISA. Statistical analysis was done using Independent T-test.

RESULTS: Exercise group showed better MMSE score (28.56 ± 1.76), and a higher concentration of BDNF (235.34 ± 12.56 pg/mL), both were statistically significant.

CONCLUSION: Physical exercise was able to maintain geriatric cognitive function performance by BDNF protein regulation.

Introduction

Increase in life expectancy is related to higher geriatrics population in Indonesia. In Indonesia, life expectancy is 70.8 years by 2015 and is predicted to reach 72.2 years by 2030. In 2013, Indonesian geriatric's population was about 8.9% of all population in Indonesia; this proportion will increase to 21.4% by 2050 and becoming 41% by 2100 [1]. This phenomenon is good progress, which reflects the more conducive level of public health. However, this advance could lead to a fatal event and has the potency to develop a new problem if inadequately managed against increasing geriatric's population. Increase in geriatric's population can worsen health burden due to population susceptibility against many disorder, such as infection, degenerative disease and memory-related disorder [2].

Memory function disorder is a major health problem in geriatric patients. This serious problem could lead to decreased geriatric function in remembering elementary subjects, like their name, family or even their place of living [3]. The decrease in cognitive function is related to geriatric personal care disturbance, especially for activity daily living such as bathing or eating. It is a very serious problem that will reduce the geriatric quality of life, even for the family and society. In concordance to that, an approach to search the modality in increasing quality of life is needed by preventing memory function deterioration.

Physical exercise is the right answer in increasing geriatric quality of life. Physical exercise has the potency to decrease the incidence of many degenerative and chronic health problem, such as cancer, diabetes and coronary heart disease. Recent studies showed that physical activity is responsible for lowering dementia incidence in geriatric population [4], [5], [6], [7], [8]. On the other side, many animal trials showed the potency of physical exercise in preventing memory loss. However, the cellular and molecular mechanism of physical exercise in hindering memory loss is still being studied, particularly about the doses and duration of physical exercise.

Therefore, this study aimed to observe the effect of physical exercise in various doses and duration on memory function by analysing the role of Brain-Derived Neurotrophic Factor (BDNF) as a regulatory protein affected by exercise.

Methods

Study design and objects

This study was an analytical observational study with cohort design in the geriatric population at Kambang Iwak Palembang elderly community. Thirty participants were included in each group, classified as exercise and non-exercise group. The exercise group was the study subjects which fulfilled criteria: age more than or equal to sixty years, no history of cardiovascular disease, metabolic syndrome or neurological disorder, enduring physical exercise in the form of jogging for at least fifteen minutes a day. On the other hand, the non-exercise group had the same criteria as an exercise group with no physical exercise. The observation was done for sixty days. The study was approved by the Research Ethics Committee of Faculty of Medicine Universitas Sriwijaya (No.193/kptfkunsri-rsmh/2018).

Cognitive function assessment

Cognitive function assessment was done by using the Mini-Mental State Examination (MMSE) questionnaire. MMSE questionnaire consisted of 11 items from five domains: 10 points related to time and place orientation, 6 points related to memory, 5 points related to numerical calculation, 7 points related to language and two points related to comprehensive/ judgement ability. True answer got one point, and the wrong answer got zero points. The assessment was done by interviews to study subjects.

BDNF level assessment

Three mL of blood from each study subjects were taken from the brachial vein and inserted into EDTA tube and centrifuged at 3000 rpm for ten minutes. Before the blood was taken, study subjects must undergo starvation phase for ten hours. Afterwards, supernatant from the serum was inserted into the tube and stored at -80°C temperature. BDNF serum measurement was done by following the manual procedure of Human *Enzyme-Linked Immunosorbent Assay* (ELISA) kit BDNF (Elabscience®)**.**

Statistical analysis

Descriptive analysis (mean±SD) was done by SPSS 24.0 program. Bivariates analysis to MMSE and BDNF was done by using Independent T-test. Level of significance was set at p = 0,05.

Results

Baseline characteristics

Table 1 showed no significant differences between exercise and non-exercise group. Study subjects had no statistically different age and BMI, which showed that age and BMI in both groups were identic. Blood glucose and cholesterol level data showed that both groups had no history of diabetes mellitus or hypercholesterolemia. Systolic and diastolic blood pressure on both groups reflected that participants did not have hypertension.

Table 1: Baseline Characteristics

Variable	Exercise-Group	Non-Exercise-Group	p-Value
Age (yrs)	65.13 ± 7.32	65.25 ± 8.31	0.21*
Height (m)	1.59 ± 0.03	1.59 ± 0.21	0.15*
Weight (kg)	69.97 ± 8.54	69.17 ± 7.97	0.34*
BMI (kg/m^2)	27.67 ± 3.12	27.42 ± 4.32	0.54*
Blood Glucose (mg/dL)	105.23 ± 7.67	106.83 ± 10.67	0.11*
Cholesterol (mg/dL)	176.13 ± 8.97	177.03 ± 11.12	0.09*
Systolic Blood Pressure (mmHg)	135.34 ± 6.23	136.01 ± 8.52	0.23*
Diastolic Blood Pressure (mmHg)	78.98 ± 7.31	78.01 ± 8.11	0.35*

*Independent T Test. p > 0.05.

Cognitive function

There were significant differences between exercise and non-exercise group related to MMSE scoring. MMSE is an application to assess cognitive function. Higher MMSE score shows better cognitive function. The exercise group had higher MMSE score compared to the non-exercise group and was statistically significant (Table 2).

Table 2: MMSE Scoring

Variable	Exercise-Group	Non-Exercise-Group	p-Value
MMSE (Mean ± SD)	28.56 ± 1.76	25.06 ± 1.32	0.02*

*****Independent T test. p < 0.05.

BDNF concentration

BDNF concentration in the exercise group was higher than the non-exercise group and was

statistically significant (Tabel 3). Higher BDNF level reflects better maintenance of individual cognitive function.

Table 3: BDNF Concentration

Variable	Exercise-Group	Non-Exercise-Group	p-Value
BDNF (Mean(pg/mL) ± SD)	235.34 ± 12.56	112.01 ± 10.12	0.01*

*Independent T-test. p<0.05.

Discussions

This study showed relevant result between cognitive function, which was assessed by MMSE and BDNF level. MMSE score in the exercise group was higher than the non-exercise group. This result showed that the exercise group was able to maintain cognitive function. This study revealed that BDNF level was higher in the exercise group compared with the non-exercise group. Molecular exploration demonstrated that BDNF possesses a role as a regulatory protein in preserving cognitive function. Elevation of BDNF level was thought to maintain cognitive function in a good state. Physical exercise, jogging for 15 minutes a day, was able to conserve cognitive function in the geriatric population by BDNF regulation. Our study strengthens physical exercise role, in the form of jogging in retaining cognitive function by BDNF regulation. This study revealed an answer about the role of physical exercise to individual memory quality, especially in the geriatric population. Brain-Derived Neurotrophic Factor (BDNF) is a neutrophil family protein member that is responsible for neuronal growth, differentiation and survivability. An elevated level of BDNF that caused by physical activity is thought to increase neurogenesis dan synaptogenesis and preventing the neuronal loss, which is contributing to cognitive function and decreasing psychiatric disorder. The study showed that peripheral BDNF concentration can reflect central nervous system health, with lower BDNF concentration in patients with psychiatric dan metabolic disorder [9], [10], [11], [12]. There are possibilities that many different aspects play the role of physical exercise effect and central nervous system health. Suggested mechanism is an increase in cerebral blood flow, changes in neuroendocrine response, changes in endocannabinoid and neurotransmitter release, which lead into BDNF release and initiate structural changes in central nervous system, in order to preserve synaptogenesis, neurogenesis and maintaining cognitive function [13], [14], [15].

In conclusion, physical exercise in the form of jogging for at least fifteen minutes every day was able to maintain geriatric cognitive function performance by BDNF protein regulation.

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Nutrition Status Related to Clinical Improvement in AFB-Positive Pulmonary Tuberculosis Patients in Primary Health Centres in Medan, Indonesia

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Keywords: Tuberculosis; Body mass Index; Albumin; Sputum Conversion; Indonesia

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Abstract

BACKGROUND: Tuberculosis (TB) remains a major public issue in Indonesia, including in North Sumatra province. Despite reported good efficacy of TB treatment in the region, the success of treatment depends on many factors, including nutritional status.

AIM: To determine the relationship between Body Mass Index and Albumin level with sputum conversion in AFB-positive pulmonary TB patients.

METHODS: The study was done in two primary health centres in Medan between October and November 2018. A total of 39 newly diagnosed TB patients with confirmed AFB-positive were included in the study. Participants received TB treatment according to the national guidelines.

RESULTS: The proportion of participants with below normal, normal and above normal BMI status were 13 (33.3%), 21 (53.9%) and 5 (12.8%), respectively. Level of albumin was determined as normal in 25 participants (64.1%), and the remaining as low. Normal BMI status was significantly associated with increased albumin level ($p < 0.05$). At 2 months follow-up, the sputum conversion was observed in 24 individuals (61.5%), and the conversion was significantly associated with normal BMI and/or normal albumin level ($p < 0.05$).

CONCLUSION: We concluded that nutritional status is an important factor in the success of TB treatment.

Introduction

Tuberculosis (TB) is a lungs disease caused by Mycobacterium tuberculosis bacillus. The source of transmission is from AFB-positive pulmonary TB patients. When coughing or sneezing, the patients spread germs in the air through sputum (droplet nuclei). One cough produces around 3000 sputum splashes. Thus, TB is included in 10 deadly diseases in the world. According to the data from North Sumatera provincial health office in 2016, the new cases of TB reached 23,097 numbers with 5,714 death rates. Medan is the biggest TB infected area compared to other places in North Sumatera. Since 2010, TB cases in Medan experiences a continuous increase. In 2010, there were approximately 1,425 new cases, with 38,615 Case Detection Rate (CDR). However, there were 1,425 cure rates in the same year.

Sputum conversion is a strong predictor and the beginning of success in TB therapy. It is determined by the discovery of mycobacteria in sputum culture taken on the second month of treatment. Sputum conversion on TB cases is formed at the end of the first month (60 – 80%), the end of the second month (95%), while 9% do not convert [1]. The success factors in the intensive phase of treatment are patients' life and work, the access to health service, food supply and behaviours (the obedience in medicine consumption, alcohol consumption, smoking habit and nutritional status) [2].

One of the most efficient and economical measurement to describe nutritional status is the Body Mass Index (BMI). The research from Tama et al., (2016) [3] shows that AFB-positive pulmonary TB patients with BMI < 18.5 kg/m^2 possess the higher cumulative probability of conversion failure compared to those patients with BMI > 18.5 kg/m^2. The speed of sputum conversion (hazard rate) on patients with a BMI < 18.5 kg/m^2 is lower compared to patients with BMI > 18.5 kg/m^2. Sputum conversion will take a longer time if the increase of patients' body weight at the end of the intensive phase is < 1 kg. Therefore, TB patients with low BMI at the beginning of treatment should be monitored for their nutritional improvement. This nutritional improvement must be the focus of attention during treatment, considering the increase of body weight in the last intensive phase is a vital contribution to the success of sputum conversion.

Albumin is one of the indicators of nutritional status, both at the beginning of a malnutrition case or during improvement [4]. To TB patients, albumin in serum will experience a significant decline, assumedly because of nutrition factors (low food supply, anorexia, catabolism increase), enteropathy and acute phase of protein reaction [5]. The decline of total protein and albumin are often caused by the decrease of appetite, malnutrition and malabsorption of TB patients [6]. According to the research by Simbolon et al., (2016) [7], it is found that there are 69.76% TB patients with albumin content < 3.5 g/dL, and the rest 30.24% possess albumin ≥ 3.5 g/dL. This result indicates that there more patients with albumin < 3.5 g/dL, which is caused by chronic inflammation, where albumin production decrease while albumin damage keeps increasing until a situation called hypoalbuminemia or the deficiency of albumin in blood [8].

This research aimed to find a relation between nutrition status clinical improvement in AFB-positive pulmonary TB patients In Medan, North Sumatera, Indonesia.

Methods

The research was conducted in a quantitative method using the correlation study design and cross-sectional approach. The research took place in Amplas and Teladan health centre median from October 01, 2018 until November 30, 2018. The population of this research is the new TB patients with AFB-positive pulmonary at Teladan and health centre Medan. Samples are all AFB-positive pulmonary patients who meet all the inclusive and exclusive criteria. Inclusive criteria include patients < 16 years old; diagnosed as the new AFB-positive pulmonary patients; New TB patients with regular treatment at Teladan and Amplas health centre; Patients agree to participate in the research and fill the informed consent. The exclusion criteria include pregnant women; new TB patients followed by DM or HIV; TB category II. Total samples are 39 patients.

The sputum examination on patients was conducted in the laboratory of Amplas and Teladan health centre by Ziehl Neelsen method. The sputum collection was a method (Sewaktu-Pagi-Sewaktu): (1) when the patients suspected TB comes to Teladan and Amplas health centre. (2) the morning after the patients suspected TB is waking up before eating and drinking. (3) When the patients suspected TB returns to Teladan and Amplas health centre while carrying morning sputum. Sputum taken is marked with patients' names. Anti-Tuberculosis medicine used were Rifampicin, INH, Pyrazinamide, Streptomycin, Ethambutol with the dosage adjusted to patients' weight. The AFB-positive pulmonary examination was conducted before medication, the second week, the eighth week after the intensive phase of medication. Patients' sputum conversion data were collected from TB 01 patients' card. The measurement of weight and height was conducted in the health centre when patients came for treatment and general anamnesis of history of the disease, gender, age, education, occupation etc. The BMI data were collected from the patients' medical records.

The examination of albumin was conducted by taking 3 cc of venous blood and left for 10 minutes without anticoagulant. After 10 minutes, blood was centrifuged in 3000 rpm speed for five minutes. Serum at the upper part was separated with a micropipette to be examined. The albumin measurement was conducted in Prodia Laboratory Medan in bromcresol green (BCG) method. Patients' albumin data was collected from the test result.

Univariate and Bivariate analysis was conducted using SPSS. Univariate analysis was aiming to describe the characteristics of Body Mass Index variable, Albumin Level and AFB-positive pulmonary Sputum Conversion results. The bivariate analysis was aiming to know the relation between Body Mass Index and Albumin Level with Sputum Conversion Sputum. Statistical analysis using SPSS 20, for analysis relationships, we use the chi-square test.

Results

Characteristics of Respondents

From 39 respondents, there were 22 males (56.41%) and 17 females (43.59%). Their ages range from 18-70 years old with average 43.23 ± 15.45. There were 28 TB patients at a productive age (16-55 years old). Respondents data based on education

background were divided into 27 High School graduates (69.23%) and 28 working people (71.79%).

Table 1: Characteristics of Respondents

Characteristics	n	%	Average ± SD
Gender:			
Male	22	56.41	
Female	17	43.59	
Age:			43.23 ± 15.45
16-35 years old	13	33.33	
36-55 years old	15	38.46	
56-75 years old	11	28.21	
Education:			
Elementary	4	10.26	
Junior High School	7	17.95	
Senior High School	27	69.23	
University	1	2.56	
Work Status:			
Working	34	87.18	
Not Working	5	12.82	

Body Mass Indeks (BMI)

Mass Index Respondents' bodies ranged from 15.14 - 32.19 with a mean of 20.82 ± 4.05. From (Table 2), it is known from 39 respondents there are 13 people (33.33%) patients have BMI < 18.5 kg/m^2, 21 people (53.85%) patients have BMI < 18.5-25 kg/m^2 and 5 people (12.82%) patients had BMI > 25 kg/m^2. These results get the majority of respondents at the time of diagnosis having a Body Mass Index > 18.5-25 kg/m^2 (normoweight).

Table 2: Sample Distribution Based on BMI

Characteristics	N	%	Average ± SD
Body Mass Index (kg/m^2)			20.82 ± 4.05
Skinny (< 18,5 kg/m^2)	13	33.33	
Normal (>18,5-25kg/m^2)	21	53.85	
Fat (> 25 kg/m^2)	5	12.82	
Total	39	100.00	

Level of Albumin

The albumin level in respondents was ranging between 3.10 g/dL – 4.50 g/dL with average 3.73 ± 0.39. From the table, it is seen that from 39 respondents, there were 25 patients (64.10%) possess albumin ≥ 3,5 g/dL and 14 patients (35.90%) possess < 3,5 g/dL. This result shows that there were more respondents with albumin ≥ 3.5 g/dL.

Table 3: Sample Distribution Based on Albumin Content

Characteristics	n	%	Average ± SD
Albumin content (g/dL)			3.73 ± 0.39
≥ 3.5 g/dL	25	64.10	
< 3.5 g/dL	14	35.90	
Total	39	100.00	

Results of Sputum Conversion

Of the 39 respondents diagnosed with AFB-positive pulmonary TB as many as 24 people (61.54%) experienced sputum conversion at the end of the intensive phase of treatment, and 15 people (38.46%) did not experience sputum conversion. These results get more respondents who experienced sputum conversion at the end of intensive phase treatment.

Table 4: AFB-positive pulmonary Sputum Conversion Results

Characteristics	n	%
Conversion	24	61.54
Non-Conversion	15	38.46
Total	39	100.00

Relationship between Body Mass Index and Sputum Conversion

From the results of cross-examination (Table 5), it was found out from 24 pulmonary TB patients who experienced sputum conversion there were 4 people (16.67%) with underweight BMI, 16 people (66.67%) with normoweight BMI and 4 people (16, (67%) with an obese BMI. Of the 15 pulmonary TB patients who did not experience sputum conversion. There were 9 people (60%) with underweight BMI, 5 people (33.3%) had a normal BMI, and 1 person (6.7%) had obese BMI. From the results of the chi-square test, it is known that the value of p-value is 0.02, meaning that there is a significant relationship between the Body Mass index and sputum conversion.

Table 5: Cross Tabulation of the Body Mass Index and Sputum Conversion

Sputum	Body Mass Index (n, %)						Total		*p*-value
conversion	Underweight		Normoweight		Obese				
Conversion	4	16.67	16	66.67	4	16.67	24	61.54	0.020
No-Conversion	9	60.00	5	33.33	1	6.67	15	38.46	
Total	11	33.33	21	53.85	5	12.82	39	100.00	

Relationship between Albumin Levels and Sputum Conversion

From the results of cross-tabulation (Table 6), it was found out of 24 pulmonary TB patients who had BTA sputum convergence of 20 people (83.33%) patients had albumin levels ≥ 3.5 g/dL and 4 people (16.67%) patients have albumin levels < 3.5 g/dL. Of the 15 pulmonary TB patients who did not experience sputum conversion as many as 5 people (33.33%) patients had albumin levels ≥ 3.5 g/dL and 10 people (66.67%) patients had albumin levels < 3.5 g/dL. From the results of the chi-square test, it is known that the value of p-value is 0.002, which means there is a significant relationship between albumin levels and sputum conversion.

Table 6: Cross Tabulation of Albumin Levels and Sputum Conversion

Sputum	Albumin Level (n, %)						*p*-value
Conversion	≥ 3.5 g/dL		< 3.5 g/dL		Total		
Conversion	20	83.33	4	16.67	24	61.54	0.002
No-Conversion	5	33.33	10	66.67	15	38.46	
Total	25	64.10	14	35.90	39	100.00	

Relationship of Body Mass Index with Levels of Albumin

From the results of cross-examination (Table 7), it was found out of 13 pulmonary TB patients with thin BMI (15.38%) patients had albumin levels album 3.5 g/dL and 11 people (84.62%) had albumin levels < 3.5 g/dL. Of the 21 pulmonary TB patients with normal BMI there were 18 people (85.71%) patients had

albumin levels ≥ 3.5 g/dL and 3 people (14.29%) had albumin levels < 3.5 g/dL. Of the five obese pulmonary TB patients, there were 5 people (100%) who had albumin levels ≥ 3.5 g/dL. From the Pearson correlation test results, it is known that the P value is 0.001, which means that there is a significant relationship between the Body Mass Index and albumin levels. This concludes if the patient's mass index increases, the albumin level will also increase.

Table 7: Cross Tabulation of the Body Mass Index with Albumin Levels

BMI	Albumin Level (n, %)						*p*-value
	≥ 3.5 g/dL		< 3.5 g/dL		Total		
Underweight	2	15.38	11	84.62	13	33.33	0.001
Normoweight	18	65.71	3	14.29	21	53.85	
Obese	5	100.00	0	0	5	12.82	
Total	25	64.10	14	35.90	39	100.00	

Discussions

Characteristics of Respondents

The number of pulmonary TB patients in the Medan Health Center was 22 men (56.41%) while the female sex was 17 (43.59%). These results get more pulmonary TB patients who are male. The results of this study are in line with the research of Puspita et al., (2016) [9] which found that the majority of men with pulmonary TB in the Lung Hospital Arifin Achmad in Pekanbaru had the highest number of men, which amounted to 48 people or 67.60%. Then the Ministry of Health data (2013) found that the incidence of pulmonary TB tends to be more male sex because it is associated with different social interactions between men and women, smoking tobacco, drinking alcohol causes a decrease in the body's defence system, so that when exposed to TB germs can quickly cause symptoms and if checked become positive for pulmonary TB.

Most pulmonary TB patients in Medan Health Center are at a productive age (16-55 years) as many as 71.79%. The results of this study are by the research of Puspita et al., (2016) [9], which states that the age group of pulmonary tuberculosis patients is in the productive age group. Furthermore, the Ministry of Health's statement in 2007 in the national guidelines for tuberculosis prevention states that as many as 75% of individuals infected with TB germs are in the productive age group (15-50 years). One of the factors that cause pulmonary TB patients is in the productive age group because they spend more time outside the home to work and interact with other people, so the risk of TB transmission becomes greater because contact with people suffering from pulmonary tuberculosis becomes more often.

The majority of pulmonary TB patients in Medan Health Center are mostly high school graduates, which are as many as 27 people (69.23%). The results of this study are similar to the research conducted by Puspita et al., (2016) [9] which found that the distribution of pulmonary tuberculosis patients treated at Lung Poly Arifin Achmad Pekanbaru Hospital based on education was found to have the most secondary education at 35 people (49.3%). Furthermore, the results of research conducted by Andhika in West Bandung Regency in 2012, obtained from a total of 42 people with pulmonary tuberculosis patients as many as 25 people (59.5%) had secondary education. Through education, an individual can understand his illness. The level of education plays an important role in public health. The higher the level of education, the higher the ability to receive health information.

The number of pulmonary TB patients who worked were 35 people (87.18%), and those who did not work were 5 people (12.82%). This result found that the majority of pulmonary TB patients in the Medan Puskesmas were working. This can occur because the work environment is the easiest place to transmit TB disease. Certain types of work have a high risk of spreading and developing TB disease, such as factory workers. The results of the study by Tama et al., (2016)[3] also found that most of the positive smear pulmonary TB patients in Persahabatan General Hospital had a working status of 79 people (65.8%). Another possibility, because patients who work have high mobility than those who do not work, so the possibility of exposure to tuberculosis germs is higher. Lifestyle such as smoking and the risk of work originating from outdoor air pollutants, especially those related to industrial exposure, also increase the risk of being infected with pulmonary TB.

Body Mass Index

From the results of the cross-tabulation test showed that of the 39 pulmonary TB patients in the Medan Health Center it was found that at most pulmonary TB patients had a BMI > 18.5-25 kg/m2 which was as many as 21 people (53.85%). A study conducted by Puspita et al., (2016) [9] which found that the nutritional status of pulmonary TB patients based on body mass index (BMI) in Lung Poly Arifin Achmad Pekanbaru Hospital was 33 people (46.5%) with normal nutritional status, 31 people (43.7%) with underweight nutritional status, 4 people (5.6%) with overweight nutritional status and as many as 3 people (4.2%) with obesity nutritional status. Furthermore, the results of Suliyanti's research on the description of nutritional status and level of protein-energy consumption in pulmonary tuberculosis patients in Medan Johor Health Center in 2013, as many as 51.7% of patients with normal nutritional status. The Wokas et al., (2015) [10] study also found more pulmonary TB patients at Prof. Dr R. D. Kandou Manado Persahabatan General Hospital which has

BMI > 18.5-25 which is as much as 48.5%.

Level of Albumin

The results showed that from 39 patients there were 25 people (64.10%) pulmonary TB patients had albumin levels ≥ 3.5 g/dL and 14 people (35.90%) had albumin levels < 3.5 g/dL. These results indicate that more pulmonary TB patients in Medan Health Center have albumin levels of ≥ 3.5 g/dL. The results of this study are in line with the research conducted by Wokas et al., (2015) [10], which found more tuberculosis patients who had albumin levels ≥ 3.5 g/dL. On the other hand, the results of the study by Simbolon et al., (2016) [7] found that more tuberculosis patients had albumin levels < 3.5 g/dL. The occurrence of differences in the results of this study with the study of Simbolon et al., (2016) [7] due to differences in the number of samples used or the possibility of patients who have albumin levels ≥ 3.5 g/dL are still in the early stages of tuberculosis.

Sputum Conversion

The results showed that AFB-positive pulmonary TB patients in Amplas and Teladan Medan Health Centers were more likely to experience sputum conversion after undergoing intensive phase treatment for two months than those without sputum conversion. Of the 39 pulmonary TB patients with AFB-positive, there were 24 people (61.54%) who experienced sputum conversion while those who did not experience sputum conversion were 15 people (38.46%). The results of this study obtained more pulmonary TB patients with AFB-positive who experienced sputum conversion after undergoing intensive treatment for two months at 61.54%. The results of this study are in line with the research of Aliyah et al., (2016) [11] that the results of sputum conversion in the intensive phase were 55 subjects (62.5%) experienced sputum conversion and in 33 subjects (37.5%) did not experience conversion. The results of the study by Tama et al., (2016) [3] obtained a cumulative probability of pulmonary TB patient survival rates of 17% and 9.2% of patients experienced failed conversions. Then the Intiyati et al., (2012) [12] study found that the recovery of pulmonary TB patients based on the results of sputum examination was mostly positive as many as 27 people (57%) and the negative as many as 20 people (43%).

The conversion number is one indicator to assess the progress and success of TB prevention. This indicator is useful to know the results of treatment quickly and to find out whether direct supervision of swallowing drugs is done correctly (Ministry of Health, 2007) [13]. To assess the success of treatment for pulmonary TB disease can be seen from the results of sputum examination at the end of treatment. AFB conversion occurs due to drug administration during treatment and is a predictor of the success of TB treatment. Although in this study pulmonary TB patients who experienced sputum conversion were greater when compared to the number of TB patients who did not experience conversion, the results were not by the WHO sputum conversion target of 85%. The healing of each person is different because the patient has a bad response or a good response after treatment. Good response is determined by changes (conversion) of sputum after 30 days of adequate treatment, but a poor response occurs if sputum changes are more than 30 days (Khairil et al., 2017) [14]. There are several factors that cause the failure of sputum conversion in the intensive phase. Among them are lack of supervision in the intensive phase, poor adherence to medication, the inappropriate dosage of medication recommendations, comorbidities, and the presence of multiple drug-resistant TB (Liu, 2008) [15].

The factors that led to the success of sputum conversion did not reach 85% as targeted by the government and WHO in this study, one of which was due to the level of positivity of sputum in pulmonary TB patients at the beginning of treatment. Of the 15 pulmonary TB patients who did not experience sputum conversion at the end of the intensive phase treatment consisted of 8 patients with AFB-2positive (2+), 3 patients with AFB-3positive (3+) and 4 people with AFB-positive (1+). The results of this study found that the majority of pulmonary TB patients with AFB-2positive (2+) and AFB-3positive (3+) at the start of treatment did not experience sputum conversion. The results of this study are in line with the research of Tama et al., (2016) [3] which states that the higher the level of positivity of a patient's sputum at the beginning of treatment, the greater the cumulative probability of the conversion rate (survival rate). Patients with a positive level of sputum 3+ have the greatest cumulative probability of survival rate, followed by 2+, 1+, and scanty (1-9 stems).

The majority of pulmonary TB patients who did not experience sputum conversion in this study had BMI < 18.5 kg/m^2. Conversion failure experienced by patients with thin BMI can occur due to Anti-Tuberculosis Drugs malabsorption. The low nutritional status of patients affects the decrease in drug concentration in blood plasma and improves kidney function to perform disposal. As a result, the effectiveness of TB treatment is not optimal so that it can increase the risk of treatment failure for TB patients; it can even increase the risk of recurrence [3]. The last factor that caused the failure of sputum conversion in this study was pulmonary TB patients with albumin levels < 3.5 g/dL. Decreasing albumin levels will cause a decrease in the number of albumin bonds with Anti-Tuberculosis Drugs so that it will have an impact on the TB healing process [16].

Relationship of Body Mass Index with Level of Albumin

From the Pearson correlation test results obtained that the value of p-value is 0.001 < 0.05 means that there is a positive and significant relationship between the Body Mass Index and albumin levels. This proves that every patient's body mass index increases; the albumin level will also increase. The results of this study are in line with the results of the study of Wokas et al., (2015) [10] which found that every increase in BMI then albumin levels would also increase. This illustrates that BMI affects albumin levels and vice versa.

Increased food intake in TB patients will increase albumin levels. The results of this study are in accordance with the theory which states that albumin is one of the largest proteins in blood plasma, where at the time of infection there is a decrease in blood plasma values, injury or stress may be the cause of increased metabolic needs to repair damaged tissue and to neutralize existing free radicals in the body. This decrease in total protein values and albumin levels can be caused by a decrease in appetite in patients, malnutrition and malabsorption often occur in tuberculosis patients.

Relationship of Body Mass Index with Sputum Conversion

According to the results of the study, 24 of the pulmonary TB patients who had sputum conversion mostly had BMI > 18.5-25 kg/m^2, which was 16 people (66.67%) while most of the 15 pulmonary TB patients who did not have sputum conversion had BMI < 18.5 kg/m^2 which is 9 people (60%). These results indicate that pulmonary TB patients who have a BMI < 18.5 kg/m^2 more do not experience sputum conversion, whereas pulmonary TB patients who have a BMI > 18.5 - 25 kg/m^2 more experience sputum conversion. The results of this study prove that one of the important factors that can influence the success of BTA sputum conversion in pulmonary TB patients is the patient's initial nutritional status when diagnosed with TB. The results of this study are in accordance with the results of the study of Tama et al. (2016) [3] which stated that smear-positive pulmonary TB patients with BMI < 18.5 needed longer time to experience sputum conversion and risked experiencing conversion failure of 1.32 - 8.86 times compared with patients who have BMI > 18.5.

The results of the study by Dillon, 1995 and by Intiyati et al., (2012) [12], which said that TB patients who were malnourished would result in inhibited production of antibodies and lymphocytes so that the healing process was hampered. The results of this study are consistent with the results of the Amaliah (2012) [17] study that pulmonary TB patients with underweight nutritional status will have a risk of conversion failure 3.5 times greater than patients with normal nutritional status. Furthermore, the results of Khariroh (2006) [18] 's study found that TB patients with thin nutritional status would be at risk of conversion failure 8,861 times greater than TB patients with normal nutritional status and TB patients with very poor nutritional status would be at risk of conversion failure 30,918 times greater than sufferers TB with normal nutritional status.

From the results of the chi-square test, it is known that the value of p-value is 0.020 < 0.05 means that there is a relationship between body mass index and sputum conversion in pulmonary TB patients at Amplas and Teladan Medan Health Centers. The results of this study are in line with the research of Intiyani et al., (2012) [12] which found that there was a relationship between nutritional status (BMI) and recovery in pulmonary TB patients in Lung Poly RSD Sidoarjo. In line with the research of Tama et al., (2016) [3], which states that the nutritional status of patients measured by BMI is known to be a predictor of sputum conversion in positive smear pulmonary TB patients. Individuals who have good nutritional status will be able to develop their immune response so that the healing process of tuberculosis can run well, whereas, in individuals whose nutritional status is poor, they are more at risk of failure in the healing process (Isselbacher et al., 1999) [19].

Relationship between Levels of Albumin and Sputum Conversion

From the results of cross-tabulation, it was found out from 25 patients who had albumin levels ≥ 3.5 g/dL as many as 20 people (80%) experienced sputum conversion and 5 people (20%) who did not experience sputum conversion. While of the 14 patients who had albumin levels < 3.5 g/dL as many as 4 people (28.57%) who experienced sputum conversion and 10 people (71.43%) who did not experience sputum conversion. From these results indicate that the majority of pulmonary TB patients who have albumin levels ≥ 3.5 g/dL will experience sputum conversion while pulmonary TB patients with albumin levels < 3.5 g/dL the majority will not experience sputum conversion. The results of this study are in line with the research of Khairil et al., (2017) [14] who found the mayor over TB patients in the Integrated TB Services of dr. Zainoel Abidin Banda Aceh Regional Public Hospitals, which has a decrease in albumin levels, will experience poor clinical improvement of 54.20%.

Furthermore, the results of the Kulsum et al., (2017) [20] study found that one of the risk factors for failed sputum conversion in TB patients was low albumin levels. The level of albumin as a protective factor or an increase in albumin will reduce the failure of sputum conversion — albumin as a means of transporting drugs such as rifampicin which is a TB therapy drug. Albumin is a plasma protein, and TB drugs bind to plasma proteins. Anti-TB drugs are

metabolised in the liver and excreted with bilirubin by bile (Mercer et al., 2007) [21].

From the results of the chi-square statistical test, it is known that the value of p-value is 0.002, meaning that there is a significant relationship between the levels of albumin and sputum conversion. The results of this study are supported by the results of a study conducted by Matos and Lemos (2006) [22] who reported that albumin has a significant relationship to sputum changes in TB patients, so it is important to take action to improve nutritional status in TB patients during the treatment process. Sputum changes in TB patients are influenced by C-reactive protein (CRP) and albumin levels. The Kulsum et al., (2017) [20] study found that blood albumin levels act as protective factors that will reduce the failure of AFB sputum conversion. Furthermore, the research of Khairil et al., (2017) [14] found that albumin levels influenced clinical improvement in TB patients in the Integrated TB Services Installation of Dr Zainoel Abidin Banda Aceh Regional Public Hospitals. Rifampicin, as a drug for pulmonary TB, is strongly linked to albumin in pulmonary TB patients. In addition to rifampicin, other pulmonary TB drugs, namely isoniazid, also bind strongly to albumin. This strong bond is expected to increase the antimicrobial effect of Anti-TB Drugs, thereby reducing inflammatory cytokines and accelerating healing (Lassen et al., 2006) [23]. Decreasing albumin levels will cause a decrease in the number of albumin bonds with Anti-TB Drugs so that it will have an impact on the TB healing process [16].

In conclusion, there was a significant correlation between Body Mass Index and albumin level on AFB-positive pulmonary TB patients at Medan health centre in this research, and there was a significant correlation between Body Mass Index and sputum conversion on AFB-positive pulmonary TB patients at Medan health centre. There was also a significant correlation between albumin level and sputum conversion on AFB-positive pulmonary TB patients at Medan health centre.

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Clinical Science

Systemic Inflammatory Response in Predicting Prostate Cancer: The Diagnostic Value of Neutrophil-To-Lymphocyte Ratio

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Abstract

BACKGROUND: Over the past decades, the study of the microenvironment of cancer has supported the hypothesis between inflammation and cancer. Previous studies have demonstrated a promising value of platelet-to-lymphocyte (PLR) and neutrophil-to-lymphocyte ratio (NLR) as a systemic inflammatory response in prostate cancer.

AIM: To evaluate their pre-biopsy values of PLR and NLR in predicting prostate cancer.

MATERIAL AND METHODS: This is a diagnostic study with retrospective design. We included all benign prostatic hyperplasia (BPH) and prostate cancer (PCa) patients who underwent prostate biopsy in Adam Malik Hospital between August 2011 and August 2015. We used PSA value above 4 ng/dL as the threshold for the biopsy candidates. The relationship between pre-biopsy variables affecting the percentage of prostate cancer risk was evaluated, including age, prostate-specific antigen (PSA) level, and estimated prostate volume (EPV). The PLR and NLR were calculated from the ratio of related platelets or absolute neutrophil counts with their absolute lymphocyte counts. The values then analysed to evaluate their associations with the diagnosis of BPH and PCa.

RESULTS: Out of 298 patients included in this study, we defined two groups consist of 126 (42.3%) BPH and 172 PCa (57.7%) patients. Mean age for both groups are 66.36 ± 7.53 and 67.99 ± 7.48 years old ($p = 0.64$), respectively. There are statistically significant differences noted from both BPH and PCa groups in terms of PSA (19.28 ± 27.11 ng/dL vs 40.19 ± 49.39 ng/dL), EPV (49.39 ± 23.51 cc vs 58.10 ± 30.54 cc), PLR (160.27 ± 98.96 vs 169.55 ± 78.07), and NLR (3.57 ± 3.23 vs 4.22 ± 2.59) features of both BPH and PCa groups respectively ($p < 0.05$). A Receiver Operating Characteristics (ROC) analysis was performed for PLR and NLR in analysing their value in predicting prostate cancer. The Area Under the Curve (AUC) of PLR is 57.9% with a sensitivity of 56.4% and specificity of 55.6% in the cut-off point of 143 ($p = 0.02$). The NLR cut-off point of 3.08 gives 62.8% AUC with 64.5% sensitivity and 63.5% specificity. These AUCs were comparable with the AUC of PSA alone (68.5%). We performed logistic regression between PSA, PLR, and NLR with result in the exclusion of PLR if calculated conjunctively. Therefore, NLR has a promising performance in predicting PCa in patients with PSA above 4 ng/dL (OR = 3.2; 95% CI: 1.96-5.11). We found as many as 80 (63.5%) patients with benign biopsy results with negative NLR value in this study.

CONCLUSION: NLR has promising value in predicting prostate cancer. A further prospective study in validating its diagnostic value was needed.

Introduction

Prostate cancer (PCa) is the second most common cancer worldwide. It accounts for more than 15% of cancer in men with ongoing rising clinical relevancies. About 70% of them occurs in the developed country [1], [2]. Trans Rectal Ultrasound Guided procedure of prostate biopsy remains the gold standard in most countries in the diagnosis of prostate cancer. Since the biopsy is mostly office procedure and not only uncomfortable but also associated with significant complications, various non-invasive strategies have been invented to prevent unnecessary biopsy [3].

Serum Prostate Specific Antigen (PSA) has been used as the screening standard for patients in suspicion of prostate cancer. PSA value of more than 4 ng/ml has been considered the threshold to biopsy in most countries [1], [2], [4], [5], [6], [7], [8]. But, recent meta-analyses showed a positive predictive value of PSA above 4 ng/ml is only 25% [6]. Besides, the invasive prostate biopsy may still miss some percentage of cancer, given that up to 20% of men will

have prostate cancer in a repeated biopsy [9]. Various imaging and bio-molecular marker have been suggested to increase diagnostic accuracy, but none of these methods is available for widespread use, either due to the availability or even high-cost issues [2], [9].

Over the past decades, our study of the microenvironment of cancer has supported Virchow's hypothesis of the relationship between inflammation and cancer. Inflammatory markers have been associated with more aggressive disease [7], [10]. Study of Cihan et al., in 2013 showed that values of lymphocytes, neutrophils, and white blood cells are significantly lower in PCa patients with statistically significant difference noted in lymphocytes value compared to healthy controls. Their ease of assessment brings the suggestion to use it in combination with other parameters in predicting the diagnosis of prostate cancer [3]. Many follow-up studies had been using neutrophil-to-lymphocyte ratio in fields of predicting diagnosis, prognosis, and recurrence after definitive management [2], [5], [11], [12], [13], [14], [15].

Though small in numbers, previous studies also demonstrated the promising value of platelet-to-lymphocyte (PLR) in prostate cancer. Kaynaret al. found an increased level of PLR in PCa compared with that in benign prostatic hyperplasia (BPH) with PSA value greater than 10 ng/ml [16]. A statistically significant higher PLR in PCa compared to BPH patients was also demonstrated by Yuksel et al., in 2015 [6].

According to our knowledge, there is still no data on the use of NLR and PLR as predictors of PCa in Indonesia. Therefore, this study is conducted to evaluate its pre-biopsy value in predicting PCa.

Material and Methods

Population of Study

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

Variables

Serum PSA was collected from recent laboratory results just before biopsy procedures were performed. We collected EPVs from their initial Trans-Abdominal Ultrasound (TAUS) of the prostate. Prostate was measured in 3-dimensional aspects, and its volume was estimated with the modified ellipsoid formulation in cm^3 (0.523 [(length x width x height)]. NLR and PLR value are acquired from the direct division of absolute neutrophil count or platelets count with their absolute lymphocyte count.

Analysis

Input and data analysis was performed using SPSS ver 20.0 software. Data will be divided into two groups according to their histopathology of prostate biopsy, the BPH and PCa group. A pathology of prostatitis will be excluded. Data related to PCa prediction such as routine blood count, NLR, and PLR of each group will be distributed in a frequency table and analysed for their value in predicting biopsy results with bivariate analysis. A p value of < 0.05 (α = 5%) was considered statistically significant. Logistic regression will be performed to multivariate analysed EPV, PSA, NLR, and PLR as predictive factors of prostate biopsy.

Results

Characteristics and Bivariate Analysis

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

Table 1: Patients Characteristics and Hematologic Parameters

Parameters	BPH (n = 126) Mean ± SD (Median)	PCa (n = 172) Mean ± SD (Median)	p
Age (years)	66.36 ± 7.53	67.99 ± 7.48	0.64*
PSA (ng/dL)	19.28 ± 27.11	40.19 ± 49.39	< 0.0001*
EPV (cm^3)	49.39 ± 23.51	58.10 ± 30.54	0.02*
Hb	12.99 ± 2.00 (13.20)	12.95 ± 2.01 (13.10)	0.754**
Leucocytes Count ($x10^3/mm^3$)	8.67 ± 3.45 (8.11)	9.19 ± 3.29 (8.46)	0.1**
Absolute Neutrophil Count ($x10^3/mm^3$)	6.09 ± 3.19 (5.45)	7.54 ± 3.64 (6.92)	< 0.0001**
Absolute Lymphocyte Count ($x10^3/mm^3$)	2.09 ± 0.83 (2.02)	2.00 ± 0.76 (1.87)	0.29**
Platelets Count ($x10^3/mm^3$)	286.16 ± 112.24 (266)	311.61 ± 120.81 (294)	0.049**
NLR	3.57 ± 3.23 (2.54)	4.22 ± 2.59 (3.67)	< 0.0001**
PLR	160.27 ± 98.96 (128.13)	169.55 ± 78.07 (151.28)	0.02**

*T-test **Mann-Whitney Test.

Comparing the laboratory results of both groups, there were statistically significant differences noted from PSA (19.28 ± 27.11 vs 40.19 ± 49.39), EPV (49.39 ± 23.51 vs 58.10 ± 30.54), NLR (3.57 ±

3.23 vs 4.22 ± 2.59), and PLR (160.27 ± 98.96 vs 169.55 ± 78.07) in each bivariate analysis.

NLR, PLR, and PSA

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

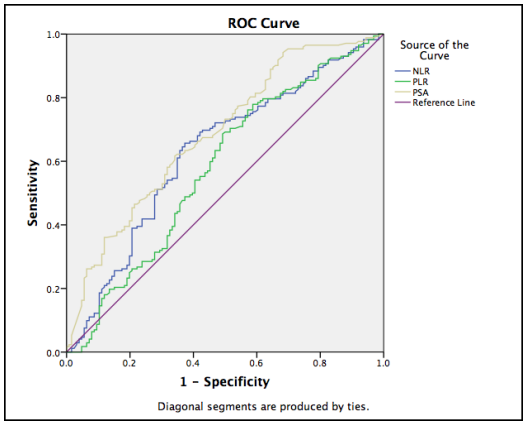

Figure 1: The ROC Curves of NLR, PLR, and PSA

The NLR cut-off point of 3.08 gives 62.8% AUC with 64.5% sensitivity and 63.5% specificity. The AUC of PLR is 57.9% with a sensitivity of 56.4% and specificity of 55.6% in the cut-off point of 143 (p = 0.02).

Table 2: The AUC of NLR, PLR, and PSA

Parameters	AUC	p
NLR	62.8%	< 0.0001
PLR	57.9%	0.02
PSA	68.5%	< 0.0001

Multivariate Analysis

We performed logistic regression between EPV, PSA, PLR, and NLR with results described in Table 2. We used the cut-off value of NLR (3.08) and PLR (143) as retrieved from ROC analysis before to be included as categorical variables in the regression. PLR was excluded from the regression as it was not statistically significant if analysed conjunctively. Thus, the equation leaves NLR as a single most important systemic inflammatory biomarker in predicting prostate cancer (Table 3).

Table 3: Multivariate Analysis: Logistic Regression

Variables	OR	95% CI	p
EPV	1.012	1.002 – 1.022	0.21
NLR (≥ 3.08)	2.856	1.734 – 4.702	< 0.0001
PLR (≥ 143)*	1.152	0.648 – 2.049	0.63
PSA	1.016	1.007 – 1.025	< 0.0001

*before excluded in Backward: LR method.

NLR, PLR, and IPCRC: A Subgroup Analysis

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

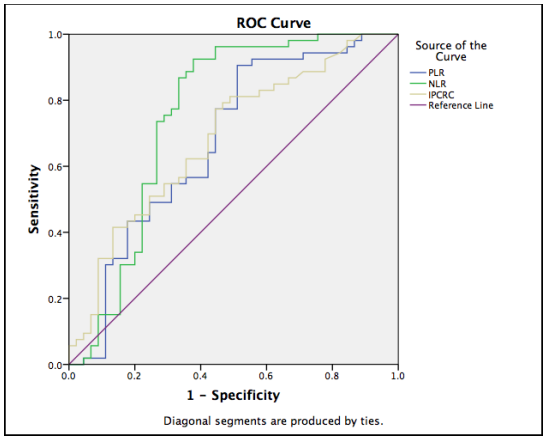

Figure 2: The ROC Curves of PLR and IPCRC Score

We found a comparable value between NLR and PLR with IPCRC in predicting prostate cancer (AUC of 75.3%, 67.6%, and 68.4%, respectively) with a statistically significant difference was noted between each value (p < 0.05) as shown in Figure 2 and Table 4.

Table 4: The AUC of NLR, PLR, and IPCRC

Parameters	AUC	p
NLR	75.3%	< 0.0001
PLR	67.6%	0.003
IPCRC	68.5%	0.002

Discussion

The body response to cancer parallels with inflammation and wound healings. In 1863, Rudolf Virchow noted leucocytes in neoplastic tissues and suggested a connection between inflammation and cancer. He suggested that the “lymphoreticular infiltrate” reflected the origin of cancer at sites of chronic inflammation. Tumour-infiltrating lymphocytes may contribute to cancer growth and spread, and the immunosuppression associated with malignant disease. In his review in 2001, Balkwill et al., still mention the theory of “Tumours: wounds that do not heal” [10] previously showed by Dvorak in 1986 [10]. This theory showed how wound healing and tumour stroma formation share many important features. Wound healing is usually self-limiting, but tumours secrete a vascular permeability factor, vascular endothelial growth factor (VEGF), that can lead to

persistent extravasation of fibrin and fibronectin and continuous generation of the extracellular matrix. Platelets in wounds are a critical source of cytokines, especially transforming growth factor β (TGF-β) and VEGF. Platelet release of such factors may also play an important role in angiogenesis. Also, malignant cells themselves secrete proinflammatory cytokines [10].

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

As supported by previous studies, we calculated the NLR values of both groups and found a significant difference of the value between PCa and BPH groups (4.22 ± 2.59 vs 3.57 ± 3.23; p < 0.0001). Gokce et al., in a larger population, also found the same significant results of the difference between both groups (p = 0.002). McDonald et al., in his study, found a correlation between increasing PSA and NLR value (ORmultiv = 1.14; 95% CI, 1.03-1.26), after adjustment for age, smoking, body mass index, education, race, co-morbidities, and use of medications [4]. These findings are also coherent with the recent study of Oh JJ et al., and Kawahara et al., in 2015 [5], [9].

In this study, though we found a significant difference of platelets count with no statistical difference in lymphocyte count between both groups, the ratio of PLR value gives the event more significant difference (p = 0.02). A similar result of this study also showed by Yuksel et al., where a significant intergroup statistical difference was found for PLR (p = 0.041) but not for lymphocyte count (p > 0.05) [6]. This also supported by the study of Li et al., who found a statistical difference of PLR value in PCa and normal/BPH patient (p < 0.05).

Our multivariate analysis revealed that not only EPV and PSA, but NLR was also an independent biomarker in predicting prostate biopsy (OR 2.856; 95%CI 1.734 – 4.702). PLR was excluded in the analysis as it is not significant statistically in the concurrent analysis. Same results were stated in Kawahara et al. who found that not only free to total PSA ratio (HR = 3.13) but also NLR (HR = 2.21) was an independent risk factor for prostate cancer [5]. In another study of Oh JJ et al., in their multivariate analyses, a higher NLR was significantly associated with prostate cancer detection after adjusting for other factors (OR = 1.372, p = 0.038). An increased accuracy noted from 0.712 to 0.725 in the addition of NLR (p = 0.005) in the multivariate model for prostate cancer detection [9]. Thus, we can conclude that NLR is increasing in prostate cancer.

IPCRC has been widely used in Indonesia as a clinical calculator to predict the diagnosis of PCa. In a subgroup analysis, we compared the NLR and PLR value with IPCRC and analysed their AUC with ROC analysis and found a comparable result of NLR with IPCRC. NLR surprisingly has a comparable AUC with IPCRC (75.3% vs 68.5%). From these findings, we can conclude that further validation is mandatory. Increasing NLR can be part of the calculation in predicting prostate cancer.

In conclusion, NLR is thus likely elevated in patients with prostate cancer. Accordingly, NLR, with or without combination with PSA, may function as a new systemic inflammatory biomarker in predicting prostate biopsy results. To be applied as routine testing and to selectively decide candidates for prostate biopsy in a patient with PSA value more than 4 ng/ml, further prospective trial and validation is mandatory.

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Clinical Science

Pattern of Prescribing NSAIDs Utilisation at Outpatient Pediatric Poly at Universitas Sumatera Utara Hospital

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Keywords: NSAIDs utilisation; Pediatric outpatient; Prescription

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Abstract

BACKGROUND: The wrong prescription pattern on NSAIDs also often results in side effects and drug interactions that cause serious and detrimental drug reactions. Drug use research is needed to describe the pattern of drug use, early signs of rational drug use, interventions to improve drug use, cycles of quality control, and continuous quality improvement.

AIM: This study aimed to determine the prescribing pattern of NSAIDs at outpatient Pediatric Poly at Universitas Sumatera Utara Hospital, Medan, Indonesia in 2017.

METHODS: This descriptive retrospective study was conducted from October to November 2016 with data from July and August 2017.

RESULT: The study showed, outpatient of pediatric poly at Universitas Sumatera Utara Hospital in Medan there were 45,000 prescriptions, and 62 (0.15%) prescriptions contained NSAIDs. The most frequently prescribed NSAIDs 53 (85.48%) of prescriptions for outpatient pediatric poly was paracetamol. The most use of NSAIDs was consumed by a female in the age group of 3 years-12 years was 35 (58.06%). The highest frequency of NSAIDs utilisation was 7 days with 25 prescriptions (40.32%). There were 17 (27.42%) prescriptions with inappropriate dose, and the most widely prescribed dosage form was syrup for 34 (54.83%) prescriptions. The most duration of treatment with NSAIDs drugs which is paracetamol reached up to seven-days 25 (40.32%). The most frequently prescribed drugs 57 (91.93%) were generic drugs.

CONCLUSION: It can be concluded that there are still inappropriate doses and frequency of NSAIDs utilisation.

Introduction

Analgesics or often referred to as painkillers are part of substances that can reduce or block pain without losing consciousness [1]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most widely prescribed and commonly used for the treatment of pain, fever, and inflammatory processes. The National Disease and Therapeutic Index said that non-steroidal analgesic and anti-inflammatory drugs (NSAIDs) are the drugs most often prescribed by doctors around the world [2].

Although analgesic drugs are generally safe to use, if they are wrong in their use, symptoms of unwanted side effects can occur. It is better to know the kinds of pain that can be treated with analgesic drugs before choosing the right pain medication. This information should be provided by pharmacists to NSAIDs users [3].

The wrong prescription pattern on NSAIDs also often results in side effects and drug interactions that cause serious and detrimental drug reactions [3]. Drug use research is needed to describe the pattern of drug use, early signs of rational drug use, interventions to improve drug use, cycles of quality control, and continuous quality improvement. The pattern of drug use can illustrate the extent to which the drug is used at certain times and in certain areas for example countries, regions, communities, hospitals, the depiction becomes important when the drug is used as part of the evaluation [4]. Because of the high number of uses of NSAIDs at pediatrics that were prescribed by doctors, researchers conducted this study to know the use of NSAIDs at Pediatric Poly of Universitas Sumatera Utara Hospital in Medan.

Methods

This study was a retrospective which conducted in March-April 2018 in the outpatient Pediatric Poly at Universitas Sumatera Utara Hospital in Medan from January to December 2017. Inclusion criteria in this study were NSAIDs prescriptions with complete data about gender, age and body weight from pediatric outpatient.

The obtained data were presented in percentages and table forms. The obtained data processed and analysed using the Microsoft Excel program, then presented in table form based on gender, age, name and NSAIDs class, duration of use, dosage form, type of medication (generic or brand name) in the outpatient of Pediatric Poly at Universitas Sumatera Utara Hospital in Medan. The NSAIDs prescriptions doses were evaluated by comparing the doses according to “Specialite Drug Information” by Indonesian Pharmacist Association, “Drug Doses” by Frank Shann, and “Handbook of Pediatric Dose” by Association Indonesian Pediatric Physician.

Results

Based on the observation of the prescription sheets for Pediatric Poly taken from January 2017 until December 2017, there were 4,500 prescription sheets was obtained and 62 (0.15%) prescription sheets met the inclusion criteria.

Table 1: The usage of NSAIDs based on gender

No.	Gender	n (%)
1.	Male	33 (46.78)
2.	Female	29 (53.22)

The number of patients that use NSAIDs based on gender and ages can be seen in Table 1 and 2.

Table 2: The usage of NSAIDs based on age

No.	Age	n (%)
1.	0 - 1 month	0 (0)
2.	2 month - 2 years	16 (24.19)
3.	3 years - 12 years	35 (58.06)
4.	13 years - 17 years	11 (17.75)

Table 1 showed that the used number of NSAIDs based on gender was 33 (46.74%) for male and 29 (53.22%) for female.

Based on ages (Table 2), the used number of NSAIDs were 16 (24.19%) for 2 month-2 years old, 35 (58.06%) for 3-12 years old and 11 (17.75%) for 13-17 years old.

Table 3: The usage of NSAIDs based on NSAIDs names

Name of Drugs	n (%)
Sanmol	3 (4.83)
Aspilet	3 (4.83)
Paracetamol	53 (85.48)
Aspirin	2 (3.22)
Paracetamol Forte	1 (1.61)

The pattern of NSAIDs name and length of use of NSAIDs in outpatient pediatric poly at Universitas Sumatera Utara Hospital can be seen in Table 3 and 4 respectively.

Table 4: The usage of NSAIDs based on the duration of use

Duration	n (%)
1 day	1 (1.61)
2 days	3 (4.38)
3 days	17 (27.41)
4 days	3 (4.83)
5 days	7 (11.29)
6 days	1 (1.61)
7 days	25 (40.32)
8 days	0 (0)
9 days	0 (0)
10 days	2 (3.22)
14 days	2 (3.22)
23 days	0 (0)
Fever symptom	1 (1.61)

Various dosage forms of NSAIDs drugs have been made to provide convenience to patients in taking medication and also to maintain drug stability. The usage of NSAIDs based on dosage forms can be seen in Table 5.

Table 5: The usage of NSAIDs based on dosage forms

Dosage Forms	n (%)
Tablet	22 (35.48)
Syrup	34 (54.83)
Drop	3 (4.83)
Powder	3 (4.83)

Table 5 showed that the most widely used drug dosage forms were syrup as much as 34 (54.83%).

Table 6: The usage of NSAIDs based on the type of drug (generic and brand name)

Type of Drugs	n (%)
Generic drugs	57 (91.93)
Brand name drugs	5 (8.07)

The use of drugs both in generic or brand name is an option in a prescription, but the most important thing is the accuracy of the doses to get the optimum therapeutic effect. The pattern of NSAIDs use based on brand name and evaluation of doses can be seen on Table 6 and 7 respectively.

Discussion

Relieving pain is the most desired by patients, especially in children. NSAIDs are widely used in pediatric patients. NSAIDs are the most extensive prescription, especially in cases of inflammatory pain

due to their strong effects. But NSAIDs has side effects, especially in children. The side effects that can occur are damage to the gastrointestinal tract, heart, kidneys, while the vital organs in children are still under development [5].

Table 7: The evaluation of NSAIDs dose

Month	Match n (%)	Mismatch n (%)
Jan	5 (8.06)	1 (1.61)
Feb	4 (6.45)	2 (3.22)
Mar	5 (8.06)	1 (1.61)
Apr	4 (6.45)	0 (0)
May	4 (6.45)	0 (0)
Jun	4 (6.45)	0 (0)
Jul	4 (6.45)	2 (3.22)
Aug	2 (3.22)	0 (0)
Sep	5 (8.06)	3 (4.83)
Oct	5 (8.06)	3 (4.83)
Nov	2 (3.22)	5 (8.06)
Des	1 (1.61)	0 (0)
n (%)	45 (72.58)	17 (27.42)

NSAIDs effectively control the symptoms of many of the diseases although they have little effect on the underlying causes. Their effect is mainly on the mediators of the inflammatory process. Unfortunately, these mediators have important physiological roles in the maintenance of health, particularly in the gastrointestinal tract and the kidney, so that their inhibition results in many unwanted reactions of varying severity [6].

Based on the result, the number of male patients using NSAIDs was 29 (46.78%), and the number of female patients was 39 (53.22%) showed that the use of NSAIDs based on gender was not too different. This is the same as the research done by Soeroso (2007), which states that the use of NSAIDs is higher in female, as seen from the percentage of incidences of pain more often experienced by women.

Table 4 showed that the longest use of NSAIDs was prescribed for 7 days as many as 25 prescriptions. According to the guideline, the use of Mefenamic Acid by the guidelines stating that the use of Mefenamic Acid is not more than 1 week. Paracetamol should not be given more than 4 g per day because of the possibility of causing liver damage [7]. Of the 62 prescriptions for using sign an "If sick/necessary" as many as 1 prescription or as much as 1.61%, this is still very low while analgesic use does not need to be taken if the patient does not feel pain because the use of long-term NSAIDs can cause side effects. The most common side effects are stomach-intestinal disorders, blood damage, liver and kidney damage and also allergic skin reactions [8]. According to Table 4, there was the inappropriate frequency of using NSAIDs; Table 4 showed that there was some physician that prescribed NSAIDs more than 7 days and its use still needs to be considered again.

The syrup dosage form is mostly like by children because of the good taste. In general, the use of oral drugs is more widely used, because oral drug use is the most enjoyable, easy and safe use [9]. The use in syrup and drop preparations is used in paracetamol, which is used in infants and young children.

As indicated in Table 6, the majority of prescription drugs are generic drugs of 57 (91.93%) prescriptions and brand name drugs of 5 prescriptions (8.07%). Based on these data the use of generic drugs was higher than brand name drugs. This finding indicated that the Indonesian Health Policy of using generic drugs had been well implemented. The government has committed to making healthcare affordable as stated in the National Health Policy. Generic drugs typically cost 30% to 60% less than the brand name drugs, and widespread use of generics has the potential to reduce the price of other brand name drugs by creating more competition. Generic medicines are as effective as brand name medicines [10].

Based on Table 7, overall there were 17 (27.42%) of NSAIDs doses were inappropriate which is less than the therapeutic range (underdose) or administrated over than therapeutic range (overdose). The NSAIDs prescriptions doses were evaluated by comparing the doses according to “Specialite Drug Information” by Indonesian Pharmacist Association, “Drug Doses” by Frank Shann, and “Handbook of Pediatric Dose” by Association Indonesian Pediatric Physician. The accuracy of the dose is a very important factor. If the dose is administrated less than the therapeutic range (underdose), there will be a therapeutical failure, but if administrated over than the therapeutic range (overdose) there will be symptoms such as nausea, vomiting, diarrhoea, and anaphylactic shock. It can be concluded that there was inappropriate drug use in terms of dose and frequency of use in NSAIDs utilisation.

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Association of Inflammation Mediator in Mucosal and Tissue of Chronic Rhinosinusitis with Recurrent Nasal Polyp

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Abstract

BACKGROUND: Chronic rhinosinusitis with polyps (CRSwNP) have a high risk of recurrence and patients often experience repeated surgery. There are several types of inflammatory patterns in CRSwNP, such as Th2 inflammation (eosinophilic) and Th1/Th17 inflammation (neutrophilic).

AIM: This study aims to determine the expression of IL-5, IL-8, IL-17A and TGF-β in recurrent CRSwNP using the most convenient and non-invasive examination tool such as brushing the mucosal polyp and find out its correlation with polyp tissues.

MATERIAL AND METHODS: A cross-sectional comparative study was carried out on 15 samples of mucosal brushing and polyp tissue. Expressions of IL-5, IL-8, IL-17A and TGF-β on mucosa were measured using the Enzyme-Linked Immunosorbent Assay (ELISA) examination and tissues using Immunohistochemical (IHC) examination.

RESULT: The result showed that Only IL-5 has a significant relationship between mucosa and tissue with moderate positive correlation ($p < 0.05$; $r = 0.527$).

CONCLUSION: This study concluded that mucosa brushing could be used as a simple and non-invasive examination to observe the expression of IL-5 in recurrent CRSwNP. IL-5 is one of the cytokines that mark the Th2 (eosinophilic) inflammatory pattern where eosinophilic polyps are closely related to recurrence.

Introduction

Chronic rhinosinusitis with polyps (CRS) is a chronic inflammatory disease of the sinuses and paranasal sinuses that last for more than 12 weeks, CRS can be accompanied by polyps (CRSwNP) or without polyps (CRSsNP) [1], [2]. This disease could reduce the quality of life of patients and cause an economic burden because of the high cost of treatment, especially because of the high rate of recurrence, causing patients to experience repeated surgery [3], [4], [5]. In the United States, chronic rhinosinusitis accounts for almost 16% of adults per year, which consumes health funds of around 5.8 billion US/year [6]. The prevalence of CRS in Indonesia is quite high. Based on data from the ENT polyclinic at M. Djamil Central Public Hospital, Padang from October 2011 to September 2012, 106 new cases of CRS were found in which 87 cases were CRSWNP without polyps and 19 cases of CRSwNP.

There have been many theories and research, the exact aetiology of nasal polyps and the causes of recurrence is still unknown [6]. The role of several mediators (cytokines) such as interleukin, growth factors and chemokines in the inflammatory

process had been widely investigated. In addition, the effect of a combination of cytokines, which serves as a signal to the cell to provide response and growth factors, produced by T lymphocytes, fibroblasts, epithelial cells such as the Granulocyte Macrophage Colony Stimulating Factor (GM-CSF), Interleukin-3 (IL-3) and Interleukin-5 (IL-5) plays an important role in the formation of polyps [7].

Several recent studies have reported that there were differences in CRS immunoprofile between Caucasian and Asian races. The American and European populations have Th2 inflammatory pattern (eosinophilic inflammation) whereas in the Asian population have Th1/Th17 inflammatory pattern (neutrophilic inflammation [3], [8], [9].

The differences in histological and inflammatory patterns between CRSwNP in the Caucasians and Asians and the high recurrence rate of CRSwNP require further study to find out a comfortable and non-invasive examination tool that can detect polyps early. We hope the polyp can be diagnosed early without having to go through a polypectomy that makes the CRSwNP patient uncomfortable and spend more cost for treatment, such as brushing the mucosa of the polyp. Based on that, we will study the correlation of cytokine expression (IL-5, IL-8, IL-17A, and TGF-β1) in CRSwNP recurrence between mucosa (brushing) and tissue (biopsy) of nasal polyps.

Material and Methods

Sample

Samples obtained from CRSwNP patients who visited the Ear, Nose and Throat (ENT) clinic in the Public Central Hospital Dr M Djamil Padang and several hospitals in West Sumatera on August 2016 until September 2018. A total of 15 CRSwNP patients met the inclusion and exclusion criteria in this study. Research approval was requested from the respondent before the operation. Samples were taken from CRSwNP patients who did not use anti-allergic drugs during the wash out period before brushing (chlorpheniramine for 3 days, cetirizine, fexofenadine, loratadine for 5 days each and corticosteroids for 2 weeks) and aged between 18 and 55 years.

Sampling

Brushing performed on nasal polyps mucosa with the nasoendoscopy in a circular motion by using a modified gynecologic cytology brush. Before brushing the polyp, a cotton tampon containing lidocaine and adrenaline installed with a ratio of 4:1 for 10 minutes on the nasal cavity. Brushing was done on the mucosa of the polyp in a circular motion ten times clockwise. Samples obtained from brushing were inserted into a sterile bottle containing PBS liquid and immediately taken to the Biomedical laboratory in the Faculty of Medicine, Andalas University and stored at a temperature of -80°C.

Retrieval of nasal polyp tissue is performed during surgical removal of the polyp by FESS (Functional Endoscopy Sinus Surgery). Polyp tissue samples when FESS performed were put into neutral formalin liquid and immediately taken to the Anatomy Pathology Laboratory of the Andalas University Medical School to make paraffin blocks.

ELISA

In this research, human IL-5, IL-17 and TGF-β1 were used from R&D and human IL-8 from BT lab for examines nasal mucous polyps.

IHC

Immunohistochemical staining techniques using the Labeled Streptavidin-Biotin Complex (LSAB) method were carried out by manual procedure. The results in the form of preparations were measured and calculated by looking under a microscope to assess the expression of IL-5, IL-8, IL-17, and TGF-β1. In this study, IL-5 (GeneTex) antibodies were used as polyclonal IgG from rabbits. Positive values are the results of assessments of the brown intensity of epithelium and stroma seen in the light microscope.

Statistical Method

We use SPSS program version 17.0.0.0. The analysis aimed to determine the correlation of IL-5, IL-8, IL-17A, and TGF-β1 between tissue (IHC) and mucosa (ELISA) in recurrent CRSwNP.

Results

Research had been conducted on 30 samples from 15 CRSwNP patients who underwent FESS surgery for the second time. This study aims to determine the correlation of the expression of inflammation mediators between tissue and mucosa in recurrent CRSwNP. The clinical characteristics of CRSwNP patients can be seen in the table below.

Table 1: Characteristics of respondents based on gender and age

Characteristics	Recurrent CRSWNP (n = 15)
Sex	
Male	12 (80%)
Female	3 (20%)
Age	41.40 ± 10.23

In Table 1, the percentage of males was higher than that of women; there were 12 male respondents and 3 female respondents with a ratio of 4:1. The average age in the recurring CRSwNP group was 41.40 ± 10.23.

Expression of IL-5, IL-8, IL-17, and TGF-β1 between mucosa and recurrent polyp tissue

The mean expression of IL-5 on the mucosa of recurrent CRSwNP was 2.75 (± 2.02) while the tissue was 78.80 (± 15.01).

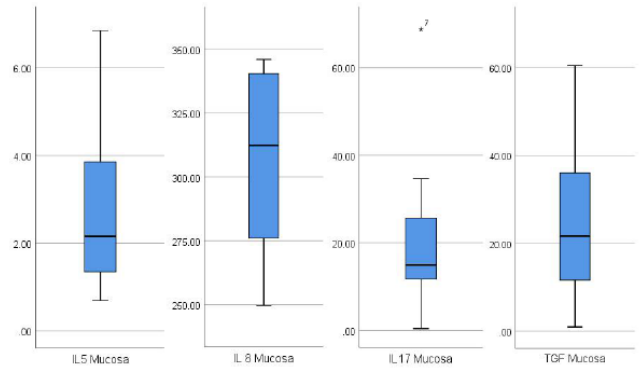

Figure 1: Expression of IL-5, IL-8, IL-17, and TGF-β1 mucosa with ELISA (pg/dl) in recurrent CRSwNP showed in the box plot graphs

In figure 1, IL-5 was the only cytokine that had a significant correlation with moderate positive correlation; it can be concluded that the enhancement of expression of IL-5 in the mucosa accompanied by an increase of the expression of IL-5 in the tissue ($p < 0.05$; $r = 0.527$). The mean of IL-8 in the mucosa was 304.35 (± 34.86) while in the tissue was 78.33% (± 18.79), but there was no significant correlation found ($p > 0.05$).

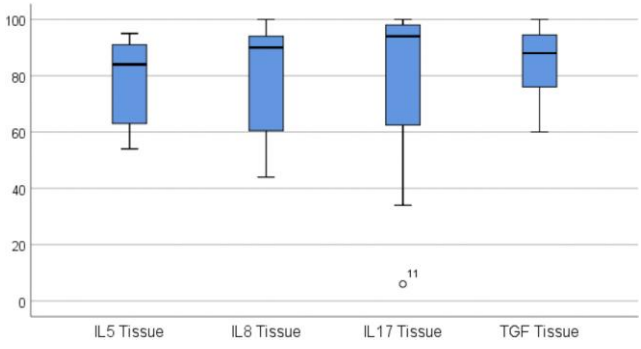

Figure 2: The box plot of expression of IL-5, IL-8, IL-17, and TGF-β1 tissue with IHC (per 100 cells) in recurrent CRSwNP tissue

Like IL-8, IL-17A also did not have a significant correlation, the mean mucosa was 20.13 (± 16.78), and the tissue was 77.13% (± 29.64). The mean of TGF-β1 in the recurrent mucosal CRSwNP was obtained at 24.51 (317.03) while the tissue was 85.33% (± 12.38) with a non-significant correlation ($p > 0.05$).

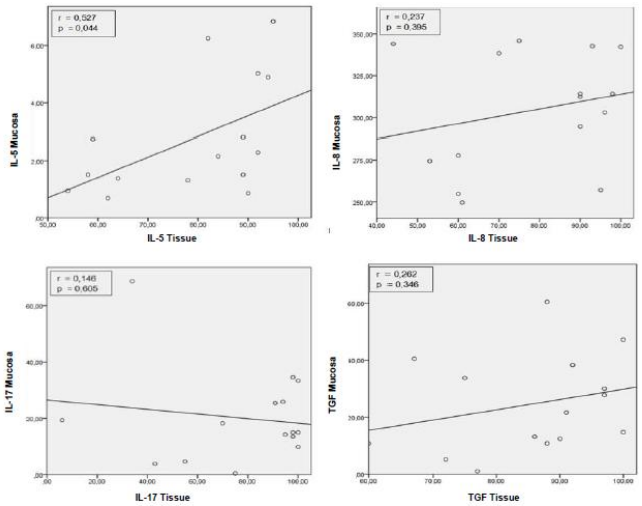

Figure 3: Correlation of expression of IL-5, IL-8, IL-17, and TGF-β1 between mucosa and tissue in CRSwNP recurrent polyps

Of all the cytokines examined, only IL-5 had a significant correlation with a moderate positive correlation ($p < 0.05$; $r = 0.527$).

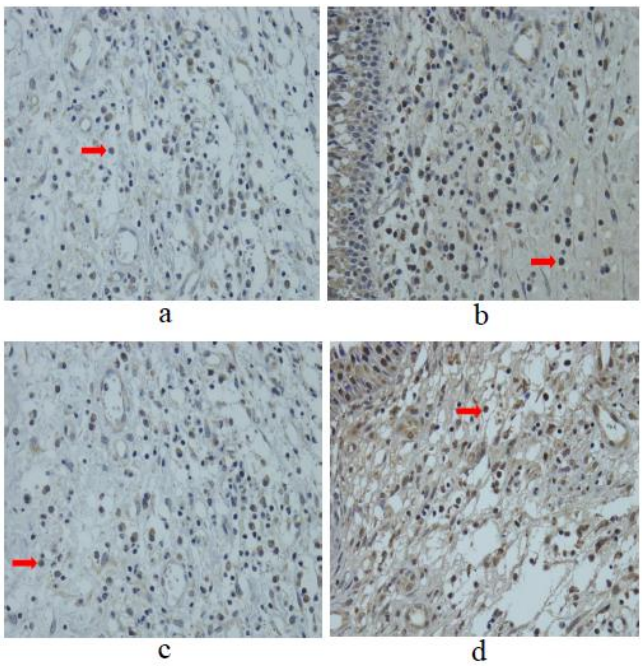

Figure 4: Description of cell expression that produces cytokines in recurrent CRSwNP tissue with 40 X 10 enlargement: a) description of IL-5 expression; b) description of IL-8 expression; c) description of IL-17A expression; d) description of TGF-β1 expression. Red arrows indicate cells that contain positive cytokines

Discussion

Interleukin-5 in the mucosa and recurrent tissue CRSWNP has a significant relationship ($p < 0.05$) with a moderate positive correlation ($r = 0.527$), we can conclude that the enhancement of expression

of IL-5 in the mucosa accompanied by an increase of the expression of IL-5 in the tissue. Interleukin-5 is a cytokine produced by T helper 2 (Th2) and ILC2 cells, which is important in the differentiation, maturation, toxicity, activation and survival of eosinophil [10], [11]. Interleukin-5, together with CCL11 (eotaxin) mobilises eosinophils into the tissues. Interleukin-5 stimulates eosinophils to increase its defence and cytotoxicity. Eosinophil infiltration and granulation encourage to release the toxic products such as major basic protein, eosinophil cationic protein, an eosinophil-derived neurotoxin and eosinophil peroxidase which contribute to airway epithelial damage, mucus hypersecretion of goblet cells, and ciliary movement damage [12]. In a study conducted by Bachert, in immunohistochemical examination 70% of cells containing IL-5 were eosinophils, and there was a strong relationship between IL-5 and Eosinophilic Cationic Protein (ECP) which is a marker of eosinophil activation [10].

Many studies have reported that eosinophilic type CRSwNP has a worse recurrence and prognosis rate. Matsuwaki compared the neutrophilic type with eosinophilic type in 2008. Matsuwaki reported that patients with eosinophilic mucosa showed a high incidence of recurrence in 5 years, where the positive predictive value for recurrence was 85.7% [13]. Nakayama also reported that the presence of eosinophilic mucosa is often associated with disease severity and recurrence of nasal polyps [14]. Ikeda in 2013 compared eosinophilic CRSWNP and neutrophilic CRSWNP types, found that eosinophilic type of CRSWNP had a poor prognosis of the disease and higher recurrence, especially eosinophilic CRSWNP with aspirin intolerance [15]. Similar was also reported by Tecimer in 2014 in which the study reported a higher recurrence rate in eosinophilic nasal polyps (55%) compared with neutrophilic nasal polyps (42.8%) [16]. Van Zele reported that the type of Th2 inflammation and eosinophilic inflammation are the main risk factors for recurrence and the severity of the disease [3]. A study conducted by Lou in 2015 in the Chinese population, also reported that the proportion of tissue eosinophilia over 27% of total tissue cells could predict recurrence in nasal polyps with a sensitivity of 87.4% and specificity of 97.1% [17].

Histologically, Gevaert reported in 2004 that eosinophils were localised in blood vessels, borders, and just below the mucosal epithelium where mast cells were generally granulated and were found increased in the nasal polyp stroma. The enhancement of plasma cells and lymphocytes are also found on cellular levels [18]. The positive correlation in this study proved that in CRSwNP recurrence, the examination of IL-5 by brushing technique on the mucosa could be assessed with IL-5 on the tissue so that the techniques can be used to look expression of IL-5 in early developmental of CRSwNP and can predict the possibility of recurrence.

The mean expression of IL-8 on the mucosal CRSwNP recurrent is 304.35 (± 34.86) and in the tissues 78.33% (± 18.79) but there was no significant relationship between tissue and mucosa ($p > 0.05$). Interleukin-8 is an inflammatory cytokine that has strong neutrophil chemotaxis activity and can induce degranulation, respiratory burst, adherence, deformation, Ca2 + mobilisation and increased regulation of CD11b/CD18 neutrophils. The initial phase of interleukin-8 will be secreted mainly from glandular cells and epithelial cells. These cells tend to secrete IL-8 to attract neutrophils out of the mucosa to the luminal side, and neutrophils that have migrated can also secrete IL-8 resulting in positive feedback [19]. Besides neutrophils, IL-8 is also a chemokine that is very important for eosinophils in all types of CRSwNP and nasal polyps [20]. In the Asian population, CRSWNP polyps occur largely influenced by infectious factors where at the onset of CRSwNP polyps the amount of IL-8 is needed for mobilisation of inflammatory cells such as neutrophils and eosinophils to the nasal polyp CRS site [21], [22]. In recurrent CRSwNP, the role of IL-5 is more dominant where IL-5 plays a role in the differentiation, maturation and survival of eosinophils, which are important in the growth and recurrence of polyps.

In this study, IL-17 expression in the mucosa was 20.13 (± 16.78), while in the tissue was 77.13% (± 29.64). Interleukin-17 is produced by cells induced by proinflammatory cytokines (such as IL-1, IL-6 and TNF-α), chemokines (CXCL1, CXCL2, CXCL5 and CXCL8), and adhesive molecules (ICAM-1 and VCAM-1) [24]. The main source of IL-17 in nasal polyps is lymphocytes. Interleukin-17 can modulate the life of neutrophils in nasal polyps that are not cystic fibrosis. In the study of Derycke et al., it can be proven that IL-17 can prolong the life of neutrophils in non-fibrous nasal polyps where IL-17 can reduce apoptosis [23]. In this study, IL-17 was expressed primarily by macrophages. IL-17 binds to the IL-17 receptor with the involvement of tumour necrosis factor and receptor factors which activate nuclear factor κB and subsequently modulate proinflammatory cytokines. Jiang et al. explained that interactions between IL-17 and IL-17 RD could contribute to the growth of nasal polyps, such as thickening of lamina basal cells and glandular hyperplasia [24].

In this study, the expression of mucosa TGF-β1 was 24.51 (± 17.03) and in tissues was 85.33% (± 12.38). Transforming Growth Factor Beta1 is a mediator related to tissue remodelling. Transforming growth factor-β1 (TGF-β1) is a chemoattractant for fibroblasts, stimulating fibroblast proliferation, and enhancing collagen laydown by fibroblasts. Of the TGF-isoforms, TGF-β1 synthesised by infiltrating eosinophils may contribute to stromal fibrosis and basement membrane thickening, which are characteristic of nasal polyp [25]. Transforming Growth Factor Beta1 plays an important role in the balance of fibrinolysis and fibrogenesis, which means it affects the extracellular matrix. This imbalance is

due to the low levels of TGF-β1 in nasal polyps and the inhibitory effects on MMP-9 activity through the release of TIMP-1 [26].

In IL-8, IL-17 and TGF-β1, there was no correlation between the expression of cytokines in the mucosa and tissue in recurrent CRSwNP, because the relationship was not statistically significant ($p > 0.05$). The absence of a significant association with IL-8, IL-17A, TGF-β1 is probably due to the variability of the inflammatory expression in nasal polyps. Stevens reports that from examining 20 cytokines of inflammatory mediators between nasal lavage and biopsy tissue of nasal polyps, only IL-10 had a significant correlation and positive correlation. This finding supports previous observations where there was regional variability in the expression of cytokine inflammatory mediators in sinonasal cavities [27], [28]. Pawankar also explained that the histological characteristics of the mucosa and inflammation cells of the exudate depend on allergic status [29]. Besides of that, in this research, CRSwNP was not differentiated based on the dominant histopathological type of inflammatory cells, eosinophilic CRSwNP and neutrophilic type CRSwNP. Eosinophilic type CRSwNP is CRS with a Th2 inflammatory pattern where IL-5 is one of the signalling cytokines [30]. Neutrophilic type CRSwNP is a polyp which initially influenced by infectious factors and commonly found in Asian ethnicities. In neutrophilic type CRSwNP, the inflammatory pattern that plays a role is Th1 and Th17 characterised by increased expression of IL-8 and IL-17A [31]. Also, in this study, the locus of polyp tissue sampling could not be taken at the same location for each sample.Samplingof tissue polypththe e could not distinguish between the stalk, body (distal, medial and proximal) and peak of the polyp. The difference of sampling location could make different expression due to the discrepancy activity processes in each part of the polyp.

In this study, it was found that only IL-5 has a correlation between mucosa and tissue in recurrent CRSwNP, we can conclude mucosal brushing examination can describe the expression of IL-5 in tissues. Further research is needed to find out the correlation of cytokine between the mucosa and tissue mainly based on the histology type of CRSwNP and to look for an easier and more convenient examination tool to diagnose recurrent CRSwNP especially at the beginning of course of the disease.

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Atorvastatin in Combination with Pegylated Interferon and Ribavirin Provided High Rate of Sustained Virological Response in Patients with Genotype 3 Hepatitis C Virus

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Keywords: Hepatitis C virus infection; Statins; Pegylated interferon alpha; Ribavirin; Sustained virological response

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Abbreviations: CHC: chronic hepatitis C; NS: not statistically significant; S: statistically significant; BMI: body mass index; AST: aspartate transaminase; ALT: alanine aminotransferase; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance.

Abstract

BACKGROUND: Chronic hepatitis C virus infection represents a more frequent cause of liver cirrhosis and hepatocellular carcinoma. Statins, inhibit HCV replication in vitro, enhance the antiviral effect of the already known antiviral drugs and reduce their resistance.

AIM: To determine the impact of additional therapy (treatment with Atorvastatin 20 mg) to the standard antiviral therapy (pegylated interferon alpha-peg-IFN α and ribavirin) on achieving sustained virological response (SVR).

MATERIAL AND METHODS: In the study which is comparative, open-label, prospective-retrospective, 70 patients diagnosed with chronic hepatitis C virus infection who met criteria for treatment with standard antiviral therapy combined with anti-lipemic therapy (Atorvastatin 20 mg) were included. Patients in the study were divided into two groups: one group of 35 patients receiving combination therapy (Atorvastatin + peg-IFN α + Ribavirin) and another group of 35 patients received only standard antiviral therapy. Those parameters were followed in all patients: genotyping, quantification of the virus, histological assessment of liver inflammation and fibrosis degree (before starting treatment), the presence of steatosis, laboratory analysis: hematology, liver, lipid and carbohydrate status, insulin blood level (the calculation of HOMA-IR) and body mass index (BMI) calculation. The overall treatment of the patients depends from the virus genotype, thus, patients with genotype 1 and 4 received 48 weeks standard antiviral therapy, but patients with genotypes 2 and 3 received 24 weeks of antiviral therapy. SVR was considered an undetectable level of HCV RNA levels 24 weeks after completion of antiviral therapy. The results were statistically analysed, and all results for $p < 0.05$ were considered statistically significant.

RESULTS: Combination therapy leads to a slightly higher percentage of SVR (85.71%) in patients with chronic hepatitis C versus standard therapy (74.29%), but in a group of patients with genotype 3 this rate of SVR amounting to 95.83%. Combination therapy leads to significant improvement of lipid and glucose status after treatment, and in terms of side effects, there was no appearance of serious adverse events that would be a reason for discontinuation of the therapy.

CONCLUSION: Combination therapy Atorvastatin + pegylated interferon alpha + Ribavirin leads to high rate of SVR of 95.83% in patients with chronic hepatitis C, genotype 3. Statins can be used safely in patients with chronic hepatitis C.

Introduction

Hepatitis C viral (HCV) infection is one of the main causes of liver diseases worldwide. The severity of the diseases is quite different, in the spectrum from acute hepatitis, through liver cirrhosis to hepatocellular carcinoma [1]. The end stage of liver disease has a definite need for liver transplantation as the only curative method [2]. The hepatitis C virus uses the host's lipoprotein metabolism for its life cycle [3]. HCV circulates into the bloodstream as lipoviroparticle composed of triglyceride-rich lipoproteins containing both, apolipoproteins B and E, viral RNA, and structural nuclear viral protein [4], [5].

During the HCV life cycle, from the viral entry into the cell, until the liberation of the virions, the virus modulates the cellular fat metabolism [6]. To facilitate its replication, the hepatitis C virus increases

lipogenesis, resulting in accumulation of the triglycerides and the cholesterol in hepatocytes [7], [8].

Cholesterol is synthesised in hepatocytes through a mevalonate pathway, which is promoted by several enzymes, including HMG-CoA (3-hydroxy-3-methyl-glutaryl-coenzyme A) reductase (HMGR). Normally, HMGR expression is consistent with the intracellular cholesterol level, but despite significant cholesterol accumulation, HMGR expression is substantially enhanced in the HCV-infected liver. The Mevalonate pathway for de novo synthesis of cholesterol is also responsible for the synthesis of farnesyl pyrophosphate and geranylgeranyl pyrophosphate (GGPP), which are essential for viral replication [9]. The hepatitis C virus induces a paradoxical condition in which cholesterogenesis is stimulated, but the current cholesterol synthesis is interrupted, or disturbed, by diverting to the synthesis of the intermediate GGPP needed for viral replication [10]. Due to the suppression of cholesterol synthesis, cholesterol levels and its fractions in HCV infected patients are lower.

Statins are a class of competitive inhibitors of HMG-CoA reductase, which are used for controlling cholesterol levels in patients with hyperlipidemia, but have a strong pleiotropic effect, modulating the inflammation, the angiogenesis, the apoptosis and the cell growth [11], [12], [13]. It is of importance to emphasise the effect of statins on the inhibition of HCV replication, which determined their significant anti-HCV effect [14]. Statins block HCV replication by inhibiting the de novo synthesis of cholesterol and inhibition of geranylgeranyl pyrophosphate [15], [16]. Thus, statin therapy can reduce the level of HCV RNA in the blood despite the fact that cholesterol synthesis is suppressed, then it leads to a rise in cholesterol levels after SVR is reached, and it has also been observed that patients with a higher cholesterol baseline are more likely to respond positively to antiviral therapy [17], [18].

Several studies have shown that statins inhibit HCV replication in vitro, that they improve the antiviral activity of already known antiviral drugs and that they reduce their resistance [19], [20]. In vivo studies have shown that statin monotherapy in conventional doses does not inhibit HCV replication [21]. In contrast, statins increase the rate of sustained virologic response when combined with pegylated interferon and ribavirin [22], [23], [24].

In addition to the positive effect of statins, along with antiviral therapy with pegylated interferon and ribavirin over the virologic response, it should be noted the influence of statins on the process of fibrosis, thus preventing the development of a more advanced form of liver disease [25]. However, the possible protective role of the occurrence of HCC, although it has not been proven in randomised studies, should not be neglected.

The purpose of the study is to determine the impact of additional therapy (atorvastatin 20 mg treatment) to standard antiviral therapy (Pegylated interferon alpha-peg-IFN α + Ribavirin) on achieving a sustained virologic response

Material and Methods

The study is comparative, open, prospectively-retrospective, which was conducted at the University Clinic of Gastroenterohepatology in Skopje in the period from 2015 to 2017. A total of 70 patients were included with diagnosed chronic hepatitis C virus infection that met criteria for treatment with antiviral therapy in combination with antilipemic therapy. Patients were divided into two study groups:

1. A group of 35 patients who, besides antiviral therapy with peg-IFN α and Ribavirin, received additional antilipemic therapy Atorvastatin 20 mg, orally once daily for the entire duration of the study.

2. A group of 35 patients who received standard antiviral therapy with peg-IFN α subcutaneously administered once a week (180 μg for peg-IFN α2a and 1.5 μg/kg for peg-IFN α2b), for a duration of 24 or 48 weeks depending on the genotype (48 weeks for genotype 1 and 4, and 24 weeks for genotype 2 and 3) and Ribavirin administered orally at a daily dose of 800 to 1200 mg depending on the genotype and body weight.

Inclusion criteria

Patients aged over 18 years, regardless of gender and race, verified C hepatitis virus infection (seropositive HCV patients, and PCR-confirmed HCV RNA positivity), naive or pre-treated patients with standard antiviral therapy, respondents with ability to follow the instructions given by the expert and meet the requirements of the provided study treatment, signed informed consent.

Exclusion criteria

Active intravenous drug addicts, the presence of other types of viruses (HBV or HIV), dialysis patients, patients with other etiology of liver disease (autoimmune hepatitis, Wilson's disease, haemochromatosis, primary biliary cirrhosis, primary sclerosing cholangitis, α1 antitrypsin deficiency), hepatic decompensation, previous liver transplantation, alcohol abuse (> 20 g/day), presence of HCC and allergy data on the above-mentioned drugs.

The following examinations were made in all patients: - genotyping (determination the genotype of the virus); - quantification of HCV RNA (viral load or number of copies); - liver biopsy under ultrasound control for histological assessment of the degree of inflammation and fibrosis, using the Knodel scale and HAI (histological activity index). According to the presence of fibrotic changes in the liver, patients were divided into three groups: Group 1 - the absence of fibrosis; Group 2 - the presence of fibrosis; and Group 3 - verified liver cirrhosis; - ultrasound examination of the abdomen for assessment of hepatic steatosis. Estimation of steatosis is done before starting with antiviral therapy and after completing the therapy. In addition, steatosis was graded from 0-2 degrees: 0 - absence of steatosis; degree 1- mild steatosis, with quite easily increased echogenicity of the liver parenchyma and clear visualization of the diaphragm and walls of the intrahepatic vessels; and degree 2 - severe steatosis with impaired or no visualization of the diaphragm and blood vessel walls; - laboratory analysis (haematological, hepatological, lipid, glucid status, insulinemia with HOMA-IR calculation); - autoantibodies to exclude autoimmune liver disease; - thyroid status (TSH, fT4); - Body Mass Index (BMI) calculation, according to the formula: weight in kg/(height in meters)^2.

All patients were evaluated for the virological response achieved (12 weeks after initiation of treatment, at the end of treatment and 6 months after the treatment), and for a sustained virologic response was considered an undetectable level of HCV RNA in the blood, 24 weeks after completion of therapy.

Before the start of the study, the protocol, informed consent and other accompanying documents were reviewed and approved by the Ethics Committee for Research over Humans at the UKIM Medical Faculty Skopje. Patients signed written informed consent for participation in the study, which was conducted in accordance with the Declaration of Helsinki.

Statistical analysis

The obtained data were processed with statistical computer program SPSS 17 for Windows. Various statistical tests were used, such as:

- descriptive statistics (arithmetic mean, standard deviation, standard error, median and interquartile interval) for a description of quantitative features,

- frequencies and percentages for a description of categorical features,

- independent parametric and nonparametric tests (Student t-test for independent samples, Chi-square test, Fisher exact test, Mann-Whitney test) were used to compare the analysis groups,

- for comparison of the analysed parameters in the analysed period, dependent parametric and nonparametric tests were used (Student t-test for dependent samples, Wilcoxon Matched Pairs test, ANOVA Repeated-measurements, Friedman ANOVA test),

- for all analyses, the value of < 0.05 was considered statistically significant, and $p < 0.01$ for highly significant.

Results

The results of our study showed a non-significant difference between the groups before the treatment regarding the gender, age, drug abuse, the genotypic distribution, viral load, histology activity index, presence of fibrosis and steatosis, transaminase activity, lipid and carbohydrate status. Table 1 shows all the baseline characteristics of the whole group, of both analysed patient groups, and the comparison of the two groups.

Table 1: Baseline characteristics and comparison between two groups of patients with CHC treated with or without atorvastatin + antiviral therapy

Variable	N (%)	Group I n (%)	Group II n (%)	P value
Gender, n%				
Male	50 (71.43)	27 (77.14)	23 (65.71)	
Female	20 (28.57)	8 (22.86)	12 (34.29)	[A]0.29
Age, years, mean ± SD	70	36.37 ± 7.7	36.23 ± 9.1	[B]0.94
Drug abuse, n (%)				
Yes	40 (57.14)	23 (65.71)	17 (48.57)	
No	30 (42.86)	12 (34.29)	18 (51.43)	[A]0.15
Genotype n (%)				
Subtype 1	22 (31.43)	10 (28.57)	12 (34.29)	
Subtype 2	1 (1.43)	0	1 (2.86)	Fisher exact,
Subtype 3	46 (65.71)	24 (68.57)	22 (62.86)	0.7
Subtype 4	1 (1.43)	1 (2.86)	0	
Viral load, n%				
Low	37(52.86)	20 (57.14)	17 (48.57)	
High	33 (47.14)	15 (42.86)	18 (51.43)	[A]0.47
Liver biopsy Knodell HAI, n %				
1	12 (17.91)	6 (17.65)	6 (18.18)	
2	22 (32.84)	13 (38.24)	9 (27.27)	
3	13 (19.4)	8 (23.53)	5 (15.15)	
4	11 (16.42)	5 (14.71)	6 (18.18)	
5	4 (5.97)	1 (2.94)	3 (9.09)	[C]0.24
6	1 (1.49)	0	1 (3.03)	
7	1 (1.49)	0	1 (3.03)	
9	1 (1.49)	1 (2.94)	0	
11	1 (1.49)	0	1 (3.03)	
18	1 (1.49)	0	1 (3.03)	
Presence of fibrosis, n%				
No fibrosis	50 (71.43)	28 (80)	22 (62.86)	Fisher exact,
Fibrosis present	17 (24.29)	7 (20)	10 (28.57)	0.165
Cirrhosis	3 (4.29)	0	3 (8.57)	
Steatosis, n%				
No steatosis	32 (45.71)	14 (40)	18 (51.43)	Fisher exact,
Mild	34 (48.57)	19 (54.29)	15 (42.86)	0.6
Severe	4 (5.71)	2 (5.71)	2 (5.71)	
BMI, mean ± SD	25.52±4.4	25.77 ± 4.2	25.27 ± 4.6	[B]0.64
AST (10-34 U/L), mean ± SD	72.53±75.2	63.6 ± 45.3	81.46 ± 96.3	[C]0.44
ALT (10-45 U/L), mean ± SD	108.86±89.7	99.97 ± 68.9	117.7 ± 106.8	[C]0.73
Trigliceride (0.0-2.0 mmol/L), mean ± SD	1.15 ± 0.6	1.17 ± 0.6	1.14 ± 0.6	[C]0.9
Total Cholesterol (0.0-5.5 mmol/L), mean ± SD	4.27 ± 1.1	4.16 ± 1.3	4.38 ± 0.9	[C]0.41
HDL-C (0.9-2.0 mmol/L), mean ± SD	1.15 ± 0.3	1.12 ± 0.3	1.17 ± 0.3	[C]0.5
LDL-C (2.2-3.7 mmol/L), mean ± SD	2.54 ± 0.96	2.47 ± 1.1	2.61 ± 0.8	[C]0.53
Fasting glucose (3.6-6.5 mmol/L), mean ± SD	5.38 ± 0.7	5.35 ± 0.7	5.41 ± 0.7	[C]0.7
Fasting insulin (2-17 μiu/ml), mean ± SD	12.45 ± 10.8	12.95 ± 11.1	11.98 ± 10.7	[C]0.72
HOMA-IR, mean ± SD	2.96 ± 2.7	3.12 ± 3.04	2.81 ± 2.5	[C]0.67

Group I (with Atorvastatin); group II (without Atorvastatin).

The virological response was determined after 12 weeks of treatment (early virologic response, EVR), at the end of treatment (end of treatment

response, ETR), and 6 months after treatment (sustained virologic response, SVR) (Table 2).

Table 2: Virologic response

Variable	N (%)	Group I N (%)	Group II N (%)	P value
Virologic response EVR				
Yes	45(93.75)	22 (95.65)	23 (92)	Fisher exact, p =
No	(36.25)	1 (4.35)	2 (8)	1.0
Virologic response ETR				
Yes	53(85.48)	25 (89.29)	28 (82.35)	Fisher exact, p =
No	9(14.52)	3 (10.71)	6 (17.65)	0.49
Virologic response SVR				
Yes	56(80)	30 (85.71)	26 (74.29)	P = 0.23
No	14(20)	5 (14.29)	9 (25.71)	

Group I (with Atorvastatin), group II (without Atorvastatin); p (Chi-square test): EVR (early virologic response-12 week of the beginning of therapy); ETR (end of virologic treatment response); SVR (sustained virologic response – 6 months after the end of treatment).

The early virologic response resulted in 45 (93.75%) patients, 22 of those (95.65%) were in the Atorvastatin group, and 23 (92%) were in the group without anti-lipemic therapy. End of treatment response resulted in 53 (85.48%) patients, 25 of those (89.29%) were in the Atorvastatin group, and 28 (82.35%) were in the atorvastatin-free group; and sustained virological response, respectively, was achieved in 56 (80%) patients, 30 of those (85.71%) were in the anti-lipemic therapy group, and 26 (74.29%) were in the group without additional anti-lipemic therapy. The statistical analysis showed the non-significant difference between the group taking anti-lipemic therapy and the group not taking anti-lipemic therapy, all in co-relation to the frequency of early virological response (p = 1.0), end of treatment virological response (p = 0.49) and 6 months after the treatment (p = 0.23).

In the group of participants receiving Atorvastatin, a sustained virologic response was achieved significantly more frequently in patients with genotype 3 compared to those with genotype 1 (95.83% vs 60%, p = 0.019), Table 3.

Table 3: Virologic response 6 months after the treatment

HCV genotype in pts with Atorvastatin	SVR n (%)	NVR n (%)	P value
1	6 (60)	4 (40)	Fisher exact,
3	23 (95.83)	1 (4.17)	P = 0.019*

SVR (sustained virologic response); NVR (no virologic response); *p < 0.05.

Figure 1 shows the difference between the rate of achieved SVR, depending on the type of therapy, as well genotype 1 and 3, whereas although the statistical analysis did not confirm a significant difference (p = 0.7, p = 0.34) between the groups, the rate of achieved SVR (95.83%) in the group of patients with genotype 3 who received Atorvastatin is remarkable.

Table 4 shows the values of cholesterol, HDL, LDL, TG in three points (before treatment, at the end of treatment and 6 months after treatment) in both groups of participants.

Before the start of the treatment, the two groups of subjects had non-significantly different cholesterol level values (p = 0.41). At the end of the treatment, in the group of patients receiving additional antilipemic therapy, significantly lower cholesterol values were measured compared to the standard therapy treatment group. (p = 0.0008). Even after 6 months of completed treatment, patients who received Atorvastatin in addition to antiviral therapy had significantly lower cholesterol values than patients receiving only antiviral therapy (p = 0.038).

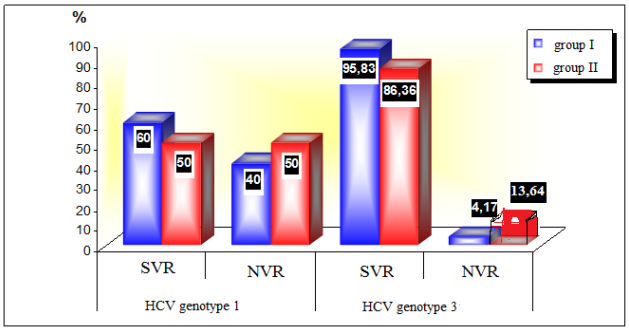

Figure 1: SVR (sustained virologic response) NVR (no virologic response) group I (with Atorvastatin), group II (without Atorvastatin)

Before the start of the treatment, participants from the two groups had non-significantly different values of HDL (p = 0.5). For a value of p = 0.02, a significant difference in HDL level values at the end of the treatment between the groups was confirmed. In 6 months after completed treatment, differences in HDL level values between the two groups remain statistically non-significant (p = 0.36).

Table 4: Cholesterol, HDL, LDL, TG in three points (before treatment, at the end of treatment and 6 months after treatment) in both groups of participants

	Group I		Group II		
	N	Mean ± SD	N	Mean ± SD	P value
Cholesterol (mmol/l)					
BT	35	4.16 ± 1.3	35	4.38 ± 0.9	^{A}0.41
ET	35	3.83 ± 1.3	35	4.43 ± 0.7	^{B}0.0008**
AT	35	4.47 ± 1.4	35	4.89 ± 1.1	^{B}0.038*
HDL (mmol/l)					
BT	35	1.12 ± 0.3	35	1.17 ± 0.3	^{A}0.5
ET	35	0.97± 0.3	35	1.1 ± 0.3	^{B}0.02*
AT	35	2.0 ± 0.35	35	1.2 ± 0.2	^{B}0.36
LDL (mmol/l)					
BT	35	2.47 ± 1.1	35	2.61 ± 0.8	^{A}0.53
ET	35	2.16 ± 1.1	35	2.56 ± 0.6	^{A}0.06
AT	35	2.77 ± 1.2	35	3.04 ± 1.1	^{A}0.33
TG (mmol/l)					
BT	35	1.17 ± 0.6	35	1.14 ± 0.6	^{B} 0.9
ET	35	1.39 ± 0.6	35	1.71 ± 2.3	^{B} 0.8
AT	35	1.32 ± 0.6	35	1.32 ± 0.7	^{B} 0.9

Group I (with Atorvastatin), group II (without Atorvastatin); ^{a} (Student t test); ^{b} Mann-Whitney test); *p < 0.05; **p < 0.01; BT - before treatment; ET-end of treatment; AT-after treatment (6 months).

In the whole period of analysis, statistically non-significant differences were shown in the mean values of LDL level between the group of patients treated with and without Atorvastatin (p > 0.05).

The differences between the two groups in terms of baseline (pre-therapy) and TG control values (at the end and 6 months after therapy) were statistically non-significant (p = 0.9, p = 0.8, p = 0.9 respectively).

Table 5 presents the values of fasting glucose, fasting insulin and HOMA IR in three points (before treatment, at the end of treatment and 6 months after treatment), in both groups of participants

Table 5: Fasting glucose, fasting insulin and HOMA IR in three points (before treatment, at the end of treatment and 6 months after treatment), in both groups of participants

			Group II		
	N	Mean ± SD	N	Mean ± SD	P value
Fasting glucose (mmol/L)					
BT	35	5.35 ± 0.7	35	5.41 ± 0.7	[A]0.7
ET	35	4.92 ± 0.7	35	5.28 ± 0.8	[A]0.049*
AT	35	5.19 ± 0.7	35	5.34 ± 0.6	[A]0.415
Fasting insulin (mmol/L)					
BT	31	12.95 ± 11.1	35	11.98 ± 10.7	[B]0.72
ET	26	9.53 ± 7.6	35	15.15 ± 10.9	[A]0.032*
AT	25	13.55 ± 10.6	35	10.86 ± 7.4	[A]0.33
HOMA IR					
BT	31	3.12 ± 3.04	35	2.81 ± 2.5	[B] 0.67
ET	26	2.15 ± 2.1	35	3.51 ± 3.4	[B]0.01*
AT	25	3.19 ± 2.7	35	2.38 ± 1.7	[B] 0.45

Group I (with Atorvastatin), group II (without Atorvastatin); [a] (Student t test); [b](Mann-Whitney test); *p < 0.05; BT-before treatment; ET-end of treatment; AT-after treatment (6 months).

Before the start of the therapy, there was no significant difference in the average values of fasting glucose between group I and group II (p = 0.7). At the end of the treatment, the average fasting glucose values were significantly lower in the group of patients who, in addition to standard antiviral therapy, received antilipemic therapy (4.92 ± 0.7 vs 5.28 ± 0.8; p = 0.049), while control values after 6 months of completed treatment were non-significantly lower (5.19 ± 0.7 Sun 5.34 ± 0.6; p = 0.415).

Values of fasting insulin before treatment start were non-significantly different between the two groups of patients (p = 0.72). At the end of the treatment, in the group with Atorvastatin, mean fasting insulin values were of 9.53 ± 7.6, which were significantly lower compared to the group without Atorvastatin, where mean values were of 10.86 ± 7.4 (p = 0.032).

Six months after the completed treatment program, non-significantly higher mean fasting insulin values were evaluated in the Atorvastatin group compared to the group without antilipemic therapy (p = 0.33).

Both groups of participants had non-significantly different values of the HOMA-IR index before the start of the treatment and 6 months after the treatment (p = 0.67, p = 0.45 consequently), and significantly different at the end of the treatment (p = 0.01). In a group, I at the end of the treatment, significantly lower values for HOMA IR was obtained, in contrary to group II.

Table 6 shows the distribution of the frequency of adverse events that occurred in patients during the treatment.

Table 6: Distribution of the frequency of adverse events that occurred in patients during the treatment

Side effects	Group I N (%)	Group II N (%)	P value
Thrombocytopenia	14 (40)	16 (45.7)	[A]0.63
Leukopenia	17 (48.6)	18 (51.4)	[A]0.81
Anemia	4 (11.4)	2 (5.7)	[B]0.34
Flu like symptoms	12 (34.3)	4 (11,.4)	[A]0.023*
Loss of appetite and weight loss	18 (51.4)	13 (37.1)	[A]0.23
Hair loss	7 (20)	1 (2.9)	[B]0.027*
Hypothyreosis	2 (5.7)	1 (2.9)	[B]0.5
Hyperthyreosis	1 (2.9)	1 (2.9)	[B]0.75
Nausea, vomitus	1 (2.9)	1 (2.9)	[B]0.75
Fatigue, malaise	6 (17.1)	4 (11.4)	[B]0.49
Cutaneous allergic reaction to the drug	1 (2.9)	0	[B]0.5
Dry skin	2 (5.7)	0	[B]0.25
Vertigo	1 (2.9)	0	[B]0.5
Infection	1 (2.9)	0	[B]0.5
Anxiety	10 (28.6)	7 (20)	[A]0.4
Depression	1 (2.9)	0	[B]0.5
Skin changes	3 (8.6)	1 (2.9)	[B]0.31
Headache	1 (2.9)	1 (2.9)	[B]0.75
Insomnia	1 (2.9)	2 (5.7)	[B]0.5

Group I (with Atorvastatin); group II (without Atorvastatin); [a]p (Chi-square test); [b]p (Fisher exact test); *p < 0.05.

The most common adverse reaction in the group which was receiving Atorvastatin was reduced appetite and decreased body weight (51.4%), followed by leucopenia (48.6%) and thrombocytopenia (40%). Haematological alterations were the most common adverse reactions in the group treated with standard antiviral therapy only-leukopenia and thrombocytopenia (51.4%, 45.7% respectively). A statistically significant difference between the two groups of patients was confirmed only about the frequency of occurrence of flu-like symptoms (p = 0.023) and hair loss (p = 0.027). Patients in the first group significantly more frequently had the appearance of flu-like symptoms and hair loss (34.3%, 20% consequently), compared with the second group of participants (11.4%, 2.9% respectively).

Discussion

Statins that inhibit the enzyme on the mevalonate pathway, HMG CoA reductase, have shown to play an important action not only in fat metabolism but also in the modulation of hepatic steatosis and fibrosis, and are presumed to have an important anti-proliferative, anti-angiogenic and antioxidant effect, with a potentially protective action against the development of HCC. In vitro studies, such as studies of Ikeda et al., [26]; and Aiziki et al., [27], have clearly shown that the statins inhibit the replication of viral RNA, more likely by inhibiting geranylgeranylation of cellular proteins, rather than by inhibiting cholesterol synthesis, showing almost the same efficacy as the most potent clinical treatment. However, studies have shown that not all statins have the same impact. According to them, atorvastatin, fluvastatin and simvastatin have stronger anti-HCV activity, lovastatin moderate activity, and pravastatin does not possess such activity, although it inhibits HMG-CoA reductase.

In vitro study of Ikeda et al., have shown that statins can be used as adjuvant therapy for interferon as well, just as ribavirin together with interferon shows synergistic antiviral activity [28].

In vivo studies, however, showed different results. Statin monotherapy did not lead to an improvement in the virologic response, probably due to the synergistic effect of statins with interferon, as demonstrated in the study of O'Leary et al., [29] and Forde KA et al., [30].

Most studies have analysed the effect of Fluvastatin on the virologic response, and the number of studies that analysed other statins is lower. There are studies that are potentiating the positive impact of Fluvastatin as an additional therapy of standard antiviral therapy over virologic response, especially for genotype 1, such as the study of Selic Kurincic et al., [31]. The study of Atsukawa et al., [32], showed the reduction of viral relapse in patients with genotype 1b, as well as the studies of Georgescu et al., [33], Sesaki et al., [34] and Kondo et al., [35]. In contrary to those, Shavakhi et al., published results where additional therapy with Atorvastatin in patients with genotype 1 over 12 weeks does not lead to a better SVR [36]. The study of Malaguarnera et al., showed that the use of Rosuvastatin has a significant influence over lipid metabolism, inflammatory status and fibrosis, but also no significantly improves the effect of standard therapy on the virologic response [37]. The author Zhu et al., in his meta-analysis, concluded that additional statin therapy to previous standard IFN-α and ribavirin therapy improves SVR, RVR and EVR without additional adverse events and thus can be considered as an adjuvant treatment to IFN-α and ribavirin [38].

Our results showed a higher percentage of the achieved sustained virologic response as a marker for the success of treatment in the statin group (85.71%) versus 74.29% of the SVR achieved in the antiviral therapy group only. But when we additionally made a comparison between Genotype 1 and 3 as the most common genotypes in our study, we obtained an even higher percentage of SVR achieved in genotype 3 and combined treatment, of a high 95.83% versus 83.36% achieved in genotype 3 and standard antiviral therapy. Although no significant difference has been obtained ($p = 0.34$), this is a remarkable result of a sustained virological response achieved in patients with genotype 3 (practically only one patient out of 23 did not achieve SVR). This high percentage of virological response is achieving with the new direct antiviral drugs (DAA), mainly for other genotypes but not for genotype 3 [39], 40]. In the study of Sette H Jr et al., the rate of sustained virological response is lowest in patients with genotype 3, which is 90.7%, while in patients with genotype 1 it is 95.8%, in genotype 2 100%, and in genotype 4 also 100% [41]. Our results in the group of patients with genotype 1 have also shown the difference between the two groups in terms of therapy, but in this case, we are talking about a much lower SVR rate (60% versus 50%, $p = 0.7$). In the Atorvastatin group, patients with genotype 3 who have achieved sustained virological response are significantly higher, compared to patients with genotype 1, $p = 0.019$, which coincides with the conclusion of Zhu et al., in their meta-analysis, that additional statin therapy should be used in other genotypes other than genotype 1, as in our case, in genotype 3. The early virological response (EVR) and end-of-treatment virologic response (ETR) analysis also showed that they were evident in a higher percentage in patients belonging to the statin group, 95.65% and 89.29%, compared to the patients put into the standard antiviral therapy group (92% and 82.35%), but the comparison between the two groups of patients was statistically non-significant ($p = 1.0$ and $p = 0.49$, respectively).

Several parameters were analyzed in our two investigated groups (one group with antilipemic therapy, the other without it), and then compared to each other, such as: gender, age, genotype, the presence of steatosis, fibrosis and liver inflammation, body mass index, transaminase activity, but also more laboratory parameters that reflect the fat and sugar metabolism during antiviral therapy, actually before and after therapy. Differences between the two groups were observed about lipid and glucose status. Specifically, patients receiving additional anti-lipemic therapy had significantly lower total cholesterol levels at the end of treatment and 6 months after, compared to the other group ($p = 0.0008$ and $p = 0.038$). Significantly lower results were detected for HDL but only at the end of the treatment ($p = 0.02$), but higher mean value 2.0 ± 0.35 and $p = 0.36$ was observed 6 months later, and the results for LDL were non-significantly lower ($p = 0.06$ and $p = 0.33$). Considering this lipid profile, we can conclude that antiviral therapy supplemented with Atorvastatin, leads to a decrease in total cholesterol and LDL 6 months after treatment, and an increase in HDL levels. According to these results, patients who received statins with antiviral therapy will have fewer chances to suffer from cardiovascular disease.

About the glucose status, statistically significantly lower values of fasting glucose, fasting insulin, and HOMA IR index at the end of therapy in the Atorvastatin group were obtained, $p = 0.049$, $p = 0.032$ and $p = 0.01$ respectively. This result suggests that additional statin therapy does not lead to a worsening of fasting glucose values and insulin resistance, as written in some studies [42], but rather to their improvement. This improvement in the glucose profile may also be due to the achieved sustained virological response in 85.71% of treated patients with Atorvastatin and antiviral therapy, thereby reversing the diabetogenic effect of the virus, as confirmed in other studies [43], [44]. Improving glucose status means reducing the chance of developing fibrosis and more advanced form of liver disease. Other parameters (gender, age, genotype, presence of steatosis, fibrosis, degree of inflammatory activity, and

transaminase activity) compared between the two groups did not show significant differences.

The safety profile of drugs is also of particular importance. Adverse events commonly occurring in both groups were: decreased appetite and decreased body weight, leucopenia, thrombocytopenia, flu-like symptoms, anxiety, fatigue and malaise, anaemia, hair loss, thyroid disorders, and others. In one patient due to a skin allergic reaction, therapy with peg-IFN α2a was discontinued and started with peg-IFN α2b after which there were no side effects, and the treatment was completed according to the genotype 3 protocol. In the remaining patients, adverse events were not life-threatening and were not a cause for discontinuation of therapy. In our study, a statistically significant difference between the group of patients with Atorvastatin and without was confirmed only about the frequency of occurrence of flu-like symptoms ($p = 0.023$) and hair loss ($p = 0.027$). These symptoms lasted for short period of time and were reversible, indicating that statins can be safely used in patients with chronic hepatitis C. Verpaalen et al., in their review writes about the positive impact of statins in the treatment of HCV infection, but also their excellent safety profile and low cost [45], which coincides with our conclusions.

In our study, the influence of statins on the progression of fibrosis, cirrhosis and the occurrence of HCC was not analysed. But since the goal of antiviral therapy is precisely preventing the onset of cirrhosis and HCC in this group of patients, we should not underestimate the significance of the statins in this field. Simon TG et al., in their review, indicates the association of atorvastatin and fluvastatin with a dose-dependent reduction in the incidence of cirrhosis and HCC in patients with HCV infection [46]. We should consider all those facts when planning a strategy for the treatment of patients with Chronic Hepatitis C.

In conclusion: 1) Combined therapy of Atorvastatin + Pegylated interferon alfa + Ribavirin leads to a high rate of sustained virological response of 95.83%, in patients with chronic hepatitis C, genotype 3; 2) Combined therapy leads to an improvement in lipid and glucose status after the treatment; and 3) Adverse events do not differ between the Atorvastatin group and the standard antiviral therapy group and do not lead to discontinuation of therapy. Therefore, statins can be safely used in patients with chronic hepatitis C.

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Laryngotracheal Stenosis: A Retrospective Analysis of Their Aetiology, Diagnose and Treatment

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Abstract

BACKGROUND: Laryngotracheal stenosis created as a result of a long-term consequence of prolonged endotracheal intubation is a state of narrowing of the airway, which, depending on the degree of narrowing, can be from an asymptomatic to a potentially life-threatening condition.

AIM: To understand the severity of postintubation laryngeal stenoses, their diagnosis, endoscopic evaluation, endoscopic and surgical treatment and their success in a multi-year period realised in the University Clinic for Ear, Nose and Throat in Skopje, Macedonia.

MATERIAL AND METHODS: Through a proper history, physical examination, endoscopic and imaging evaluation of the ear, nose and throat in the Clinic, in the period of 8 years, that is, from 2010 to 2017, laryngotracheal stenosis was diagnosed in a total of 36 patients. During this period, 24 male or 66.7% were diagnosed, as were 12 female subjects or 33.3% of diagnosed patients. Of the analysed total of 36 patients, by Mayers cotton classification, 14 or 38% are patients with grade 1 stenosis, 5 or 13% are grade 2, and 10 or 27% are grade 3, while 7 or 19% are grade 4 stenosis

RESULTS: It is essential for all laryngotracheal stenoses to exist or to provide a breathing path that depends on the degree of stenosis. Further course of treatment also depends on the characteristics of the stenotic zone that is visualised endoscopically. At the Clinic for ear nose and throat in Skopje, endoscopic treatment was performed through a series of dilatations within 21 patients, or 58 % of the examinee, as well as the administration of mitomycin and corticoderates in 21 patients or 52% of the examinee. The applied actions and procedures had shown 100% outcome on stenoses not longer than 2 cm, who have a fibro-inflammatory scar and by Myer cotton classification 1 and second stadium. Patients that have failed endoscopic treatment, surgical treatment are a method of choice. Surgical treatments have been performed in 4 patients with realised end of the anastomosis, and 3 crycotracheal reconstructions, which is decannulated. After an extensive follow-up of these patients, depending on their condition, multiple endoscopic evaluations have been decannulated to 21 patients or 58%, and after a series of multiple unsuccessful endoscopic treatments, a condition with tracheal stoma occurs in 8 patients or 22% of the examinee.

CONCLUSION: The observations indicate that the methods used, which are explained previously, have good effects in terms of the achieved outcomes. Due to limited resources, it is necessary to improve new methods and approaches in the treatment of stenoses, depending on their type and severity, thereby improving patient outcomes. Also, to reduce laryngotracheal stenoses, appropriate tubes and low pressure of the caffeine in the endotracheal tubules should be used.

Introduction

Laryngotracheal stenosis is a disease that is characterised by mucosal inflammations and bounded fibrosis resulting blockage of the upper airway and life treating conditions. Cricoid is the narrowest portion of the larynx. The first tracheal ring is partially anchored to the lower limit of the cricoid cartilage and, sometimes, can be attached to it [1]. Small changes of the diameter of the laryngotracheal stem and length of the narrowed segment create important variation in airflow that results in immediately progressive dyspnea requiring urgent interventions [2]. Symptoms occurred when airway change of 30%, and distress of change of 80%. This condition affects patients their ability to; breathe, modification of voice or swallowing problems [3].

It is serious problems that affect adult and children. Treatment depends on the aetiology of the occurrence. It is often caused by autoimmune diseases, infection, scar formation post-intubation or

post tracheotomy and idiopathic causes.

The most common cause remains mechanical ventilation. As one of the most common causes, mechanical ventilation leads to mucosal injuries through a cuff pressure ischemic damage to the trachea, post tracheostomy injury or combination of the two. High pressure of balloon cuff or tube surpasses the capillary pressure, with loss of the regional blood flow, leading to ischemia or necrosis. Local ischemia promotes and stimulates fibrinolytic pathway, which generates the proliferative phase characterised by angiogenesis, collagen deposition and granulation tissue formation, and the third phase maturation and remodelling phase with the creation of membranous web-like stenosis.

Idiopathic subglottis stenosis is defined as laryngotracheal stenosis of unknown origin. Diagnose is made as exclusion and lack of history of traumatic intubation, tracheostomy and negative serologic autoimmune markers. It is a rare, fibroinflammatory process which leads to narrowing the airway of the subglottis, first and the second tracheal ring. When the patient is evaluated by the laryngologist, the scar is mature, circumferential, laryngotracheal stenosis. Mostly affected by woman, probably because of the anatomic predisposition of smaller female subglottis, frequent coughing causing mechanical trauma, subtle vascular disease. It a slowly progressive condition, mild symptoms present in mouths [4], [5], [6].

Autoimmune laryngotracheal stenosis – It's not an isolated phenomenon; it's a present of other organ system involvement. It is known as Wegener granulomatosis, relapsing polychondritis, sarcoidosis. Wegener granulomatosis refers to pulmonary disease. Necrotising vasculitis, cANCA positive and renal disease. Autoimmune laryngotracheal stenosis can have two stages. The first stage is inflammatory is distinguished with erythematic, oedema of the epithelium and granulation tissue. The second stage is mature scar marked as rigid white scar with cicatricle line of fibrosis. If the autoimmune disease is already diagnosed, that the appearance of laryngotracheal stenosis is as a result of inflammatory exacerbation in the larynx. The follow-up course and treatment depend on whether the pre-diagnosed autoimmune disease has been diagnosed or not [7], [8], [9].

Congenital subglottic stenosis defined as the diameter of the cricoid of less than 3.5 mm. Congenital subglottic stenosis is the third most common congenital anomaly of the larynx, which accounts for 15% of all cases. Etiopathogenesis: as a defect in the formation of cone elastics and cricoid cartilage, creating an elliptical cricoid, flaccid cricoid, or excessively submucosal cough. Congenital subglottic stenosis can be classified into two types: Membranous congenital subglottic stenosis (granulation tissue, fibrous connective tissue, hyperplastic submucosal glands.) This type is the most common and mild form of congenital subglottic stenosis. The cartilaginous congenital subglottic stenosis results from abnormal cricoid cartilage.

This condition is the most common laryngeal anomaly that requires tracheotomy in infants. Male children are affected twice as often as females [10], [11], [12].

Material and Methods

The observation period lasted from 05.2012 until 05.2018 and included a patient who was treated in the University Clinic in Skopje, Republic of Macedonia.

Prolonged analysis from their diagnosis to the level corresponding to subsequent treatment was carried out in a total of 36 patients.

Results

In the following diagram, it is demonstrated the percentage of a patient with laryngotracheal stenosis that has been examined. Of them infant, toddlers and preschooler represent 5 patients or 20%, on the other hand, children and adolescent 9 persons or 37% others represent adults and seniors.

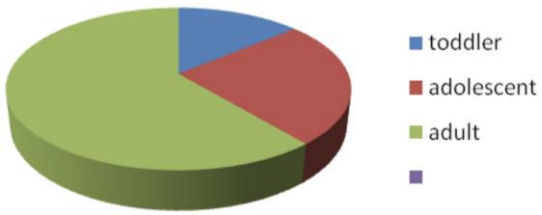

Figure 1: Patient with laryngotracheal stenosis

Evaluation of on each is done, including their medical data (age and sex, aetiology, morphologic description of the laryngotracheal stenosis, degree of airway narrowing, location, transitions zone abruptness of stenosis. Clinical presentation, laboratory results, imaging, treatment, diagnosis, complication and hospitalisation time). All age group are included.

Cough is one of the most common symptoms refer to 83%, as well as the history of intubation 66%, and change in voice 61%. Patients with acquired stenosis have been diagnosed for several days to 10 years or more after the initial injury.

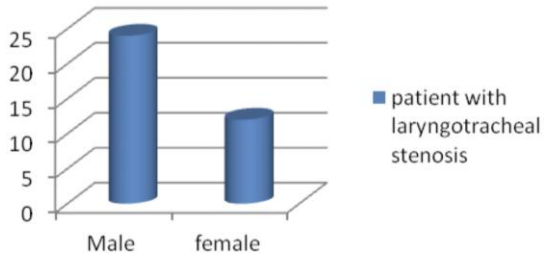

Figure 2: Gender distribution

The majority of cases have been diagnosed within a year. Many patients before the discovery of stenosis are diagnosed as asthma and recurrent bronchitis. A high index of suspicion is justified by the onset of respiratory symptoms and indications.

Table 1: The symptoms that mostly occur in our examinees

Difficulty swallowing	6 m-9m	20	55 %
Change in voice	4 m-6m	22	61 %
reflux	5 m	7	19 %
Degree of limitation of daily activity	5 m	13	36 %
Degree of chest recession on breathing	5 m	18	50 %
dyspnea	3m-6 m	15	41 %
Vacuities	2 years	2	5 %
History of intubation	3m- 6m	24	66 %
Verified Wegener	1	1	2 %
Recurrent bronchitis	6m-9 m	10	27 %
Asthma	6m-9m	15	41 %

The manifestations of congenital subglottic stenosis usually occur in the first few months of life. Patients with mild congenital stenosis are usually asymptomatic, and they are diagnosed after severe intubation or while undergoing endoscopy for other reasons. Stenosis is usually not evident until the child develops an acute inflammatory process, which further compromises the subglottis. The clinical presentation of a child during these periods is no different from infectious laryngotracheobronchitis (large). Biphasic stridor with or without symptoms of respiratory distress is the most common symptom of presentation. A child may have a fake cough, but the cry is usually normal. Anatomically highly susceptible to congenital subglottic stenosis, these symptoms are repeated or prolonged after the normal duration of the infectious break (1-3 d). Asymptomatic children who are difficult to intubate, extubate, or debunk represent another clinical scenario that causes suspicion of congenital stenosis. Children with Down syndrome are at increased risk of having congenital subglottic stenosis and can be presented in this way.

According to the way of development, the most common aetiology is posted polytraumatic condition in a total number of 24 patients or percentage 66% of the 27% occurs post-intubation or 10 patient and 38% occur post tracheotomy. Other conditions are presented, but there are in much smaller percentages. In Table 2 is presented the most common aetiology. Accurate setting of the aetiology of the incidence of laryngotracheal stenosis will lead to an accurate diagnosis.

Table 2: The most common aetiology

Aetiology	Total number	Percentage
post-polytraumatic	24	66 %
Post combustion	4	11 %
post suicidal	3	8 %
Congenital	2	5 %
Idiopathic	2	5 %
Inflammatory conditions	1	2 %
post-intubation	10	27 %
post – tracheostomy	14	38 %

Investigations

Laboratory investigations in the absence of a history of previous trauma or when suggested by other findings, evaluate for inflammatory or infectious causes, including the following: granulomatosis Wegener, recurrent polychondritis, syphilis, tuberculosis, sarcoidosis, leprosy, diphtheria, Scleroma and presence of antibodies.

Pulmonary function test

First pulmonary function test is spirometry to acknowledge reduce air volume or airflow.

Flow-volume loops may help monitor restenosis following the intervention.

Imaging Investigations

Standard chest radiography can often provide a great deal of information regarding the flow of air through the trachea and the location and degree of stenosis, especially anteroposteriorly.

MRI is useful in assessing the length and width of stenotic regions using coronary and sagittal cross-sections.

CT scanning is not as useful as MRI, because its views are usually only in the axial plane. CT cannot, however, differentiate between the true lumen and the overlying secretions, which introduces a defect in the presence of blood, mucus and crusting. The degree of stenosis can be underestimated on CT scan because the imaging is a form of sampling unless fin cuts are made ½ mm with sagittal and/or coronal reconstructions may be useful, the apex of the stenosis can be missed. CT scans help to confirm airway compromise and act as a guideline to its severity.

Endoscopy is the main tool of evaluation and treating laryngotracheal stenosis. It establishes endoluminal therapy, observation of laryngotracheal dynamic and direct visualisation of luminal pathology.

Flexible endoscopic examination of the upper aerodigestive tract will allow assessment of vocal cord function, evidence of reflux, demonstrate pooling of secretions and may also determine the site and degree of airway stenosis. A detailed assessment of vocal cord function and swallowing is vital if laryngotracheal surgery is to be considered Visualization of the larynx with a flexible or rigid telescopic (90- or 70-degree range) in the clinic is

crucial for the assessment of airway lesions. Flexible bronchoscope allows evaluation of the dynamic airway, the trachea and bronchi. But when flexible bronchoscope is placed through narrow stenosis, the patient airway becomes obstructed.

Table 3: Presentation of the degree of airway narrowing

Grade I	Obstruction from 0-50%	14	38%
Grade II	Obstruction from 51-70%	5	13%
Grade III	Obstruction from 71-99%	10	27%
Grade IV	No lumen 100%	7	19%
	Grading	Patient	Total

Suspension laryngoscopy allows the use of rigid optical endoscopes and flexible bronchoscopes to access the airway. Advantages allow two hands-frees for instrumentation; the patient is sedated, a series of investigations can be made, dilatators, laser, stent, can be inserted and used with relative ease. Measurements required: location of the stenosis in relations of the vocal fold and the light of stenoses segment.

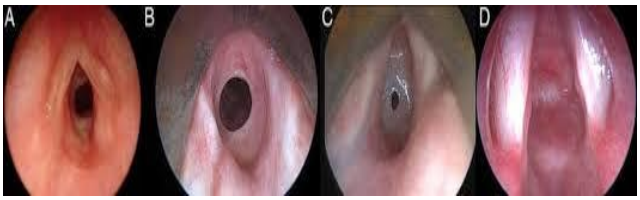

Figure 3: Classification of the laryngotracheal stenosis

Leight of stenoses segment is measured by gentle traction of the endoscopic vision first distal level of the stenosis then proximal end mark with pen allowing biaxilar calibration and radial extent of the area being measured.

Table 4: Length of laryngotracheal stenosis

Grades	*McCaffrey classification of the laryngotracheal stenosis, based on their light*	Patient	Total
Grade 1	Subglottic or tracheal leasion < 1 sm	15	41 %
Grade 2	Subglottic lesion > 1 sm	4	11 %
Grade 3	Subglottic/tracheal lesion without glottis	12	33 %
Grade 4	Glottis lesion	5	13 %

A multidimensional system is necessary for the detection of laryngotracheal systems including the degree of airway narrowing, vertical light, Four types of consistency (soft, hard, cartilaginous, and mixed), Location (glottis, subglottic area and upper trachea).

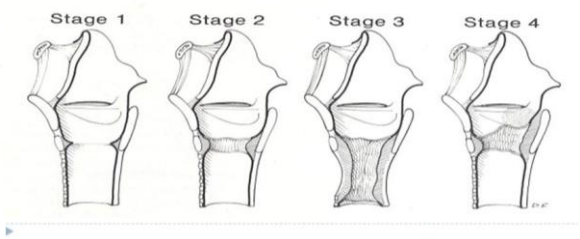

Figure 4: McCaffrey classification system (1992)

In the follow-up tables, our aim to show the multidisciplinary approach in measuring the laryngotracheal stenosis, in these tables, by Myer cotton classification most common is patient with grade I and 38%. On the other hand, Mc Caffey classifications 41% are presented with grade one. Based on their location, subglottis and upper trachea are the most common location in developing laryngotracheal stenosis.

Table 5: Location of laryngotracheal stenosis

Subsite location	Patient	Total
Glottis	5	13 %
Subglottic area	12	33 %
Upper trachea first third	16	44 %
Upper trachea second third	0	0 %
Two locations or more	3	8 %

Treatment

There are 3 types of treatment categorised: 1) endoscopic dilatation of the tracheal stenosis; 2) endoscopic resection of the stenosis (medical therapy after surgery); and 3) open surgery resection end to end anastomosis.

The principle of endoscopic dilatation is maximal preservation of the tracheal mucosa and minimal trauma during procedure. Evaluation of the location and severe in stenosis, the inter arytenoid area for stenosis or long dual fissures and the position of the vocal cords is mandatory.

Transoral exposure of the tracheal scar using rigid tracheobronchoscopy is necessary for balloon dilatation or controlled radial expansion device. Airway balloons are passed through the rigid endoscope, expanding balloon is placed in the middle of the stricture. As the balloon expands, the energy is transmitted of the area of least résistance. The balloon is inflated in enlargement with periodical collapsing to observe progress. Periods of appearing for about 4minuts occur between balloon dilatation intervals. Desideration may occur. Patient with fibro-inflammatory stenosis required fewer procedures rather than mature stenosis.

The follow-up and several endoscopic interventions can take up to 8, for 3 years result in longer intervention free trials. Most patients are high surgical risk patient, and in the poor general condition, it allows minimal invasiveness.

Disadvantages' is associated with a higher rate of recurrence, restenosis and repeated surgery.

In post tracheostomy stenosis. The stricture is often anterior, sparing the posterior membranous portion of the airway. Because of that balloon dilatation is often noneffective.

Complications that can occur during balloon dilatation is no complications, perforation, wall rupture, postoperative airway obstruction oedema, overtreatment and restenosis, postoperative bleeding,

aspiration pneumonia, pneumothorax.

In table 6 shown, our goal is to draw the treatment of the balloon dilatation and the best outcome from it.

Table 6: Treatment of the balloon dilatation and the best outcome from it

classifications	Balloon dilatation	Radial Incision (3, 6, 9, 12)	applications Intra-steroids	Application of Mitomycin C	No. of endoscopic interventions	decannulation	stoma
Myer cotton scale	Grade I	14	14	14	4	14	0
	Grade II	5	5	5	6	5	0
	Grade III	10	10	10	8	2	8
	Grade IV	0	0	0	0	0	7
Scar type	Fibro inflammatory scar formation	19	19	19	6	19	0
	Mature scar	17	17	17	9	2	0
location	Subglotis	12	12	12	64	6	6
	Upper trachea	16	16	16	96	10	6
Vertical leight	grade 1	15	15	15	5	15	0
	Grade 2	4	4	4	6	6	0
	Grade 3	12	12	12	6	12	12

It can be said that the best treatment of balloon dilatation has fibro-inflammatory stenosis with a 100% outcome, as well as grade 1 and 2 of laryngotracheal stenosis. Decanilman is produced after more permanent treatments with a balloon dilatator, on the other hand, Grades 3 and 4 and patients who cannot be realised balloon dilatation open surgery is the option of treatment.

Applications of intralesional steroid injections have shown to have benefited in Wegener granulomatosis and sarcoidosis also it has a positive impact on fibro-inflammatory laryngotracheal stenosis. Mitomycin C is a chemotherapeutic agent that prevents collagen synthesis and scar formation.

In the last few decades, the increased use of carbon dioxide (CO2) laser have been seen, which offers advantages of delayed formation and maturation of collagen in the wounds, which allows for re-epithelialization before the formation of a scar and minimal tissue injuries. The laser allows precise control of areas whose scars are removed and excused hemostasis from preserving the mucosa used for reparation. Negative treatments with burns and injury from laser CO2 rays as for the surgeon and hospital staff, thermal injury, which may result in perioperative oedema and postoperative scarring of the headache.

The idea of placing a stent is to support the airway wall, restore airway patency. Silicone stents are effective in splitting the laryngotracheal stenosis for palliation airway narrowing in nonsurgical candidates.

Stents are placed in stenosis greater than 70% and no longer than 5 mm for 4-6 weeks to aid healing. Disadvantages are granulation tissue formation of the proximal and distal end of the T-tube and stent.

An open approach is indicated after the failure of the endoscopic approach when the degree of stenosis is severe, or factors are unfavourable for this approach. Choosing the specific technique is based on the length of the resection, the need for cartilage tissue and the need for mucosal coverage, placement of a stent is necessary for some procedures. In advanced stages of the laryngotracheal stenosis, especially in the matriculated stenosis, the treatment of choice is resection and end to end anastomosis.

Table 7: The modalities of the operative treatment and their outcome

classifications	no.intervention failed endoscopy		Stent	Cricotracheal resection	End to end anastomosis	decannulation	stoma	Contracture of stoma
Myer cotton maturation	Grade III	10	3	1	2	3	7	2
	Grade IV	7	4	2	2	4	3	1
	Mature scar	17	7	3	4	7	10	
Location	Subglotis	6	3	3	0	3	3	
	Upper trachea	6	4	1	4	4	2	
Vertical leight	Grade 3	12	6	1	5	6	6	
	Grade 4	5	1		0	1	4	

Pediatric laryngotracheal stenosis is researched all rib graft if possible, tracheal or cricotracheal resection and an end to end anastomosis. On the other hand, adult laryngotracheal stenosis treatment options are tracheostomy, tracheal resection or cricotracheal resection associated with great morbidity and mortality. Anterior and/or posterior cricoid split with stenting and graft is rarely used in our country.

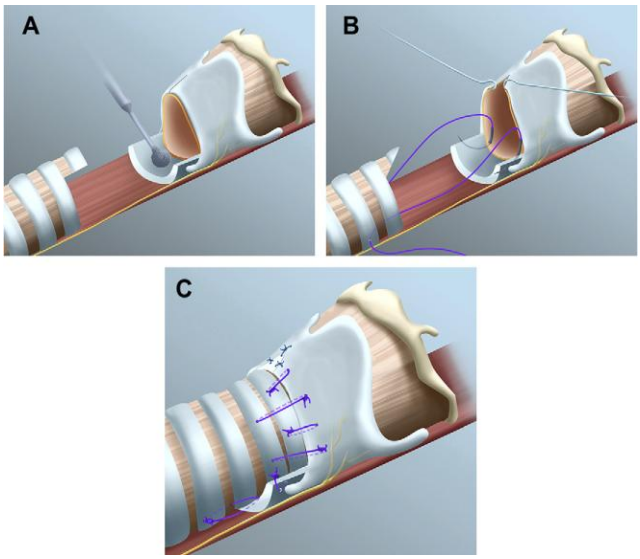

Figure 5: Cricotracheal anastomosis

Tracheal resection is preserved for a patient with a long segment complete or complete collapse of the cartilaginous tracheal support. Cricotracheal resection is a procedure that requires the removal of subglottic scar tissue, with anastomosis of a healthy trachea with the healthy larynx. The procedure is consisting of elevation of perichondrium from cricoid

cartilage anteriorly to avoid recurrent nerve injury, removal of subglottic stenotic tissue, removing soft tissue in posterior cricoids. Laryngofissure may be made to increase the lumen in the laryngeal box, a tracheal submucosal flap in the treatment of posterior subglottic stenosis, full reconstruction with end-end anastomosis.

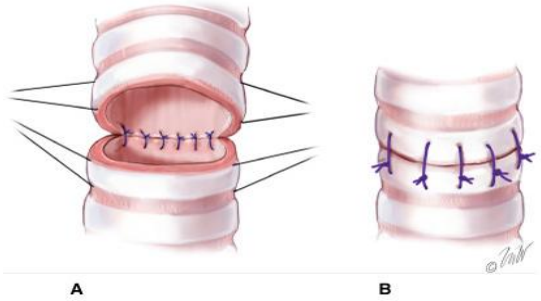

Figure 6: End to end anastomosis

Over resection of the anterior tracheal rings during tracheostomy, at decannulation can cause scarring and contracture of the stoma site. It looks like a lambda shaped stenotic deformity tie the lateral regions fragment resulting wound contracture. Endoscopic is more recommended in this condition rather than open tracheal resection and anastomosis.

Patients with grade 3 and 27% of respondents are on 30% of them subject to laryngotracheal resection with excellent outcome, the remaining patients due to their accompanying comorbidities are with a tracheostomal aperture or the remaining 70%.

Table 8: The outcome of the treatment

	Balloon dilatation	Open surgery
Curative	21	7
Improvement	0	0
Failure	8	0

Patients with grade 4 and 19% of respondents use 57% of them subject to laryngotracheal resection with excellent outcome, the remaining patients due to their accompanying comorbidities with a tracheostomal aperture or the remaining 43%.

Table 8: Postoperative complications in the patient

Type of complication	Number of complications	percentage
Granulations	10	27 %
Restenosis	7	19 %
Wound infection	0	
Dysphonia	7	19 %
Sepsis	0	
Detachment of anastomosis	0	
Injury of the recurrent nerve	0	
Bleeding / hematoma	0	
Stent migration	4	11 %
Death	0	0

Mature scar distinguishes itself with a positive treatment of 70% about its total number.

Subglottic/tracheal stenosis that do not involve glottis or grades 3 at McCaffer classification is 12 patients. Of them, 6 patient laryngotracheal resection and anastomosis were performed or 50% leading to decannulation in all of them. Some recommend prolonged postoperative neck flexion with the longue or chin-to-breast sewing in the end-to-end anastomosis.

In the postoperative treatment: antibiotics are given for 1-3 weeks, depending on the degree of recovery and general health of the laryngotracheal mucous membrane. According to the surgical intervention administration of antibiotic is needed. Authors advocate the elimination of antibiotics when a stent is used. Intensive anti-reflux therapy is needed Injecting steroid sprays (not nasal sprays) are sometimes useful for reducing the granulation tissue of the surgical wound.

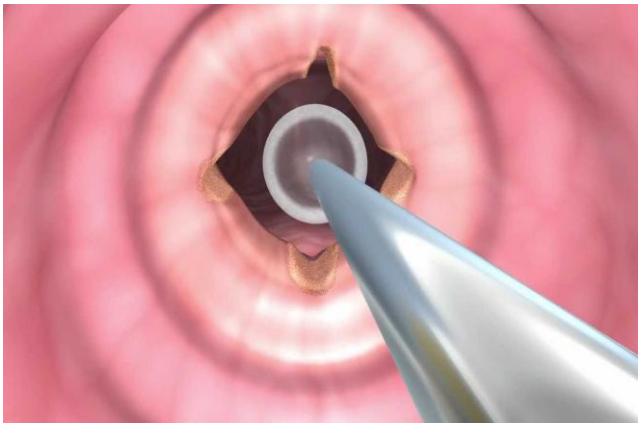

Figure 7: Ballon dilatation with radial incisions

After 6 weeks, control is taken to determine the condition of treatment and the need for further procedure. Whenever is required or not, tracheostomy was implemented.

Some authors want immediate extubation after reparation; some support 1-2 days of postoperative intubation in intensive care units; and some require extubation in the operating room, rather than in intensive care units.

Discussion

These evidence-based procedures made it possible that most patients required open surgery can be managed with airway augmentation rather than resection [13], [14].

Assessments and initial endoscopic treatment provide beneficial information in recognition of the degree of tracheal cartilaginous support and carefully chosen patients in whom tracheal resection could be acknowledged [15]. We should mention that active obstructive autoimmune inflammatory disease under

any conditions should not undergo open surgical intervention because of its ability to expand. Only intralesional steroid injection is replicated.

Follow-up is recommended at least 1-2 years of evaluation postoperatively as cicatricial changes continue to be remodelled and matriculated. Follow-ups included: symptomatic and/or flow-volume estimates loop, imaging in suspicious relapses, endoscopy before decannulation if tracheotomy is present.

Out of the total number of respondents 21, in the following table 8, they were decannulated from a total of 36 or 77 per cent, with successful treatment for the treatment of these patients.

Postoperative complications can occur. The most common complication is granulation in 27 %, followed by restenosis and dysphonia. Stent migration is present in 57% or 4 patient of the total number of the patient, whose stent is set or 7 patients.

Another complication is not shown in our examinee.

In conclusion, endoscopic treatment is minimal invasive allow restoration of vocal function to a sizable proportion and decannulation can occur during the initial visit. It's a treatment of intraluminal inflammatory process Intra steroid, balloon dilatation and reduction of granulation without open surgical intervention can be an effective treatment for acute post-intubation airway stenosis.

Movement of the tube superiorly, inadequately high tube placement, granulation tissue is some of the causes of tracheostomy tube injuries that cause traumatic laryngotracheal stenosis. These events can lead to mucosal ulceration, perichondritis, chondritis and cartilage necrosis [16].

The spectrum of injuries depending on their depths can cause mucosal injuries to full thickness injuries inducing cartilage

It should be mention that active obstructive autoimmune inflammatory disease under any conditions should not undergo open surgical intervention because of its ability to expand. Just intralesional steroid injection and follow up treatment

Patient with laryngotracheal stenosis generates more negative intrathoracic pressure due to their increased inspiratory force, potentiating reflux after the development of laryngotracheal stenosis.

A multidisciplinary approach is recommended, as the decision for a specific intervention is guided by the urgency of each patient and demands a high degree of competence and collaboration between otolaryngologist, interventional pulmonologists and thoracic surgeons

Treatment indices are designed to improve the compromised airway and progress to decay. Rapid intervention before forming contracture or cartilage damages is the preferred method of choice in early diagnostics.

To prevent the formation of subglottic stenoses, the appropriate size and technique for endotracheal intubation are recommended. Prolonged endotracheal intubation of more than 7-10 days is associated with a high-risk factor for laryngotracheal stenosis.

A highly set tracheostomal opening results in a higher risk of laryngotracheal stenosis.

Higher pressures on cuffs lead to increased ischemia on the mucous membrane of the laryngotracheal stem and thus result in a higher percentage of stenos [17], [18].

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Case Report

Spontaneous Clearance of Chronic HCV: The Key Ending Left in the Dark

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Abstract

BACKGROUND: Hepatitis C is the second leading cause of liver cirrhosis and hepatocellular carcinoma. Although the discovery of direct-acting agents made the disease curable, HCV elimination can be achieved solely by the host's immunologic arsenal.

CASE REPORT: We report the case of a 29-year-old woman with chronic hepatitis C infection - elevated transaminases, positive serology. HCV was detectable on two occasions, and histology showed mild disease - A1F1. Upon follow up and without any treatment, the patient achieved spontaneous clearance confirmed by two consecutive undetectable HCV RNA tests. Spontaneous HCV clearance rarely occurs – 0.5% per person-year. This is sometimes accompanied by special circumstances like additional disease or medical interventions. Host factors like gender and interleukin-28B polymorphisms have been known to contribute to clearance. Viral factors like HCV RNA levels are also a factor. The characteristics of host-viral interplay – age of acquisition and fibrosis stage – cannot be overlooked.

CONCLUSION: All of the abovementioned factors contribute to the complex immunological interaction between virus and host and the result, although rarely can be spontaneous clearance.

Introduction

Hepatitis C (HCV) is an enveloped, single-stranded RNA virus capable of causing acute and subsequent chronic infection [1] affecting mainly but not only the liver [2]. Although 20-40% of the acutely infected clear the virus spontaneously, about 75% of the individuals develop chronic infection [3]. Chronic HCV has a 170 million worldwide burden and is the second leading cause of liver cirrhosis and hepatocellular carcinoma [4].

The discovery of direct-acting agents, an undisputed breakthrough of modern medicine, has changed the course of the disease. The natural progression has given way to treatment with a near-to-100% success and a WHO Program for the worldwide elimination of HCV as a public health threat by 2030 [5]. Nevertheless, recovery can be achieved by the host's immunologic arsenal [6]. There's more to be found, as the mechanisms and predictors of spontaneous clearance in the chronic setting remain as blurred as the rarity of the event itself.

Case Report

We report the case of a 29-year-old woman who presented to our clinic with an anti-HCV (+) positive test done 2 months earlier. The patient was unaware of her HCV infection. She has a history of intravenous drug use, currently on Methadone therapy and a sexual partner (also currently on Methadone therapy) with an HCV infection that was diagnosed at the same time as her diagnosis. She has no

concomitant diseases or therapy and is a smoker.

Further laboratory testing verified elevated transaminases (ASAT 386 IU/ml ALAT 740 IU/ml) and a low total bilirubin elevation 21.4 μmol/l. No concomitant HBV or HIV infection was discovered. HCV RNA was 7 247 IU/ml, genotype 3. After four months she presented with normal transaminases and still detectable HCV RNA. This time the genotype was 1b. As per the national consensus for treating chronic viral hepatitis, a percutaneous liver biopsy was performed with an 18G true cut needle showing slight activity and fibrosis-METAVIR A1F1.

The unusual change in genotype arose some concerns, and before any treatment, a third HCV RNA test was done (nine months after the first one) with an undetectable result. Spontaneous clearance of chronic HCV RNA was discussed, and a last HCV RNA test was performed after 3 months (1 year after the initial) confirming the elimination.

Discussion

Spontaneous elimination of chronic hepatitis C occurs rarely: a Scottish cohort demonstrating incidence of 0.19-0.36 per 100person-years [7]; a Japanese study demonstrated a clearance rate of one 0.5%/year/person [8], and a third study placed the number at 0.75% per person-year (1.15 per 100person-years) in Alaska natives [9]. In most published cases loss of HCV RNA is associated with special circumstances like pregnancy and parturition [10], [11], [12], HBV superinfection [13], alcoholic hepatitis [14] or hepatocellular carcinoma [15]. Medical interventions can also alter the natural course of the disease – HCV clearance has been reported after surgery [16] including transplantation indicated for HCV cirrhosis [17] or the initiation of HAART for HIV co-infection [18]. Checkpoint inhibitors used in oncology show a promising effect when used in the setting of a concurrent chronic viral infection [19].

As in the presented case, perhaps more often than reported, HCV RNA is eliminated without any of the abovementioned events. Host (genetic) and virologic factors have been distinguished, influencing an interplay with several characteristics found to be clinically important. Female gender contributes to better prognosis in HCV infection, with HCV RNA rates higher for males than females [20]. Polymorphism on chromosome 19 - the interleukin-28B gene (IL28B) in particular the IL28B-CC genotype is associated with spontaneous clearance in the chronic setting. The latter is especially important in the setting of pregnancy/parturition [10] or HIV coinfection [18]. A genome-wide study detected an important polymorphism on chromosome 6-the HLA class II region near DQB1*03:01 [21]. Although the study did not distinguish between chronic and acute spontaneous resolution, the gene has a role in chronic disease. It has a higher incidence in patients who are asymptomatic carriers compared to those with HCV associated cirrhosis [22]. Younger age of infection is associated with a higher chance of spontaneous HCV clearance [7], [9]. Same can be said for the absence of significant fibrosis (evaluated by ultrasound) [8], although spontaneous elimination has been observed in the cirrhotic setting [14], [15]. There is a connection between the age of infection and fibrosis-younger age of HCV acquisition (< 40 years) is predictive of a slower progression to fibrosis [23]. As far as viral factors go, neither of the known genotypes has been associated with a higher rate of spontaneous clearance in the chronic setting, but low baseline HCV RNA is often discussed [6], [9].

The abovementioned “criteria”, in which chronic HCV spontaneous elimination takes place, have their logical connection when looking into the immunology behind the events. Usually, an acute viral infection triggers a robust T-cell response. With antigenic clearance, immunological reactivity declines (protecting potential autoimmunity) and memory T-cells form [24]. In chronic infections, such as HCV, memory T-cells enter a state of exhaustion, unable to repeat the initial rapid response while antigens persist [25]. Up-regulation of PD-1 (programmed-death-1 receptor) on virus-specific T-cells marks and explains the state of exhaustion. The same receptor is influenced by the innovative checkpoint inhibitors used in cancer therapy [26]. PD-1 blockade improves T-cell proliferation, altering the state of exhaustion, and induces a significant reduction in HCV viremia [27], [28] even to a level below quantitation [28]. But T-cell exhaustion is not only medically reversible. Heterologous viral infections can also trigger the immune system [29]. Both alcohol consumption and parturition have been shown to induce a switch of responses [12], [14]. Humoral immune responses play a role in the decline of HCV RNA-neutralizing antibodies appears [30]. Despite the lack of clearance due to neutralising antibodies alone, there is a connection between them and the natural resolution of HCV [31].

The presented case and discussion perhaps only scratch the surface on the topic of spontaneous clearance in chronic HCV. Further work is required to uncover its true complex mechanisms. Nevertheless, the search continues. Despite the therapeutic success, true elimination will require more – a vaccine development [32].

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Case Report

Facing of Family Doctor with Hantavirus Infection

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Abstract

BACKGROUND: Hantavirus infection is manifested as an urgent, severe and life-threatening disease caused by Hantavirus. The virus affects human endothelial cells. The natural reservoir of the Hantaviruses is chronically infected rodents. Human infection is accidental. Occurs by intake of contaminated food or inhalation of contaminated secretion from infected rodents' excretions have an increased risk of contamination. The most affected persons are people who work in nature. The virus causes haemorrhages, fever and acute renal failure. The disease appears more frequently in endemic regions with the lethality of 6-15%. The disease can surprise doctors with severity, urgency and undefined clinical picture. Fast clinical evaluation, proper and urgent diagnosis and treatment can improve the safe life of these patients.

CASE REPORT: We report a case of 45 -year-old male patient worked as a shepherd on mountain Babuna near the city of Veles in the Republic of Macedonia at the end of the summer in the year 2017, presented with prolonged hemorrhagic fever with renal syndrome. The clinical presentation and lab findings support the diagnosis of Hantavirus infection with acute renal failure.

CONCLUSION: It is necessary to raise the awareness of the family doctors for the hantavirus disease, especially in countries with sporadic cases, as in our country. It needs for prompt and timely diagnosis, timely hospitalisation and initiation of therapy.

Introduction

Hantavirus infections (order *Bunyavirales*, family *Hantaviridae*) attract more attention in the world. For the first time were reported entities in Korean wore, beside river Hantan. Three thousand soldiers were faced with febrile fever, acute renal failure, shook, with the lethality of 7%. The cause of the disease is *Hantavirus* – single strain RNA virus with 21species and more than 30 genotypes. *Hantavirus* affects human endothelial cells and causes vascular instability [1], [2]. The natural reservoir of the *Hantaviruses* is chronically infected rodent. Virus and the host have a long period of co-evaluation without the existing disease [3]. Infection is accidental. Infection occurs by ingestion of contaminated food with excretions (urine, faeces and sputum) or inhalation of contaminated dust from an infected rodent. People (shepherds, foresters, woods) with close contact with rodents' excretions have an increased risk of contamination. The most common types of *Hantaviruses* in Asia are Amur and Seoul virus with the lethality of 17%. Seoul *Hantavirus* (SEOV) causes mild to moderate hemorrhagic fever with renal failure in Russia, South Korea and China. Few tens to a few thousand, human infections were diagnosed in China and four sporadic human cases in the United Kingdom, France Netherlands [4] and Germany [5], [6]. Dobrava, Tula, Puumala and Saaremaa viruses are most frequent with the most severe clinical pictures on Balkan [7]. In our region, the most frequent is Dobrava *Hantavirus*. The infection from human to human is very rare, with the exception of Ande virus in South Argentina. Puumala virus stands as the main contributor to hemorrhagic fever with renal syndrome (HFRS) in Europe, while

the Dobrava virus is the causative agent of the most severe HFRS causes in central Europe. The mortality rate for Dobrava viruses is more than 10% [8]. Bruges virus is a novel *hantavirus* found harboured by the European mole (Talpa europea) which is the host of Nova virus. These findings highlight the complexity of *hantavirus* evolution and the importance of further investigation of *hantavirus* reservoir relationship [3]. There are three types of the disease with different clinical manifestations, but often some of the symptoms are found in the three types of disease, especially in the first two types: 1. NE (nephritic enteropathy), 2. HFRS (hemorrhathe gic the fever with renal syndrome), and 3. HPS (Hemorrhagic pulmonary syndrome). HFRS last from 7-36 days with the lethality of 6-15% and is the most frequent [9]. The *Hantavirus* causes systematic damages of capillaries and venules. It induces hemorrhagic manifestation and vascular disturbances, which causes acute renal failure as a result of interstitial haemorrhages and infiltrates [2]. The clinical expression of the disease is classified in 5 phases: febricity, hypotension, oliguria, diuresis and convalescence [10]. The first phase is characterised by the predominance of fever. The first 3 to 4 days are characterised by the appearance of the chest and abdominal pain, fever, myalgia, photophobia, malaise, diarrhoea, vomiting, diffuse redness on the face. Symptoms in this period are very non-specific and can be difficult to differentiate from a simple virus infection accompanied by diarrhea. In the fourth and fifth day of illness, appear diffuse petechial hemorrhages, enanthema on the hard palate, hemorrhages in conjunctives, involvement of the temporal visual field, coughing, hematuria and proteinuria.

The second phase is characterised with predomination of hypotension: The hypotension is developed in the 3-6 days of disease with strong expressed malaise, shook, leukocytosis and thrombocytopenia, with a wide range of renal impairment – (acute tubulointerstitial nephritis) or (necrotising glomerulonephritis, and IgA nephropathy). The severity of thrombocytopenia in a patient with HFRS may predict disease severity and critical patients' survival [11]. This phase is also nonspecific and difficult to recognise and reminiscent of dehydration, caused by prolonged fever and diarrhoea. The third phase is with the appearance of oliguria: In that phase, if it is not done on time a complete blood account analysis which is characterised with thrombocytopenia and increased values of blood creatinine, it is not possible to recognise the disease. If the disease is not recognized at this stage of the disease, the likelihood of a fatal event is possible due to advanced acute renal failure. The eighth day of the disease is also characterised by hemorrhagic manifestations.

The fourth phase is characterised by diuresis. If the patient survived, an intensified diuresis occurs on the eleventh day of the illness.

The fifth phase is convalescence, which lasts 2 weeks to 6 months. All five phases are not strictly delineated. Sequels are rare with chronic renal failure and hypotension. Extrarenal symptoms in this disease are presents as acute myopia, convulsions, myocarditis, gastrointestinal bleeding, liver, pancreas, thyroid gland and pulmonary damages.

The diagnosis is established based on the clinical picture and laboratory investigations. The main factor on which depends on the severity of the disease is the degree of endothelial permeability and genetic predisposition, HLA-B8, DRB1*0301, C4A*Q0, or DQ2 alleles, HLA B35. Thrombocytopenia appears at the early stage of the disease. Detection of specific IgM antibodies confirms the diagnosis [12]. Increased levels of procalcitonin could be predictive of disease severity, secondary bacterial infection and mortality in patients with HFRS caused by *Hantavirus* infection [13]. There is no applicative etiological therapy. Supportive therapy, such hemodialysis, correction of bleeding and platelets cells, blood pressure, antibiotic treatment of bacterial infection, anticoagulant therapy and supervision. Ribavirin and interferon have limited results. Prevention is particularly important. It is recommended to avoid places with an increased presence of mice or other rodents to reducing contact with contaminated excretions. Preventive measures in the houses and the environment by eliminating the food sources are useful. These measures could make home and workspaces unattractive to rodents [14]. There are needs of increased efforts for preparing effective and reliable vaccine with recombinant RNA technology. The potential effect of the inactivated *Hantavirus* vaccine remains controversial. It appears in the research in the Republic of Korea; the vaccine is moderately effective for patients (older patients) at high risk for HFRS [15] Current vaccines are ineffective, with development of neutralising antibodies [16]. Until today there is no suitable vaccine with inactivated *Hantaviruses* that will provide adequate protection in humans.

Case Report - Our Experience with Hantavirus Infected Patient - (Hemorrhagic Fever and Renal Syndrome)

The present article reports a case of 45 old male patients, presented with *Hantavirus* infection disease. He worked as a shepherd on the Babuna Mountain near Veles in the Republic of Macedonia. History is negative for any disease of interest. Epidemiological history is positive, and it is connected with eating contaminated watermelon. He visited his family doctor after 6 days of the onset of symptoms. The first symptoms were high temperature, > 38.5°C, which lasted 5-6 days, prolonged vomiting, diarrhoea for 5-6 days, dorsal, strong lumbar and sacral pain,

abdominal pain, myalgia, pronounced fatigue, reduced coordination, and cough**.** The patient's entire condition was unspecific and reminded of gastrointestinal infection and the common cold. By first physical examination in the family doctor's office was found the existence of elevated temperature > 39°C, bradycardia, diffuse redness of the face, photophobia, hypotension, petechial haemorrhages of hard palate and conjunctiva and temporally disturbed vision, reduced coordination, slow speech, hoarse voice and cough. Lab analyses were performed in the first visit of the patient. The measured blood pressure was 100/70 mmHg.

Table1: Laboratory results of the first and second day of ambulatory examinations

	Hb	Er	Le	Gr	HCT	PLT	Glycemia	Urea	Cratinine	ALT	AST
First day	195 g	7.15	11.44x10^{9}/L	83.8	69	45	6.9	11.8	159	43.5	68
Second day	195	7.26	19x10^{9}/L	85.3	70	45	8.1	17.4	374	44.5	69
Ref. values	120-174gr/l	4.00-5.50x10^{12}/l	5.00-10.00x10^{9}/ l	40-70%	36-52%	150-400x10^{9}/l	4.2-6.5 mmol/l	1.7-8.5 mmol/l	56-120 µmol/l	< 45 U/l	< 45U/l

The Laboratory results of the first ambulatory day are presented in Table 1. Blood analysis showed high values of haemoglobin, erythrocytes and hematocrit (haemoconcentration), increased number of white blood cells, low platelet counts, increased values of blood sugar, creatinine, urea and alanine transaminase (ALT), aspartate transaminase (AST) values. Urine analysis showed mild proteinuria and hematuria. We suggested urgent hospitalisation because of the complexity of the symptoms, high fever and signs of renal failure. He rejected to be hospitalised, but finally, he decided to visit as with his wife the next day. We started intravenous rehydration and symptomatic treatment.

During the review of the patient's condition, the deterioration of the health status was observed, with frequent vomiting, hypotension, oedema on the face, vision disorder, pronounced malaise, enanthema of the soft palate, conjunctivas bleeding and appearance of the oliguria. The measured blood pressure was 90/60 mmHg. The second-day lab analyses were done. Prompt worsening, with the persistence of thrombocytopenia, haemoconcentration, hyperglycemia, uremia, high levels of creatinine values, liver and pancreas damages were observed.

The patient was immediately referred to the General Hospital, Department of Internal Medicine in Veles, for further hospital treatment. Laboratory analyses, chest and abdominal x-ray were performed. An abdominal x-ray was normal, without signs of acute surgical disease. The chest x-ray was normal. Because of the appearance of strong abdominal pain, vomiting of bloody content and unclear clinical picture of the disease was made unsuccessful gastroscopy attempt in the local hospital. Shortly after that, complete anuria appeared (creatinine value 541 µmol/l, thrombocytes 29 x 10^{9}/l, leucocytes 22.5 x 10) and the patient was forwarded as an emergency patient to the University Clinic for Nephrology (UCN) Skopje where were done lab analysis and serological analysis. Serological analyses (ELISA) done at the Laboratory for Virology and molecular diagnostics, Institute of Public Health, showed the existence of IgM antibodies against *Hantavirus*.

The serological analysis was not performed again. After establishing the diagnosis and started hemodialysis patient was transferred at University Clinic for Infectious disease and febrile conditions (UCIDFC) Skopje. Consultation with Transfusion department was done twice times, where was analysed coagulation.

Table 2: The results of the blood coagulation analyses

Transfusion department	First analysis	Second analysis-after 2 days	Reference values
Number of platelets	33	96	150-450x10^{9}/l
Hematocrit	43.2%	20.5%	35-50%
Prothrombin time	11.3	12.32	9-14.2 sec.
Activated partial thromboplastin time	39.59	45.01	27.9-37.7%
Thrombin time	24.64	21.73	16.1-24.1 sec.
D-dimer	4306.42	2553.13	0-500 ng/ml

The first analysis showed consumptive thrombocytopenia with activation of secondary thrombolysis and hemolytic anaemia. The second analysis was done after started anticoagulation treatment. Hemodialysis was performed three times at UCN. The patient was hospitalised 21 days at UCIDFC, and he had important improvement of the general condition and renal function. Lab results during hospitalisation are presented in Table 3.

Table 3: Lab analyses performed at UCIDFC

Days of the hospital treatment	4-th day	6-th day	8-th day	11-day	14-th day	17-th day	20-day	21-th	Reference values
Hb	128	76	103	100	93	96	103	106	120-174 g/l
Er	4.1	2.5	3.4	3.2	3.1	3.2	3.4	3.5	4.00-.50.50x10^{12}/l
Le	25.8	20.2	19.5	15.0	10.4	5.3	7.9	8.2	5.00-10.00x10^{9}/l
PLT	46	100	90	119	150	129	99	303	150-450x10^{9}/l
Hematocrit	36	22	31	30	29	31	33	33	35-50%
Gr	74	65	77	76	69	52	58	49	40-70%
Glycemia	6.7		5.7	5.7	6.3	5.6	5.3	3.5	4.2-6.5 mmol/l
Urea	26	36.7	18.8	17.9	14.1	6.3	5.3	3.5	1.7-8.5 mmol/l
Creatinine	604	630	563	364	340	128	89	87	56-120 µg/l
ALT	27		80		95				<45 U/l
AST	35		64		69				<45 U/l
LDH	944	984	1157		958				225-450 U/l
CK	162	190	31		51				24-190 U/l
K	3.5	3.7	3.8	3.6	3.9	4.3		4.9	3.6-5.5 mmol/l
Na mmol/L	125	125	133	135	144	145		143	135-155 mmol/l
Ca mmol/L	1.71	2.3	1.98	1.92	1.95	2.17		2.39	2.02-2.60 mmol/l
ABS-pH	7.41	7.47	7.43						
Albumins			26		27				38-51 g/l
Globulins			28		31				26-46 g/l
Total proteins			54		58				66-87 g/l
urine						20-25Er,6-8 Le, the mass of bacteria		10-15Le 6-8Er	
CRP	25		33		34	19			
agriculture							Klebsiella pn.esbl+		
Serology for Hantavirus IgM Antibodies							positive		

The results are connected with the severity of clinical pictures. He received supportive therapy. Correction of bleeding and platelet was done with blood transfusions and blood products on several occasions. Rehydration with intravenous infusions, anticoagulant therapy, antibiotics treatment, hemodialysis and supervision was done. The

complete patient's treatment provided complete recovery without sequels. Control examinations were performed regularly.

Phylogenetic analysis of the *Hantavirus* was performed at the Medical School Aristotle University of Thessaloniki, conforming Dobrava serotype.

Established Diagnosis: Hemorrhagic fever with Renal Syndrome, *Hantavirus* infection (Dobrava serotype).

Discussion

Hantavirus infections with HFRS are periodically seen in our country at the end of the summer and autumn among persons who are working in nature. The diagnosis and treatment can be difficult, especially in the region where the disease is not frequent, and the doctors are not familiar with that disease. *Hantavirus* infections are more frequent in Korea and China. The minor peak season is from May to July, and the major peak is in the harvest season, from October to December when the ground is disturbed, and there was a lot of dust [17]. The disease has a high percentage of mortality. The mountain Babuna and region around cities Tetovo and Gostivar are endemic regions for *Hantavirus* infection, but the disease appears periodically. According to data of the Institute of Public Health in 2017, 17 patients with detected *Hantavirus* infection were reported, and calculation of fatality rate for this disease is 11.8%. In the 2018 year, 9 cases of *Hantavirus* infection were reported. Dobrava serotype was confirmed for the cases in 2017. Two patients died, and 3 patients had chronic kidneys failure**.** Due to the need for treatment and prevention of the disease, many studies have been undertaken, including the efforts on creating a new, more effective vaccine [18]. Despite the emergence of this disease, there is a space for improvement concerning making a rapid and accurate diagnosis and implementing appropriate care. The family doctors and clinicians are facing challenges in dialling with the unfamiliar disease in people who are working in nature. *Hantavirus* infection with HFRS is a very urgent disease with very prompt developing of the spectrum of unspecific symptoms which can make difficulties in establishing of the diagnosis, especially in regions where the disease is not very frequent, and it can cause delayed of the treatment. Every delayed of the treatment can be the reason for fatal consequences and sequels. Making algorithm is not possible because the disease is not very frequent, and clinical presentation is not specific.

The goals of the treatments are quick recognition, lab investigations, prompt symptomatic treatment, and hemodialysis in patients with anuria or oliguria. *Hantavirus* disease should be considered in the differential diagnosis of leptospirosis and rickettsiosis, unspecific gastroenterocolitis, severe atypical pneumonia, pneumonia influence, heart failure, etc. Accurate diagnosis and timely initiation of the therapy are critical to the management. The main diagnostic method is the serological analysis of elevated Ig-M antibodies for *Hantavirus* [12]. Risk factors for *Hantavirus* infection are winter temperature, population density and enough available food for rodent [19]. Preventive and education measures are crucial. Many attempts have been made to produce a vaccine for *Hantaviruses* for different types, but most often it is unsuccessful.

In conclusion, *Hantavirus* disease has an unclear and profuse clinical picture with different and variable symptoms in people who work in nature. The symptoms are prolonged febricity, lumbosacral pain, abdominal pain, vomiting, malaise, dehydration and signs of acute renal failure, thrombocytopenia, and often looks like a simple cold and gastrointestinal virus infection. It is necessary to raise the awareness of the family doctors for this disease, especially in countries with sporadic cases, as in our country. It needs for prompt and timely diagnosis, timely hospitalisation and initiation of therapy. There is not etiological therapy, but symptomatic treatment can safe a life. Using preventive measures and education can reduce the risk of infection. Eradication of environment of rodents' excretions is useful in the situation of lack of proper vaccines.

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Case Report

High-Risk Basal Cell Carcinomas of the Head and Neck: Selected Successful Surgical Approach in Three Bulgarian Patients!

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Keywords: Advancement flap; Island flap; Undermining surgical approach; Treatment outcome

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Abstract

BACKGROUND: Regarding localisation, basal cell carcinomas are classified in three risk groups, designated as H for high-, M as medium-, and L as low-risk area. In patients with high-risk basal cell carcinomas (BCCs), as a first-line of treatment are mentioned, different types of surgical approaches and radiotherapy. Depending on the location of the tumour, the choice of surgical technique should vary and be consistent with the patient's will for a most aesthetically acceptable result.

CASE REPORT: Three cases of patients with BCCs defined as high-risk about two different indicators-localisation and relapse after radiation therapy are presented. For the recovery of the occurred defects, three different types of surgical approaches (primary closure/undermining surgical approach, island flap and advancement flap) were used, tailored to the high-risk factors in each patient, which at the same time provided a perfect clinical outcome.

CONCLUSIONS: High-risk BCCs are a challenge for every dermatosurgeon and require serious training and knowledge both in terms of anatomy and in terms of the possibilities for reconstruction of the defects that occurred. Operations usually run in three phases, namely: 1) removal of tumour tissue, 2) intraoperative plan for reconstruction according to the size of the defect and the condition of the surrounding tissues as well as phase 3) undermining of surrounding tissues and adaptation of the wound edges.

Introduction

The main indicators are determining basal cell carcinomas (BCCs) as high-risk include size, tumour location, histological subtype, recurrent tumours and previous history of radiotherapy [1]. Also, locally advanced BCCs refer to tumours affecting and infiltrating underlying and/or surrounding tissues with the highest frequency for BCCs in the head and neck area [1]. We describe three different cases of basal cell carcinomas, two of which are defined as high-risk depending on the localisation (H-zone of the face and nasolabial fold), and the third as well, but due to the recurrent character after repeated radiation therapy. Various surgical techniques have been applied to the prospect of achieving a perfect clinical outcome.

Case Report 1

A 91-year-old man with hypothyroidism and several cardiovascular diseases is presented. The patient reported a 5-6-year onset of formations on the face and neck, which periodically bleed and were difficult to heal. The ambulatory biopsy taken showed basal cell carcinoma data, and on this occasion, the patient had a series of radiotherapy (Figure 1a and 1b). From 1 year in the neck area to the left, there has been a recurrence of BCC, for which case the patient is hospitalised again to be selected an adequate treatment regimen.

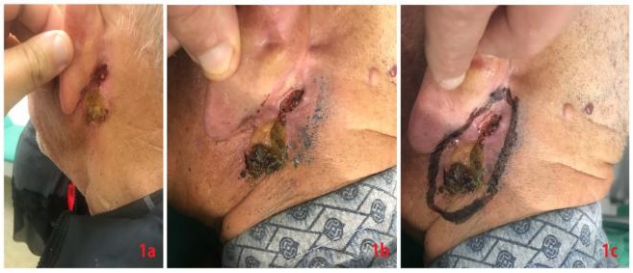

Figure 1: a), b), and c) Clinical view of an ulcer defect in the left neck area with a size of 5.2 to 3.1 cm, located retro-auricularly, with bleeding, purulent surface, raised edges and hemorrhagic crusts at the periphery

Within the clinical examination, an ulcer defect was found in the left neck area with a size of 5.2 to 3.1 cm, located retro-auricularly, with bleeding, purulent surface, raised edges and hemorrhagic crusts at the periphery, clinically suspected for recurrent BCC (Figure 1a and 1c).

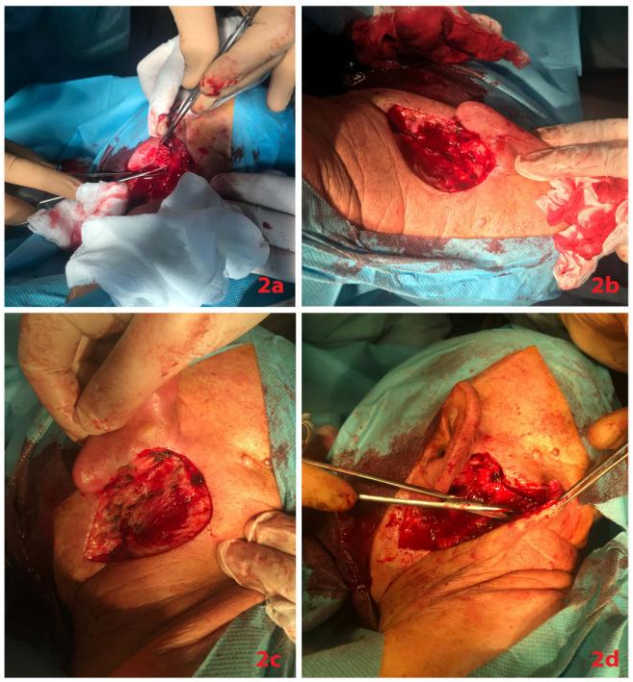

Figure 2: a), b), c), and d) Intraoperative finding: radical surgical eradication under local anaesthesia with oval excision of the lesion, followed by undermining of the wound edges

Radical surgical eradication was performed under local anaesthesia, and the lesion was removed by oval excision (Figure 2a and 2c). It was followed by undermining of the wound edges with the purpose for maximum adaptation (Figure 2d).

The resulting surgical defect was recovered by advancement flap with a rotating element from the distal part of the neck to the retro- and periauricular area (Figure 3a and 3c).

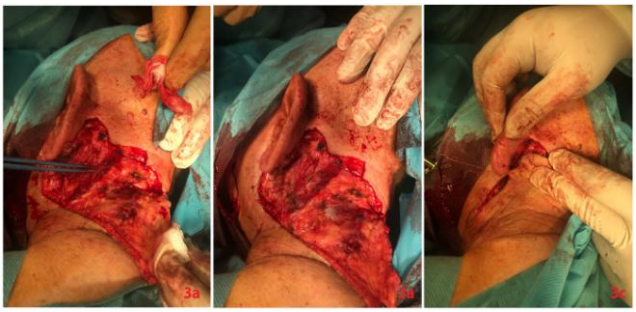

Figure 3: a), b), and c) Undermining of the wound edges and rotating element from the distal part of the neck to the retro- and periauricular area. Closure with single interrupted sutures

The closure was with single interrupted sutures (Figure 3c, 4a and 4b). The histological examination showed the presence of residual basal cell carcinoma nests in extensive ulceration, underlined fibrosis and hyalinosis, resection lines - without tumour infiltration. A quiet post-operative period was observed, without complications and the perfect cosmetic result (Figure 4c).

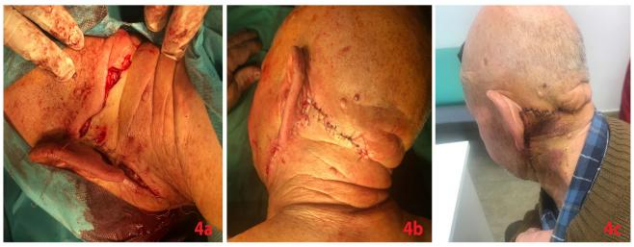

Figure 4: a), b) and c) Postoperative finding: surgical defect closed by single interrupted sutures

Case Report 2

We present a 71-year-old man in good general condition. The patient reported the presence for 7-8 years of a cutaneous neoplasm in the base of the nose, which increased over time and began to bleed. In 2018 curettage of the lesion was performed without complete elimination of the tumour, and subsequently, the patient observed lesion enlargement and persistence of the already occurred ulcerative defect. He was hospitalized for surgical

treatment and radical excision of the erosive lesion in the Department of Dermatology and Dermatological Surgery. During the dermatological examination the presence of a lesion with ulcerative character and raised edge at the periphery, located in the area of the glabella and nose base, clinically suspected for basal cell carcinoma, was established (Figure 5a and 5b).

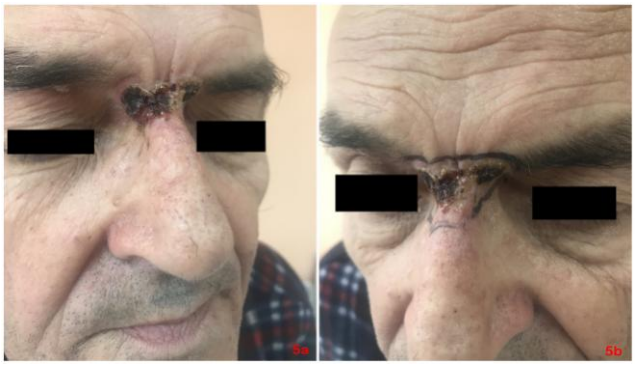

Figure 5: a) and b) Clinical view of a lesion with ulcerative character and raised edge at the periphery, located in the area of the glabella and nose base. Outlining the surgical margins

The lesion was removed by radical excision with a field of surgical safety of 0.2-0.3 mm and the occurrence of a major surgical defect, encompassing the root of the nose and the glabellar area, reaching directly to the eyebrows (Figure 6a and 6b). The wound edges were undermined for optimal adaptation, and the resulting operational defect was initially closed step by step through single interrupted sutures (Figure 6c and 6e). The subsequent histological examination revealed the presence of superficial basal cell carcinoma with multifocal growth, a maximum tumour diameter of 28 mm, no lymphovascular invasion, resection lines less than 2 mm from the tumour. A smooth post-operative period without complications was observed (Figure 6f).

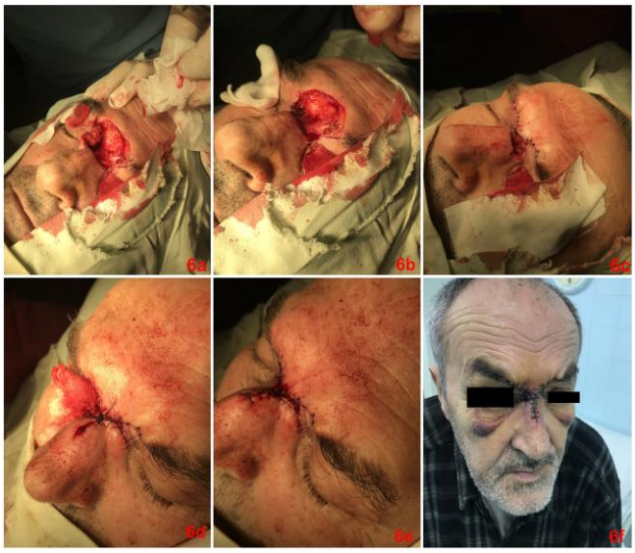

Figure 6: a) and b) Intraoperative view: radical excision of the lesion; c), d) and e) Undermining of the wound edges and closure of the resulting surgical defect with single interrupted sutures; f) Postoperative finding- perfect clinical outcome

Postoperatively prophylactically, nadroparin calcium was applied 2 x 0.4 ml/day due to high-risk localisation of the tumour and the existing risk for sinus cavernosus thrombosis.

Case Report 3

A 73-year-old man is presented with a complaint about a 1-year presence of a formation in the left cheek area, which progressively grows and periodically bleeds (Figure 7a). During the dermatological examination, an oval ulcerative lesion with a bleeding surface and the presence of hemorrhagic crusts was found, located next to the left ala nasi (Figure 7a and 7b). The so-called island flap was performed, and the lesion was removed by oval excision with an operational safety margin of 0.5 cm in all directions (Figure 7c). Next, a triangle was contoured in the distal direction along the line of the left nasolabial fold (Figure 3), followed by transposition of the undermined triangle to the ala nasi in the left (Figure 7d). The resulting surgical defect was closed by a single interrupted suture (Figure 7e and 7f). A quiet postoperative period was observed. Post-operative histological verification revealed the presence of basal cell carcinoma with multifocal growth, tumour diameter 13 mm, free resection lines. Post-operative prophylaxis was performed with application of nadroparin calcium 2 x 0.4 ml/day.

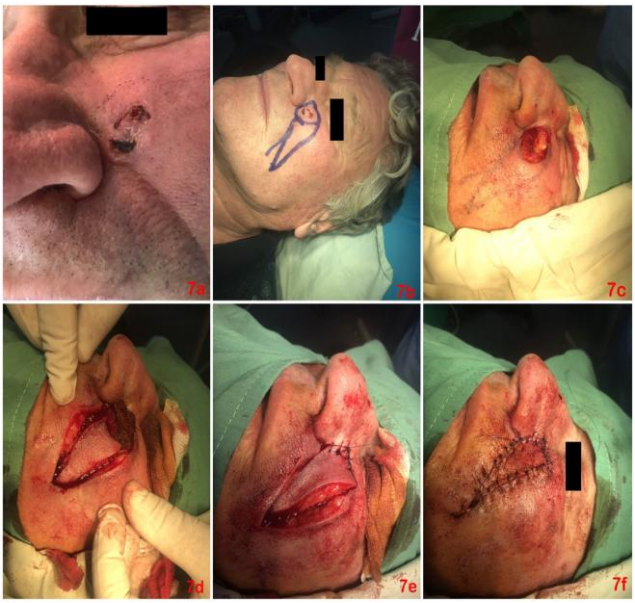

Figure 7: a) and b) Ulcerative lesion with a bleeding surface and presence of hemorrhagic crusts located next to the left ala nasi. Outlining the surgical margins; c) Oval excision of the lesion; d) Contouring of a triangle in the distal direction along the line of the left nasolabial fold; e) Transposition of the undermined triangle in the proximal direction; f) Postoperative finding- surgical defect closed by single interrupted sutures

Discussion

Depending on the location of the BCC, there are three areas of risk indicating different parts of the body skin, namely H as high- (mask areas of the face including the central face, nose, eyelids, chin, ear, genitalia, hands, feet, nipple-areola, ankles), M as medium (cheeks, forehead, scalp, neck, jawline, pretibial surface) and L as a low-risk area (trunk and extremities, excluding H and M areas). Furthermore, depending on the additional features of the affected area, prerequisites that may lead to a higher risk of relapse may occur [2]. Because of the specific anatomical features and difficult surgical techniques in the H-zone of the face (i.e. nasolabial fold, nasal alar, orbital area and auricular area), and next to it, often it comes to narrow excision margins and inability to follow the recommended surgical margins of 4mm for small, primary, well-defined basal cell carcinomas and 5-6 mm for high-risk and recurrent tumours (as in case 2) [2], [3], [4]. It is believed that incompletely excised BCCs are most likely to recur [5]. As additional factors increase the risk of recurrence, microcirculation, vasculature and host inflammatory response of the affected area, especially in high- risk areas, such as H-zone, are also reported [2].

The main difficulties for the management of BCC in the glabellar area are derived from 1) the immediate proximity to the angular vein and the serious risk of eventual thrombosis (again case 2), 2) due to the fact that at the moment the precise venous anatomy of the glabellar to the forehead region remains unknown [6] and 3) the need for an aesthetically acceptable cosmetic result.

Regarding restorative surgical technique after radical excision, the primary closure is the simplest type of defect repair, which is most suitable at the nasal root where the skin is mobile and also in the medial part of the nasal bridge, and combined with the undermining of the wound edges gives a good favourable cosmetic result (case 2) [7]. Other surgical techniques that can be used to repair surgical defects in BCC patients and allow one-stage closure was applied to the other two patients, namely the so-called island pedicle flap and advancement flap [7].

The advancement flap performed in the first patient belongs to a group of sliding flaps, where the tissue is moved or "slid" directly into the adjacent defect and thus avoided the need for “jumping” over the interposed tissue [8]. Also, unlike rotation and transposition flaps, this type of plastic does not significantly alter the direction of the primary tension vector for wound closure and the tension vector required to close an advancement flap is nearly identical to a linear closure [8]. The other advantage of the advancement flap is that it creates the possibility of reconstruction that permits scar camouflage along cosmetic subunit junctions [8].

In the third described patient we used the so-called island flap, which has two main advantages, namely that 1) the island tissue which is being transposed, is near the surgical defect and 2) it can be performed under various variants (V-Y tissue), i. E represents a flexible method which, through variations of designs, allows successful modelling of delicate areas, especially those in the facial area [7], [9].

In conclusion, in patients with high-risk BCC, the surgical approach needs to vary depending on the individual indicators that define the tumour as high-risk. The advantage of the reconstructions carried out in one stage, as in the described primary closure, island flap and advancement flap, is the avoidance of further deformations of the tissue that might occur in one or two stage interventions, while at the same time leading to an aesthetically satisfying result.

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Case Report

Seizure Disorder Exacerbated by Hepatic Encephalopathy: A Case Report

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Keywords: Hepatic encephalopathy; Seizures; Cirrhosis

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Abstract

BACKGROUND: Hepatic encephalopathy is a serious complication of cirrhosis that presents with a variety of neuropsychiatric abnormalities, including disorientation, asterixis, and coma. Seizures are an uncommon and potentially dangerous complication of hepatic encephalopathy. We present a unique case of a 42-year-old female with a history of well-controlled seizure disorder suddenly become refractory to anticonvulsant therapy following the development of hepatic encephalopathy secondary to liver decompensation.

CASE PRESENTATION: A 42-year-old female presented to our hospital following a seizure accompanied by loss of consciousness, urinary incontinence, and the prolonged postictal state. She reports her seizures were initially well-controlled with Levetiracetam 500 mg twice a day but recently began experiencing seizures every other day despite up-titration of Levetiracetam to 1500 mg twice a day over a few weeks. On arrival, her serum ammonia level was 116 µmol/L. CT brain was negative while CT liver was consistent with cirrhotic morphology. An electroencephalogram revealed irregular, diffuse, delta/theta slowing consistent with mild to moderate encephalopathy. The patient was started on lactulose 40mg and Rifaximin 550 mg twice a day. Her symptoms of disorientation and lethargy resolved over 3 days.

CONCLUSION: Though uncommon, hepatic encephalopathy should be considered in patients presenting with convulsions, especially if there is a known history of liver disease. Until the underlying liver issues are addressed, patients may not respond to traditional anti-convulsant therapy for their seizures.

Introduction

Around 30 to 45% of patients with cirrhosis develop hepatic encephalopathy (HE), and this development has been associated with a poor prognosis [1]. HE is a serious complication of cirrhosis and portosystemic shunts that presents with a spectrum of neuropsychiatric abnormalities, ranging from disorientation to coma, that can greatly debilitate patients' lives. Abnormal motor symptoms such as irregular tremor and asterixis are also frequently associated with the disorder [2], [3], [4], [5]. Seizures are uncommon yet potentially dangerous complications of HE [3], [6], [7].

Case Presentation

A 42-year-old female with a history of cirrhosis secondary to chronic hepatitis B virus infection was brought to our emergency department following a seizure accompanied by loss of consciousness and urinary incontinence. She reports a history of seizures that began 5 months prior when she was admitted to a different hospital for HBV cirrhosis and abdominal pain. During that hospital stay, the patient was given Tramadol, which she attributes the onset of her seizures too. The seizures were initially controlled with Levetiracetam 500 mg twice a day; however patient states that seizures have increased in frequency to every other day over the past few weeks despite up-titration of Levetiracetam to 1500 mg twice a day. Her other medications at the

time include Tenofovir 300 mg daily for her HBV infection and propranolol 10 mg twice a day and pantoprazole 20 mg daily for portosystemic varices seen on prior CT imaging. She also reports intermittent lower extremity oedema which she takes Furosemide 20 mg daily. She usually does not go to the ED when she has a seizure. However, she presented to our ED because her seizures have increased in frequency and been accompanied by prolonged post-ictal states. Per discussion with family, the patient had had increasingly slower mentation since the initial admission 5 months ago. Patient reports feeling more lethargic and "off" for the past few weeks.

Vitals on admission were a blood pressure of 139/97, the pulse of 93 beats/minute, and SpO_2 of 99% on room air. On clinical exam, the patient was somnolent and slow to answer questions. She was alert and oriented to person and place but not time. Scleral icterus, asterixis, and mild upper abdominal pain were present. There were diminished vibration and position sense in the lower extremities bilaterally. There was no ascites or oedema. Heart sounds were normal, and the lungs were clear to auscultation.

Laboratory studies showed a white blood cell count of 4500/mm^3, haemoglobin of 12.1 g/dL, hematocrit of 37.9%, and platelets of 112000/mm^3. Electrolytes were normal. Other labs include: total protein of 7.3 mg/dL, total bilirubin of 3.8 mg/dL, albumin of 2.6 g/dL, aspartate aminotransferase of 98IU/L, alanine aminotransferase of 71IU/L, alkaline phosphatase of 275IU/L, and ammonia of 116μmol/L (reference range 9 μmol/L-33 μmol/L). Prothrombin time was 20.6 seconds, and INR was 1.79. CT brain was without evidence of acute bleed or mass effect while CT liver was consistent with hepatic cirrhotic morphology. An electroencephalogram revealed findings consistent with mild to moderate encephalopathy (Figure 1). The patient denied the prior history of hepatic encephalopathy.

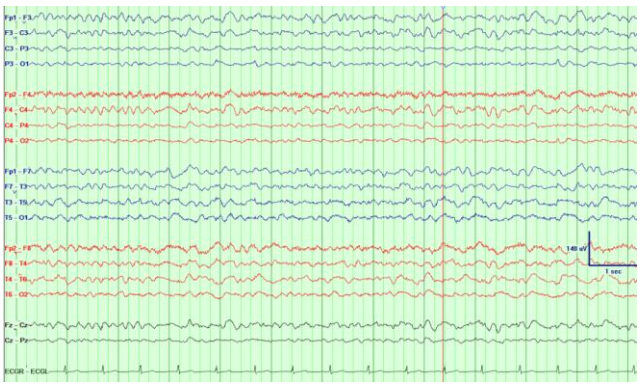

Figure 1: Electroencephalogram showing irregular, diffuse, delta/theta slowing consistent with mild to moderate encephalopathy

Based on the laboratory, imaging, and clinical findings, her altered mental status and seizures refractory to anticonvulsant therapy were attributed to hepatic encephalopathy secondary to liver decompensation from HBV cirrhosis. The patient was started on lactulose 40 mg twice a day, titrated to 2-3 bowel movements daily and Rifaximin 550 mg twice a day. Over 3 days, her mental status improved, she was no longer lethargic, and her scleral icterus and asterixis resolved. She was discharged on the fourth hospital day with her home medication and lactulose for further management of her hepatic encephalopathy. She has not experienced another seizure on 3 months follow up and continues to do well.

Discussion

Hepatic encephalopathy (HE) is a reversible consequence of advanced liver disease and/or portosystemic shunting characterised by impaired neurologic function including altered mental status, asterixis, and possible coma [2], [3], [4], [5]. The frequency of seizures in HE remains uncertain. One study found that up to one-third of their patients with HE developed seizures; however, this was largely in more advanced disease stages [8]. Other authors suggest seizures are an uncommon event [3], [6], [7]. Ficker et al. reviewed EEG tracings in patients with HE and found that when epileptiform abnormalities were present, they were associated with a poorer prognosis [6].

The pathophysiology of HE is multifactorial and complex, including changes in ammonia (NH_3) levels, inflammatory cytokines, and amino acids [2], [4], [7]. The most widely understood mechanism involves the hepatic metabolism of NH_3. The 2 primary metabolic pathways by which ammonia is handled is through the urea cycle and glutamine synthase (produces glutamine from glutamate) [4]. In patients with cirrhosis, there is hepatocellular dysfunction and portosystemic shunting, resulting in increased levels of ammonia through the systemic circulation. Astrocytes in the brain convert NH_3 and glutamate to glutamine. Hyperammonemia results in increased glutamine production and accumulation in astrocytes creating an osmotic gradient that promotes astrocytic swelling [2], [4], [7]. Elevated glutamine levels also result in the generation of reactive oxygen species through a process of hydrolysis in mitochondria, which contributes to the neuronal dysfunction in hepatic encephalopathy. A milieu of inflammatory cytokines augment the neurotoxic effects of ammonia by enhancing the diffusion of ammonia across the blood-brain barrier in addition to exerting their neurotoxic effects [4].

EEG recordings have been useful in identifying the underlying aetiology of altered mental status in patients with liver cirrhosis. A few reports

detail the use of EEG recordings to discern hepatic encephalopathy from conditions such as nonconvulsive status epilepticus (NCSE), which can be hidden within a diagnosis of HE yet requires a different course of medical management [9], [10], [11]. While other cases of status epilepticus secondary to HE have been reported [3], [12], [13], our case demonstrates a patient who initially had a history of seizures well-controlled with anti-convulsants, and no prior history of HE, suddenly become refractory to her medication. Her decompensated liver state aggravated her neurological issues, which prompted further medical management of her condition beyond regular anti-convulsant therapy. The addition of lactuolose and Rifaximin produced a clinically significant resolution of symptoms in our patient. Therefore, although rare, it is important to appreciate HE as an exacerbating factor in convulsive patients because the therapeutic management of these patients may change. Furthermore, certain anti-epileptic drugs, such as benzodiazepines, can potentially worsen HE, so it is imperative that a diagnosis of HE is not overlooked in convulsive patients [2], [3].

In conclusion, seizures are an uncommon and potentially serious complication in patients with hepatic encephalopathy. EEG recordings may be useful in ruling out other etiologies of altered mental status in patients with a history of liver disease presenting with convulsions. Patients experiencing seizures secondary to HE may not respond to traditional anti-convulsant therapy, and therefore, the underlying liver problems must be addressed.

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Brief Communication

Determination of the Product of DNA Oxidation in the Blood of Women Living in the Sub-Aral Area

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Keywords: Aral Sea region; Oxidative stress; 8-OH-deoxyguanosine; Reproductive health

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Abstract

To assess the impact of climatic and anthropogenic factors of the Aral Sea region on reproductive health, we examined 300 women living in the Kyzylorda region of Kazakhstan, which borders the Aral Sea region. The survey was based on comprehensive clinical-functional and laboratory studies accounting on regional environmental and ecological factors. The survey subject was the area of 2 settlements in Kyzylorda region. In both areas, it was revealed that the examined women of the age group 30-39 years old had increased values of oxidative stress indices comparison with Atasu village of Karaganda region.

Introduction

The Aral Sea, located on the border between Kazakhstan and Uzbekistan, was once the fourth largest inland sea in the world. Since the 1960s, the volume of water has decreased fourteen times [1], [2]. The inflow of water to the Aral Sea came from the rivers the Amu Darya from Tajikistan and the Syr Darya-from Kyrgyzstan. In the early 20th century, the demand for river water to supply local agriculture, primarily the cotton industry, led to the construction of irrigation systems [3]. As a result, a very inefficient water distribution system has emerged, accompanied by excessive use of resources. The subsequent failure to maintain the infrastructure, combined with large emissions of pollutants, had serious consequences for the people living in the areas around the Aral Sea. The literature describes numerous evidence of deterioration in the health of the local population [4]. Respiratory diseases, including tuberculosis (most of which are drug-resistant) and cancer, digestive disorders, anaemia and infectious diseases are common diseases in the region. Problems with the liver, kidneys and eyes can also be associated with toxic dust storms characteristic of the area [5].

All this has led to an unusually high mortality rate among vulnerable groups of the population: the infant mortality rate is 75 for every 1,000 newborns, and the maternal mortality rate is 12 for every 1,000 females [6]. Life in the Aral Sea area has detrimental effects on fertility, both for people who grew up in this area and for adult immigrants [7], [8].

As part of the scientific and technical program "Integrated approaches to managing the health of the population of the Aral Sea region", carried out by staff

of the Karaganda State Medical University in 2014-2016, the influence of environmental factors on the reproductive function at the molecular-cellular level of women living in the populated areas of the Republic of Kazakhstan adjacent to the Aral sea region was studied.

Oxidative stress is the result of excessive formation of reactive oxygen species regarding the degree of antioxidant protection. Physiological oxidative stress exists a priori in the body of both women and men, but with an excess of radical compounds, it can become pathological.

Oxidative stress contributes to the ageing and development of several diseases that affect the fertility of women and men. Endothelial dysfunction, secondary to oxidative stress, contributes to the development of obstetric complications, such as early and repeated pregnancy loss, pre-eclampsia, intrauterine growth retardation and premature birth [9].

Based on the preceding, the purpose of our study was to determine the quantitative content of 8-hydroxy-2-deoxyguanosine, a product of DNA oxidation, in the blood of women living in the Aral Sea Region.

Material and Methods

Women aged 18 to 49 years living in Aralsk and Aiteke-Bi, Kyzylorda region, were surveyed and divided into 3 groups: 18-29 years old, 30-39 years old, and 40-49 years old. Atasu of the Karaganda region was chosen as the region of comparison, as it is characterised by a favourable environmental situation.

The study used the method of reversed-phase variant HPLC with some modifications [13, 14]. Chromatographic methods allow to obtain the most accurate quantitative result, as well as simultaneously and quantitatively determine all available modified bases.

Results

According to the results of the analysis, the concentration of 8-OHdG fluctuated within 18.96–51.93 ng/ml in the studied samples. An elevated level of 8-OHdG was determined in women of the age group of 30-39 years old, living in the town of Aralsk (51.93 ng/ml) and the village Ayteke-Bi (48.69 ng/ml), compared with 8 OHdG 33.51 ng/ml in women living in the village Atasu of the Karaganda region.

Table 1: The content of 8-hydroxy-2'-deoxyguanosine (8-OHdG, ng/ml) in the blood of women living in the settlements of Kyzylorda and Karaganda regions (M ± m)

Region	Age, number surveyed	8-OHdG (ng/ml) M ± m
Kyzylorda region		
Aralsk. n = 150	18-29 years old. n = 50	18.96 ± 0.86
	30-39 years old. n = 50	51.93 ± 0.89*
	40-49 years old. n = 50	32.62 ± 1.25
Ayteke-Bi. n = 150	18-29 years old. n = 50	22.21 ± 0.98
	30-39 years old. n = 50	48.69 ± 0.92*
	40-49 years old. n = 50	32.54 ± 1.12
Karaganda region		
Atasu. n = 225	18-29 years old. n = 75	19.05 ± 0.78
	30-39 years old. n = 75	33.51 ± 0.98
	40-49 years old. n = 75	30.06 ± 0.89

Note the statistical significance of differences between the studied region and the comparison region: $p < 0.05$ *. Comparison of groups was carried out according to the criterion of Kruskal-Wallis

Discussion

8-OH-deoxyguanosine (8-OHdG) is a modified nucleoside produced in a DNA molecule as a result of the action of reactive oxygen species and other damaging factors. Since its discovery in 1983 [10], this compound is determined in various tissues and body fluids: in blood, urine, brain, liver, etc., as a biomarker of oxidative stress [11]. It has been established that in women with the presence of more than 30% of damaged oocytes, the level of intrafollicular 8-oxodeoxyguanosine is significantly increased, which indicates DNA damage due to oxidative stress [9].

Currently, the European Committee is organized, with which research groups from Italy, France, Slovakia, Belgium, Germany, Denmark, Sweden, Poland, Switzerland, and Spain (more than 25 laboratories) collaborate, in which work is underway to standardize DNA disorders, in particular, the level of 8-hydroxy-2'-deoxyguanosine in cellular DNA is normalized at the level of 0.5-5 damage per 106 guanosine bases [12].

As a result of the analysis, increased values of oxidative stress indices were found among women of the age group 30-39 years old living in the town of Aralsk and Ayteke-bi of the Kzylorda region in comparison with Atasu village of Karaganda region.

It should be noted that these settlements are most closely located in the Aral Sea region. This may mean that the residents of these cities are exposed to adverse environmental factors caused by the drying up of the Aral Sea. The presence in the blood of the examined quantities of markers of lipid peroxidation and DNA damage above the norm can be considered as a prognostically unfavourable sign that indicates the development of pathological processes.

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Dental Science

Fracture Localisation of Porcelain Veneers with Different Preparation Designs

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Abstract

BACKGROUND: Porcelain veneers are permanent restorations that combine good aesthetic with functionality by minimal destructive techniques.

AIM: This study aimed to investigate the influence of the preparation designs on the fracture localisation.

MATERIAL AND METHODS: Three preparation designs of porcelain veneers fabricated by a method of laying on a fireproof abutment on maxillary central incisor were examined in this in vitro study-feather preparation, bevel preparation and incisal overlap – palatal chamfer. The samples from all three groups were loaded into a universal test machine-TRITECH WF 10056 until damage occurred on the porcelain veneer. Fracture localisation was classified as an incisal, gingival or combination. Data were analysed with statistical programs: STATISTICA 7.1; SPSS 17.0.

RESULTS: In feather preparation, as a consequence of the mechanical force, the most common is the incisal localisation (66.7%), followed by the combined (33.3%), while the gingival fracture localisation is not registered. In bevel preparation, the most common fracture localisation is combined (53.6%), followed by incisal (35.7%) and subsequent gingival localisation (10.7%). In incisal overlap (palatal chamfer), combined and gingival localisation of the fracture is equally recorded in 14.3% of the samples, while the incisal is the most common localisation and is registered in 72.4%.

CONCLUSION: During the study, a statistically significant dependence was found between the localisation of the occurred changes (incisal, gingival and combination) and the three different types of preparation.

Introduction

The use of porcelain veneers as permanent restorations combines good aesthetic and functionality with minimal destructive techniques (Calamia, 1996) [1]. With a wide range of indications, they present a therapeutic alternative in a large number of patients that only want an aesthetic correction or in other situations, when they are used in combination with another type of treatment.

To gain high aesthetic and functional restorations, the mechanical resistance of porcelain veneers is of great importance. They must survive and resist the mastication forces whose average value in anterior teeth ranges from 20 to 160 N [2]. The obtained mean values of fracture resistance overcome the mastication forces [3]. The longevity and the success of the porcelain veneers depend on better stress distribution [4].

The most common causes of failure described in the literature are: debonding, colour change, fracture, marginal defects in the palate-incisal region and increased percentage of poor marginal adaptation in the palate-incisal region [5].

The fracture resilience of a material is defined as the maximum load that the material can withstand until a fracture occurs. The mechanical strength of the

ceramics is presented by the force that the material uses to oppose plastic deformations such as fractures or bending. The resistance of the material to the deepening of the cracks and breakages constitutes the mechanical hardness of the ceramics [6]. Ceramics is a rigid material with low tensile strength [7]. Another disadvantage of the ceramics is its inability to twist as well as its tendency to break when exposed to minimal deformation [8].

There are several test methods to evaluate the mechanical properties of dental ceramics, such as tensile test, pressure, bending test, hardness and diametric tensile strength test. It is important to note that different testing methods give different values for the force that causes fracture on the restoration, so making their direct comparison is not always possible. Many factors [9] impact the appearance of the fracture or the debonding of the veneer: shape, thickness, length of restoration, microstructural characteristics and elastic modulus of ceramic material, errors in the clinical phases of the work, surface defects or exposed dentin, errors in the technical manufacturing of the restoration, the magnitude and the direction of the force [10].

So, the mechanical resistance of the veneer depends on the type and the shape of the preparation, which altogether can resist the occlusal and lateral forces of the chewing pressure during mastication. Before the preparation, it is very important to decide whether the incisal edge will be reduced. There are four basic preparation designs depending on the involvement of the incisal edge: Window preparation; Feather preparation; Bevel preparation and Incisal overlap – palatal chamfer.

Until now, there are still insufficient data regarding the best type of veneer preparation. Very few studies have focused on the impact of the preparation design on the success and durability of the restoration, and at the same time, there are some very controversial results. Therefore, in this paper, we investigated the influence of the preparation designs on the fracture localisation.

Material and Methods

Three preparation designs of porcelain veneers fabricated by a method of laying on a fireproof abutment on maxillary central incisor were examined in this in vitro study-feather preparation, bevel preparation and incisal overlap – palatal chamfer.

Because natural teeth show many variations due to age and individual structure, time and place of storage of extracted teeth, and to standardise the size of porcelain veneers, we carried out the test on metal abutments.

Materials used for fabricating the metal abutments and the porcelain veneers are given in Table 1 and Table 2.

Table 1: Materials used for fabrication of metal abutments

Material	Manufacturer
Low-viscous floating additional curing vinyl (A)-pouring silicone Doubling	WP Dental
Alloy Wiron 99	Bego

An extracted human maxillary central incisor was selected on which initially we performed feather preparation, carried out according to all the principles of porcelain veneering preparations. On the same tooth, appropriate bevel and incisal overlap (palatal chamfer) preparations were carried out, and proper silicone moulds were made.

Table 2: Materials used for fabrication of porcelain veneers

Material	Manufacturer
Low-viscous floating additional curing vinyl (A)-pouring silicone Doubling	WP Dental
Carbon-free phosphate bonded precision casting investment for crowns Polivest and Polisol	Polident
Sintered into a furnace for firing and pressing dental ceramics - multiagent Press	Densply
Feltspat ceramics Duceram Kiss	Degudent
Self-adhesive resin cement relyxtm U200	3M ESPE

To standardise the experimental samples, the thickness control of the manufactured veneers was performed using a dental calliper, measuring the bucopalatal diameter of the tooth in three points. Also, the length of the veneers was controlled.

Figure 1: Testing the samples with universal test machine (Tritech of 10056) and their fracture localisation

Literature data for fracture resistance of porcelain veneers indicate a lack of standardised and unique methodology of measurement, and it is likely due to the complex geometric shape of the restorations. The samples from all three groups were fixated in a special highly alloyed stainless-steel holder and loaded into a universal test machine (cyclic/stress path triaxial system TRITECH WF

10056) until damage occurred on the experimental sample.

The pressure was directed at an angle of 45 degrees and with a speed of movement from 0.5 mm/min until the moment of the first crack/fracture or damage on the experimental sample. This force was defined as the fracture force of the veneer. The changes that occurred were registered with Olympus microscope SZ2-ILST. Fracture localisation was classified as an incisal, gingival or combination (Picture 1).

Data were analysed with statistical programs: STATISTICA 7.1; SPSS 17.0;

Results

The research includes 90 samples of porcelain veneers specially prepared for the experiment and divided into three groups:

Group I-30 porcelain veneers with feather preparation;

Group II-30 porcelain facets with bevel preparation;

Group III-30 porcelain veneers with incision overlap (palatal chamfer) preparation.

The samples from all three groups are subjected to mechanical strength testing of mechanical resistance, which leads to changes such as peel and fracture. Fracture localisation is classified as an incisal, gingival or combination.

In Group I, the mechanical force causes combined localisation in 33.3% of the samples; incisal localisation is the most common and appears in 66.7%; gingival localisation is not registered at all.

The percentage difference recorded between the combined fracture localisation versus the incisal according to the Difference test is statistically insignificant for $p > 0.05$ ($p = 0.2473$).

In Group II, a gingival fracture localisation is recorded in 10.7% of the samples; followed by an incisal localisation in 35.7%; combined localisation is the most common and appears in 53.6%.

The percentage difference registered according Difference test is statistically significant for $p < 0.05$ between gingival localization versus combined and incisal ($p = 0.0267$, $p = 0.0006$); percentage difference between combined versus incisal localization is statistically insignificant for $p > 0.05$ ($p = 0.1779$).

In group III, combined and gingival localisation of the fracture is equally recorded in 14.3% of the samples, while the incisal is the most common localisation and is registered in 72.4%.

The percentage difference recorded between combined and gingival localisation versus incisal according to Difference test is statistically significant for $p < 0.05$ ($p = 0.0000$) (Table 3).

Table 3: Legend of fracture localisation

Localisation	I Group		II Group		III Group	
	N	%	N	%	N	%
Incisal	4	66.7	10	35.7	21	72.4
Combined	2	33.3	15	53.6	4	14.3
Gingival			3	10.7	4	14.3
Total	6	100.0	28	100.0	29	100.0

During the study, a statistically significant dependence was found between the localisation of the occurred changes (incisal, gingival, and combination) and the three different types of sample preparation (Pearson Chi-square: 11.2217, df = 4, $p = 0.024182$) (Figure 2).

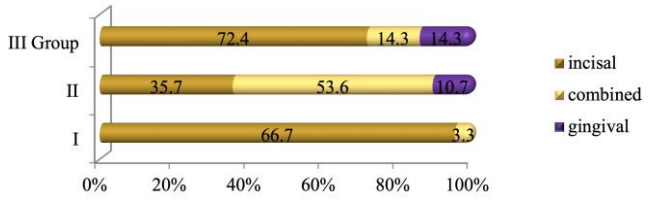

Figure 2: Fracture localisation of porcelain veneers with different preparation designs

Discussion

The use of porcelain veneers as a permanent restoration combines good aesthetics and functionality with minimal destructive techniques (Calamia, 1996) [1]. Different types of preparation designs are described in the literature for this type of restoration. The actuality of the problem, the more frequent use of the veneers in the daily clinical practice requires clarification and a clear definition of the type of preparation that will improve the features of the fixed prosthetics.

Porcelain veneers are biocompatible with stable colour and shape and minimal risk of gingival irritation (Coyne & Wilson [11], Shaini, Shortall & Marquis [12]). No other type of prosthetic therapy would save so much tooth substance such as porcelain veneers which also are characterised by great tolerance towards gingival tissues. In terms of aesthetics, porcelain veneers have all the necessary prerequisites for a highly aesthetic restoration. We can use them to correct not only the colour, but also the form and the position individually or on a group of

teeth.

In our study, porcelain veneers of the central maxillary incision were made. However, the test was performed on stainless-steel metal abutments to standardise the samples. In this way, we also eliminated some of the disadvantages of natural teeth, such as variations in size, shape and individual structure conditioned by the patient's age [13], the place and the time of storing extracted teeth, and the strength of the adhesive bond on the biomechanical behavior of the porcelain veneer [14].

Most studies investigate the mechanical resistance of porcelain veneers cemented to natural teeth. Alghazzawi T. et al., [15] used resin abutments in their in vitro study instead of natural teeth. The elastic modulus of composite abutments (E = 12 GPa) is close to that of dentine (E = 18.6 GPa) and is significantly different from the metal (E = 200 GPa). But in those cases, during the test of the mechanical resistance of the porcelain veneers, a fracture of abutments appeared. According to Hui et al., [16], the strength values that cause the porcelain veneer fracture are higher if the test is done on natural teeth rather than on the composite abutments [17], [18], [19], [20], [21], [22], [23].

To eliminate this factor, we worked on metal abutments, which reduced the possibility of a fracture to a minimum. In this case, the adhesive bond with the composite cement [3] cannot be achieved.

The fracture resistance of one restoration is influenced by the material and the technique of production. In the literature, different values of the force that causes fracture of the porcelain veneer depending on the type of ceramics used, are found. At the same time, there are conflicting results on whether one preparation design is superior to others when it comes to the fracture resistance of these gracile fixed prosthetic constructions.

According to Hui et al., (1991) [16], the stress concentration is incisal in the untreated teeth and window preparation. In cases where the incisal edge is involved, stress is distributed through the tooth.

Most in vitro tests for crown fracture [24] found that the fracture occurred at the point of pressure [25] on the test machine. It was concluded that occlusal force causes a fracture [26], [27] in porcelain crowns at the contact spot and provided the basis for many in vitro tests [28] and analyses [29], [30]. However, the type and localisation of the occurred change in clinical function [31] does not always coincide with those obtained in vitro findings [25], [32], [33].

Analysis of the type of change that occurs during the fracture of porcelain veneers showed different types of failure for different ceramic materials. In 50%, a cervical fracture of the tooth itself appeared, without the porcelain veneer being damaged. The type of change in YPSC (partially stabilised zirconium dioxide) in 100% of the samples is a cervical fracture of the tooth [34]. The cause may be the increased rigidity of the tooth and the material, causing a concentration of stress in the cervical part. Cervical fracture of incisors at static load is described by other authors [19], [20]. Zirconium veneers usually remained intact after the examination because they were able to resist the stress that is transferred from the tooth to the restoration. If strength exceeds the endurance limit of the tooth tissue, a tooth fracture occurs. Several studies have confirmed that stress is concentrated on the adhesive surface between composite cement [35] and enamel [36]. The shear strength causes movement of the veneer and effect of compressive stress in the weakest parts (incisal or gingival) [37]. This causes microcracks that propagate and affect the occurrence of fracture or debonding of porcelain veneers [38].

In conclusion, during the study, a statistically significant dependence was found between the localization of the occurred changes (incisal, gingival and combination) and the three different types of preparation.

In feather preparation, as a consequence of the mechanical force, the most common is the incisal localization (66.7%), followed by the combined (33.3%), while the gingival fracture localization is not registered.

In bevel preparation, the most common fracture localization is combined (53.6%), followed by incisal (35.7%) and subsequent gingival localization (10.7%).

In incisal overlap (palatal chamfer), combined and gingival localization of the fracture is equally recorded in 14.3% of the samples, while the incisal is the most common localization and is registered in 72.4%.

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Dental Science

Alveolar Bone Osteoclast Profile in the Periodontitis Wistar Rats Model with the Snail Slime (Achatina Fulica) Application

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Abstract

BACKGROUND: Bone damage is a result of periodontal disease that occurs due to changes in osteoclast and osteoblast activity in response to local inflammation. The bacteria Aggregatibacter actinomycetemcomitans produces Lipopolysaccharide (LPS), which can increase osteoclast activity.

AIM: This study aimed to analyse the decrease in alveolar bone osteoclasts in periodontitis rats' model with the application of snail slime.

METHODS: Wistar rats (27) with periodontitis divided into three groups, namely the control group (debridement), P1 group (debridement and application of oral snail slime) 300 Mg/Kg Body weight, P2 group (debridement, application of topical snail slime) 0.1 Mg. Osteoclast profile analysis was carried out by HE staining procedure to determine the histological feature of osteoclasts. The statistical significance was determined using the Shapiro-Wilk Test, One Way ANOVA, and Post Hoc test ($p < 0.05$).

RESULTS: Osteoclast profile in rats with periodontitis applied with snail slime significantly decreased the number of osteoclasts with both oral and topical administration, there were significant differences in the number of osteoclasts between groups (one way ANOVA, $p < 0.05$) and there were no significant differences between groups P1 and P2 (Post Hoc, $p > 0.05$).

CONCLUSION: In this study, there was a decrease in the number of osteoclasts which were slipped by snail slime in Wistar rats with periodontitis; this indicates a periodontitis healing process.

Introduction

Periodontitis is an inflammatory disease of the supporting tissues of the teeth caused by certain groups of microorganisms that usually originate from dental plaque which can result in the progressive destruction of periodontal connective tissue and alveolar bone [1]. Bone damage is a result of periodontal disease that occurs due to changes in osteoclast and osteoblast activity in response to local inflammation. Osteoclasts play a role in bone resorption with excessive expression of RANkL produced through activation of T lymphocytes and B lymphocytes in response to secretion of inflammatory cytokines such as TNFα and IL-β [2]. Inflammatory cytokines can also play a role in inhibiting bone repair by osteoblasts [3]. The bacteria *Aggregatibacter actinomycetemcomitans* produces a lipopolysaccharide (LPS) which can increase osteoclast activity. Chronic inflammatory reactions stimulated by bacteria and their products around periapical can cause bone damage [4].

Bone destruction is mediated by the host immune and inflammatory response against the microbial activity. Bone resorption is followed by

activation of cells that resorb bone called osteoclasts. Osteoclasts are bone cells that affect the degenerative process. Osteoclasts and osteoblasts regulate dynamic balance in the bone remodelling process [5]. An imbalance of bone remodelling is caused by more osteoclast cells than osteoblasts resulting in the process of bone resorption. Bone resorption is affected by osteoclast activating factors, including prostaglandins, endotoxin bacteria, and complement activator products consisting of cytokines, IL-1, IL-1, TNF-, TNF-, IL-6, and IL-11 [6]. In the process of osteoclast cell formation (osteoclastogenesis), an osteoclast bond differentiation factor occurs with its receptor. This increase in the osteoclastogenesis process results in increased bone resorption [7]. One of the natural ingredients for regenerating alveolar bone is snail slime. Snail slime reacts positively to testing the protein content that plays a role in cell regeneration and growth, including amino acids and enzymes. Protein can function and play a role in growth, defence, bodily functions and as a protective function, which is a substitute for damaged tissue and cells. Based on the purpose of this protein, it is estimated that the animal protein content in snail slime has a high biological value, namely in healing and inhibiting the inflammatory process [8].

Snail slime (Achatina fulica) has an active ingredient which plays a role in the process of wound healing. One of the active compounds found in snail slime is heparin sulfate, which functions as a factor that influences cell division. Besides, this substance also features as an aid to the attachment of proteins that function as signals for the cell division receptor stimulus on the cell membrane [9]. Addition of the concentration of heparin sulfate absorbed by the tissue will increase fibroblast proliferation. Fibroblast Growth Factor (FGF) initiates the cell proliferation process in injured tissue.

Another active compound is achasin isolate; the chemicals contained in snail slime give a positive reaction to the testing of protein content that plays a role in cell regeneration and growth. Achasin is a protein contained in snail slime, has essential biological functions, the leading organic compounds that makeup bone are proteins, and the main protein making up bone is type I collagen which is 90-95% of the leading organic matter while the rest is a homogeneous medium called basic substance [10]. Achasin isolates function as antibacterial and analgesic while Calcium plays a role in hemostatic. Calcium in periodontal tissues is found in the extracellular and intracellular matrix. In the extracellular matrix, it functions as signalling between cells and a source of calcium for hard teeth and periodontal tissues. Calcium in the intracellular matrix acts as a mediator for the actin preparation of fibroblast tissue and soft tissue [11].

Therefore, this study aimed to analyse the profile of the number of alveolar bone osteoclasts in the rats with periodontitis model with the application of snail slime.

Material and Methods

Materials

This study used 27 male Wistar rats with a weight of 200-250 grams, aged 2-3 months, while for the treatment the slime taken from as many as 50 snails were used. Snail shells were disinfected using 70% alcohol to prevent bacterial contamination. Snail meat was stimulated using the tip of the pipette to remove its slime. Then the slime which collected in the bottle was added to ethanol and then centrifuged in the Chemical Analytical Laboratory of Udayana University, Denpasar, Bali, Indonesia. Snails slime were collected from the Banjar Umaanyar garden, Nyalian village, and Banjar Angkan Klungkung district, Bali. This study was an actual experimental study with a randomised post-test only control group design conducted at the Analytical and Veterinary Laboratory of Udayana University, Denpasar, Bali.

Preparation of research subjects

Periodontitis was induced in the study sample by attaching silk ligature to the incisive mandibular cervical, then *Aggregatibacter actinomycetemcomitas* bacteria were infiltrated in the gingival sulcus as much as 2.42 x 10^8 / ml CFU equivalent to 0.5 Mac Farland one time with a dose of 0.22 ml, topical anaesthesia was previously applied (Figure 1, 2, and 3). On the third day, the rats experienced acute inflammation of the gingiva; the rats were sick and did not want to eat. On the eleventh day after the infiltration of the *Aggregatibacter actinomycetemcomitans* bacteria, the mouse gingiva appeared bluish and swelled with the appearance of gingival pocket, third-degree tooth shake, and central diastema, the gingiva also became easily bleed, indicating the occurrence of periodontitis. Rats with periodontitis were divided into three groups. The Control Group (K) only carried out debridement with distilled water, while P1 group given debridement and administration of oral snail slime of 300 Mg/Kg Body Weight, and P2 group given debridement and administration of topical snail slime of 0.1 Mg.

Figure 1: A) Preparing silk Ligature; B) Infiltration with bacteria Aa; C) Periodontitis

Observations

Observations were carried out after treatment, on day 7, day 14, and day 21 after exposure. Considerations include a clinical examination and tissue examination taken from the alveolar bone. The tissue was preserved in 10% formalin buffer, decalcified with EDTA, and then stained with Hematoxylin-Eosin. Analyses were carried out under a 400x magnification of light microscope and seen from five fields of view.

Administration of snail slime

In group P1 the administration of snail slime was given once a day for seven days at a dose of 300 mg/kg body weight, P2 group (debridement and administration of topical snail slime), was given three times 0.1 mg for seven days. The first time is at 7:00 a.m., the second is at 13:00 a.m. and the third is at 6:00 p.m. The examination was carried out after the treatment, on the 7th day, 14th day, and 21st day. The experimental animals were euthanised using ether anaesthesia; then the jaw was cut in the mandibular incisor region. The sample was prepared using 10% formalin for 24 hours, decalcified using 14% EDTA, for 30 days, dehydrated, cleared, and embedded in paraffin, then cut in the longitudinal direction with a thickness of 5 microns for the Hematoxylin-Eosin (HE) painting procedure [12]. Staining procedures were performed using HE to determine the histological feature of osteoclasts. Analysis of the number of osteoclasts was examined under an electric microscope with 400 x magnification.

Effects of giving snail slime

There was a decrease in the number of osteoclasts in rats with periodontitis given snail slime both orally and topically.

Results

Analysis of the number of osteoclasts can be seen in histological features (Figure 2, 3, and 4).

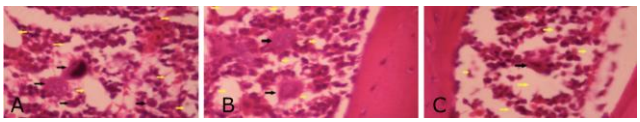

Figure 2: A), B), and C) Alveolar bone histology

Based on the clinical examination, pocket depth is reduced, 1st-degree tooth shake, there was no bleeding, no central diastema, healthy gingiva in the subject given snail slime.

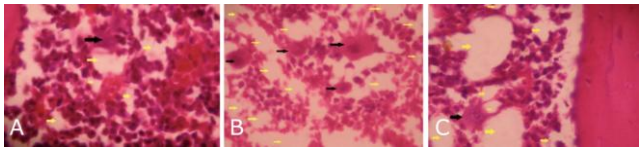

Figure 3: A), B), and C) Alveolar bone histology; Day 14 (K) Day 14 (P1) Day 14 (P2)

Figure 5 shows that the number of osteoclasts in the K group was the highest, namely on the 14th day amounted to 4.33, then the P1 group on the seventh day showed two cells and on the P2 group showed one cell.

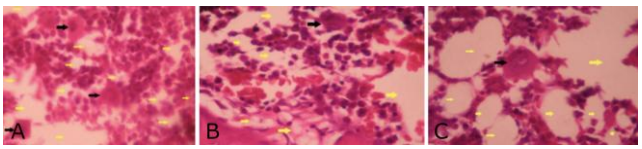

Figure 4: A), B), and C) Alveolar bone histology; Day 21 (K) Day 21 (P1) Day 21 (P2)

The normality test using Shapiro-Wilk test obtained a value of p > 0.05 indicating that the data on the number of osteoclasts was typically distributed, followed by the One-Way ANOVA test showing that the p-value < 0.05 means that there are statistically significant osteoclast numbers in all groups.

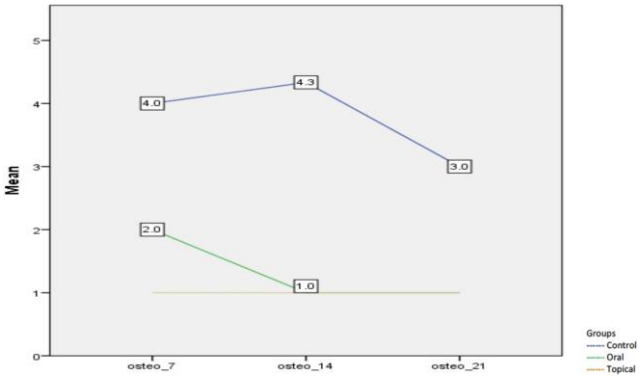

Figure 5: Diagram of the average number of osteoclasts

Table 1 shows that in the control group on day 14 the highest number of osteoclasts was 4.33 cells that differed significantly from groups P1 and P2, while the number of osteoclasts in the P2 group was one cell from the seventh day.

Table 1: Comparison of the number of osteoclasts based on the research group

Independent Variable	Day	Groups			P value
		Control	Oral	Topical	
Number of Osteoclast	7	4.00 ± 1.1	2.00 ± 0.00	1.00 ± 0.00	0.002
	14	4.33 ± 0.57	1.00 ± 0.00	1.00 ± 0.00	0.001
	21	3.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	NA

We used the post-hoc test to see if the differences in osteoclasts between treatment groups were statistically significant on day 7, 14, and 21 (Tables 2 and 3).

Table 2: Post Hoc Test Results Between Groups on the 7th Day

Independent Variable	Groups	Groups	Mean Difference	*P value*
Number of Osteoclasts (day-7)	K	P_1	2.000	0.005*
		P_2	3.000	0.001*
	P_1	K	-2.000	0.005*
		P_2	1.000	0.078
	P_2	K	-3.000	0.001*
		P_1	-1.000	NA

Table 2 shows that the difference between the seventh-day groups on osteoclast cells examination was significantly different indicated by the value of $p < 0.05$ except that the differences in groups P1 and P2 were not significant.

Table 3: Post Hoc Test Results Between Groups on the 14th Day

Independent Variable	Groups	Groups	Mean Difference	*P value*
Number of Osteoclast (day-14)	K	P_1	3.333*	0.000
		P_2	3.333*	0.000
	P_1	K	-3.333*	0.000
		P_2	0.000	1.000
	P_2	K	-3.333*	0.000
		P_1	0.000	1.000

Table 3 shows that the difference between groups on day-14 of the examination of osteoclasts was significantly different indicated by the value of $p < 0.05$ except the difference between group P1 and P2 was not significant, with a value of $p > 0.05$.

Table 4: Results on the 21st Day Post Hoc Test Between Groups

Independent Variable	Groups	Groups	Mean Difference	*P value*
Number of Osteoclast (day- 21)	K	P_1	3.333*	0.029
		P_2	3.333*	0.029
	P_1	K	-3.333*	0.029
		P_2	0.000	NA
	P_2	K	-3.333*	0.029
		P_1	-3.333	0.029

Table 4 shows that the difference between groups on day-21 of the examination of osteoclasts was significantly different indicated by the value of $p < 0.05$ except the difference in groups P1 and P2 was not significant, the p-value is not available.

Discussion

The periodontal tissue is the tissue around the teeth, which functions as a support for the teeth, consisting of gingival tissue, cementum, periodontal ligament, and alveolar bone. Periodontitis is an inflammation of the supporting tissues of the teeth, which causes progressive damage to the periodontal ligament and alveolar bone [13]. Alveolar bone loss is a hallmark of periodontitis progression, and its prevention is an essential clinical challenge in periodontal disease treatment. Bone destruction is mediated by the host immune and inflammatory response to the microbial challenge. However, the mechanisms by which the local immune response against periodontopathic bacteria disturbs the homeostatic balance of bone formation and resorption in favour of bone loss remain to be investigated [14].

The results showed that there were significant differences in the number of osteoclasts between the control groups, with groups P1 and P2 performed by the One-Way ANOVA test, so that it can be said that snail slime affects the reduction of osteoclast cells in bacterial-induced Wistar rats alveolar bone by *Aggregatibacter actinomycetemcomitans*. Achasin isolate, glyconyugugate, glycosaminoglycans, calcium, can trigger angiogenesis. Vascular endothelial growth factor inhibits and decreases mitogen activity of fibroblast growth factor. The Vascular Endothelial Growth Factor (VEGF) is a cytokine involved in Sing angiogenesis, inducing new blood vessel formation in the periodontium by VEGF activation. VEGF is known to be an angiogenesis factor produced by various types of cells, including fibroblasts, smooth muscle cells, hypertrophic chondrocytes, osteoblasts, and others. VEGF immunoreactivity is found in vascular endothelial cells, in fibroblasts adjacent to hyaline tissue, a necrotic area in the pressure area of osteoblasts, osteoclasts, and mononuclear cells [15]. VEGF can modulate recruitment, differentiation, and activation of osteoclast precursors, thereby increasing bone resorption. VEGF indirectly causes bone resorption because it promotes angiogenesis in vitro, which allows new capillaries to help recruit osteoclasts near the bone surface to the resorption area; VEGF can induce neovascularisation [16].

In the control group, the number of osteoclasts was higher than that of the treatment group. This was caused by the bacteria *Actinobacilus actinomycetemcomitans* capable of producing an increase in the number of osteoclasts. The bacteria released their products in the form of Gram-negative bacterial lipopolysaccharide (LPS) on the cell wall. LPS has strong potential as an inflammatory stimulator if injected in vivo because LPS can penetrate the periradicular tissues and act as endotoxin in its host organisms which causes inflammation in periradicular and continues with bone damage [17].

IL-1 is known to stimulate fibroblasts to produce collagenase. IL-1 is known to have the most potential to induce bone demineralisation and is synergistic with tumour necrosis factor α in stimulating bone resorption, especially in altering the connective tissue matrix. Macrophages produce IL-1 as the primary mediator of tissue destruction in periodontal disease [18]. IL-6 cytokines can stimulate bone resorption, in this case, the influence of IL-6 cytokines

on RANKL expression (ligand of receptor activator of NF-jB), RANK (receptor activator of NF-κB), and OPG (osteoprotegerin). IL-6 type cytokine families are cytokines consisting of IL-6, IL-11, leukaemia inhibitory factor (LIF), oncostatin M (OSM), ciliary neurotrophic factor, cardiotrophin-1, and neutrophil-1 / B cell stimulatory factors 3. These agents use the same receptor subunit, gp130, as a signal [19].

There was a higher number of osteoclasts in the seventh-day P1 group than in P2 on the seventh day due to topical snail slime administration which could improve bioavailability and drug efficacy by avoiding the first-pass elimination in the liver. The Pocket acts as a natural reservoir and provides easy access to drug administration day 14, but after days 14 and 21 there is no difference because it has undergone cell regeneration and osteoclast, differentiation has decreased, and bone density has increased [20].

In conclusion, snail slime (Achatina fulica) affects decreasing the number of osteoclasts in the alveolar bone of Wistar rats induced by *Aggregatibacter aactinomycetemcomitans* bacteria, either given orally 300 Mg/Kg Body Weight or topically using snail slime 0.1 Mg.

Acknowledgement

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Author Contributions

All the authors have made substantial contributions to the work reported in the manuscript.

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Evaluation and Comparison of Efficacy of Gluma® and D/Sense® Desensitizer in the Treatment of Root Sensitivity Induced by Non-Surgical Periodontal Therapy

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Keywords: D/sense®; Gluma®; Root sensitivity; Scaling and root planing

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Abstract

BACKGROUND: Dentinal hypersensitivity is one of the most common sequels of non-surgical periodontal therapy. Resulted discomfort may restrain patients from oral hygiene maintenance, thus affects the long-term success of periodontal therapy. So, it becomes a prime concern of the clinician to manage the post-operative hypersensitivity.

AIM: This clinical investigation aimed to evaluate and compare the efficacy of D/Sense® and Gluma® in preventing post-operative sensitivity after non-surgical periodontal therapy.

MATERIAL AND METHODS: The present randomised, double-blind, split-mouth study was conducted on forty-five (22 male, 23 female) systemically healthy patients, with the mean age of 40 ± 17.5 years. Visual Analogue scale was used to evaluate root sensitivity after application of tactile and cold stimuli at baseline, 1, 2, 4 and 6 weeks after scaling and root planing. After scaling and root planning, the sites were randomly divided into different groups for the application of desensitising agents. Collected data were analysed by using, analysis of variance (ANOVA) for inter-group and paired t-test for intra-group comparisons.

RESULTS: No adverse or side effects were reported by any of the patients throughout the study period. Gluma® showed a statistically significant reduction in the VAS score for root sensitivity as compared to D/Sense®, at 1, 2- and 4-weeks follow-up period ($p < 0.05$). Whereas, at 6th-week follow-up, both the solution showed almost similar score for root hypersensitivity. Intragroup comparison for D/Sense® revealed a significant difference in scores from baseline to all intervals ($p < 0.05$), except baseline to 6 weeks ($p > 0.05$). Whereas Gluma® showed a significant difference in scores from baseline to 2nd-week follow-up ($p < 0.05$).

CONCLUSION: The result of the present investigation revealed that application of Gluma® resulted in better control on iatrogenic root hypersensitivity as compared to the D/Sense® during the initial follow-up period.

Introduction

The successful treatment of the periodontal disease depends on the effective removal of bacterial deposits from the tooth surface by performing non-surgical periodontal therapy. Scaling and root planning is considered as the mainstay of non-surgical periodontal therapy. Several undesirable side effects like gingival recession and exposure of root dentin due to the removal of cementum may result from scaling and root planning [1], [2]. An enormous amount of dentinal tubules may get exposed to the oral environment, because of which patient may experience increased sensitivity of the root surface [3].

Dentin Hypersensitivity (DH), is characterised by short sharp pain arising from exposed dentin in response to stimuli typically thermal, evaporative, tactile, osmotic or chemical and which cannot be ascribed to any other form of dental defect or pathology [4]. According to the Canadian Advisory Board on Dentin Hypersensitivity, the term “pathology” has been replaced with the more suitable term “disease”. Currently, the term “Root Sensitivity” (RS) is used to describe the sensitivity originating from periodontal pathology and its management [5].

The most widely accepted Hydrodynamic theory for tooth hypersensitivity was presented by Brainstorm. According to this theory, an alteration in tubular fluid movement in response to stimuli results in depolarisation of the nerves endings [6]. A direct relationship is present between the amount of fluid flow in dentinal tubules and the amount of charge produced in pulpal nerve fibres. Researchers have found that the outward flow of fluid results in more discomfort as compared to the inward movement of the fluid. Depending on this concept, they concluded that heat stimulus produces less discomfort while the cold stimulus produces more discomfort [7].

Tooth hypersensitivity can be treated by two approaches; first by occluding the dentinal tubules and second by reducing nerve excitability or by a combination of both the mechanisms. There are a large variety of products available in the market for the treatment of dentinal hypersensitivity such as; sodium fluoride, potassium nitrate, strontium chloride, stannous fluoride, cavity varnishes, lasers, sodium fluoride, stannous fluoride, adhesive resins, potassium nitrate, and calcium phosphate [8]. According to the requirements laid down by Grossman, the desensitising agents should be; non-irritant to the pulpal tissue; painless on use; easy to apply, rapid mechanism of action; long term effect and without staining effects [9].

Gluma® (Heraeus Kulzer GmbH, Hanau, Germany) is a commercially available desensitising agent consists of glutaraldehyde and hydroxyethyl methacrylate (HEMA). Glutaraldehyde occludes dentinal tubules by coagulation of amino acids and proteins present in the dentin, whereas HEMA can work by occluding the dentinal tubules [10]. HEMA penetrate deep into dentinal tubules because of its hydrophilic nature. Whereas the blocking effect of HEMA is reversible and the dentinal tubules become exposed after some time [10]. D/Sense Crystal® (Centrix, Inc. Shelton, CT) is also a commercially available one-step, dentin desensitiser. The desensitising agent is a combination of potassium binoxalate and nitric acid that reacts with the dentin smear layer to form minute crystals of calcium oxalate and potassium nitrate [11]. These byproducts from a 3-micron thick acid-resistant layer that seal off the dentinal tubules [11]. D/Sense Crystal shows best results on the clean and dry dentinal surface, but it can be used on the moist Dentin.

A large number of desensitising agents have been recommended in the recent past for the treatment of tooth hypersensitivity. Root sensitivity is a common phenomenon after scaling and root planning; therefore, a method to avoid this problem would be in favour of the patients. Hence, the present study aims to evaluate the efficacy of D/Sense® and Gluma® in preventing post-operative root sensitivity after non-surgical periodontal therapy.

Material and Methods

This study was designed as a double-blind, split mouth, randomised clinical study for a time duration of six weeks. The study was evaluated and approved by the institutional ethical review board of King Khalid University, Abha, KSA. Forty-five systemically healthy patients of both sex, age ranging from 18 to 58 years, were randomly selected from the pool of the patients visited the dental hospital. Patients reported with chronic periodontitis exhibiting pocket depth ≤ 5mm or attachment loss 3-4 mm, indicated for scaling and root planning was included in the present study. The patients were excluded from the study if they present; current hypersensitivity, medication for systemic illness, pregnancy and breastfeeding, gastrointestinal disturbances, orthodontic appliances, faulty restored, grossly carious teeth and history of periodontal treatment within last 6 months. Written informed consent was obtained from the patients after a thorough explanation of study procedures and protocol.

Patients full filling the inclusion criteria were selected for the study after recording a comprehensive case history. On the initial visit, patients were explained about the oral hygiene procedures and only scaling, and polishing was performed. Patients were recalled after one week for complete root planing. Baseline values for root sensitivity tests were recorded just before the root planing by a single examiner. Similar tests were recorded at 1, 2, 4 and 6 weeks follow-up intervals after root planning. Root sensitivity was evaluated by the application of tactile and cold stimulus on the buccal surface of each tooth in the jaw. The tactile test was performed by passing number 23 explorer perpendicular to the long axis of the tooth on the affected area. The final score was recorded after reassurance by repeating the test three times. The cold water test was performed after complete isolation of the area of interest, and then fresh ice water was applied to the exposed root surface for 3 seconds. The final score was recorded after reassurance by repeating the test three times.

McGill Visual Analog Scale (VAS) was used to measure the root sensitivity using the score from 1 to 10. The VAS comprises of a horizontal line which is of 10 cm in length (one score for each cm). Score 0 signifies complete painlessness (extreme left end) and Score 10 represents the worst pain experienced (extreme right end). Subject indicates the degree of pain perception by choosing the digits on the ruler after the tooth being stimulated by the different stimulus explained above [12]. The VAS is supposed to be a reliable tool for grading the response because, in one patient, it is measured multiple times. Throughout the study period, there should be a minimum of 5 minutes gap between the applications of two different stimuli. In any point of time, when the

pain becomes unbearable, the stimulus was withdrawn immediately.

All the patients were recalled after one week of root planning to evaluate the degree of root sensitivity. After recording the VAS score, the jaw was randomly split into two quadrants for the application of Gluma® or D/Sense®. The solutions were applied by the second investigator to the designated sites as per the instruction provided by the manufacturer by using a small brush applicator. The first investigator was unaware of the site and application of the solution, to maintain the double blindness. Patients were recalled as per the schedule for recording the VAS score to evaluate the degree of root sensitivity. After completion of all VAS tests scores, data were assessed as mean and standard deviation of VAS. Intragroup comparison in sensitivity levels at different recall visits was done by using a Paired t-test. Mean scores were related between groups at baseline, 1, 2, 4 and 6 weeks by applying Analysis of Variance (ANOVA) at the significance level of 0.05. Statistical package for social sciences (SPSS) Version 12 was used for the statistical analysis.

Results

A total of 45 patients (22 male, 23 female), with the mean age 40 ± 17.5 years, completed the follow-up of 6 weeks without any dropouts (Table 1). No adverse or side effects were reported by any of the patients during the study period.

Table 1: Subjects distribution according to gender and age

variables	Number	Percentage
Sex		
Male	22	48.5%
Female	23	51.5%
Age		
≤ 20	2	4.5%
21-30	12	26.5%
31-40	14	31.0%
41-50	13	28.5%
51-60	4	9.0%

Table 2 displays the intergroup comparison between Gluma® and D/Sense® for root sensitivity scores after applying a tactile stimulus.

Table: 2 Comparison of sensitivity scores for Tactile Stimulus between Gluma® and D/Sense®

Tactile test	Gluma® (Mean ± SD)	D/Sense® (Mean ± SD)	Significance
Baseline	0.84 ± 0.22	0.85 ± 0.20	P > 0.05
1st week	1.21 ± 0.34	1.96 ± 0.51	P < 0.05*
2nd week	1.00 ± 0.33	1.51 ± 0.56	P < 0.05*
4th week	0.81 ± 0.35	1.02 ± 0.38	P < 0.05*
6th week	0.73 ± 0.10	0.81 ± 0.14	P > 0.05

At baseline, the scores were similar for both the groups without any significant difference (p > 0.05). Whereas at follow-up visits of 1, 2 and 4 weeks, Gluma® showed marked reduction in the VAS score of root sensitivity as compared to D/Sense® which was found to be statistically significant (p < 0.05). At the end of 6th-week follow-up, both the solution showed an almost similar effect of root sensitivity. Intragroup comparison of tactile stimulus for D/Sense® revealed a significant difference in scores from baseline to all intervals (p < 0.05) except baseline to 6 weeks (p > 0.05) whereas Gluma® showed a significant difference in scores from baseline to 1 and 2 weeks, while scores from a baseline to 4 and 6 weeks were non-significant (p > 0.05) (Table 3).

Table: 3 Intragroup comparisons for Tactile Stimulus for Gluma® and D/Sense®

	Gluma®		D/Sense®	
	Mean (SD)	*P* value	Mean (SD)	*P* value
Baseline-1st week	0.37 ± 0.21	P < 0.05*	1.11 ± 0.34	P < 0.05*
Baseline-2nd week	0.16 ± 0.17	P < 0.05*	0.66 ± 0.26	P < 0.05*
Baseline-4th week	-0.03 ± 0.19	P > 0.05	0.17 ± 0.21	P < 0.05*
Baseline-6th week	-0.11 ± 0.13	P > 0.05	-0.04 ± 0.14	P > 0.05

Intergroup Comparison between Gluma® and D/Sense® for root sensitivity scores after applying cold stimulus is presented in Table 4.

Table: 4 Comparison of sensitivity scores for cold Stimulus between Gluma® and D/Sense®

Tactile test	Gluma® (Mean ± SD)	D/Sense® (Mean ± SD)	Significance
Baseline	2.35 ± 0.41	2.37 ± 0.47	P > 0.05
1st week	3.71 ± 0.42	5.12 ± 0.81	P < 0.05*
2nd week	3.18 ± 0.36	5.35 ± 0.75	P < 0.05*
4th week	2.49 ± 0.22	3.89 ± 0.41	P < 0.05*
6th week	2.20 ± 0.21	2.57 ± 0.17	P > 0.05

At baseline, the sensitivity scores were almost similar for both the groups without any significant difference (p > 0.05). Whereas Gluma® showed a marked reduction in the VAS score for root sensitivity when compared to D/Sense® at 1, 2 and 4-week follow-up visits, differences in scores were statistically significant (p < 0.05). However, both the solution showed an almost similar effect on root sensitivity scores at the end of 6th-week follow-up. Intragroup comparison of cold stimulus for D/Sense® revealed a significant difference in scores from baseline to all intervals (p < 0.05) except baseline to 6 weeks (p > 0.05). Whereas, Gluma® showed a significant difference in scores from baseline to 2 and 4 weeks, while scores from a baseline to 4 and 6 weeks were non-significant (p > 0.05) (Table 5).

Table: 5 Intragroup comparisons for Cold Stimulus for Gluma® and D/Sense®

	Gluma®		D/Sense®	
	Mean ± SD	*P* value	Mean ± SD	*P* value
Baseline-1st week	1.36 ± 0.32	P < 0.05*	2.84 ± 0.48	P < 0.05*
Baseline-2nd week	0.83 ± 0.23	P < 0.05*	2.98 ± 0.35	P < 0.05*
Baseline-4th week	0.14 ± 0.17	P > 0.05	1.52 ± 0.29	P < 0.05*
Baseline-6th week	-0.15 ± 0.14	P > 0.05	0.20 ± 0.11	P > 0.05

Discussion

The effective treatment of periodontitis can be accomplished by mechanical debridement and

through oral hygiene maintenance by the patients. Iatrogenic root Dentin hypersensitivity is a consequence of non-surgical periodontal therapy (scaling and root planning). Dentinal hypersensitivity is a commonly encountered problem in the clinics, where patients complain of significant discomfort on eating hot, cold, acidic or sweet fluids and foodstuff [13]. Usually, patients tend to avoid brushing in hypersensitive areas due to discomfort. This may lead to the accumulation of more plaque and food debris on exposed surfaces, which often results in increased root sensitivity and the vicious cycle continues. Therefore, hypersensitivity resulting from periodontal therapy may affect oral hygiene measures and thus may affect the success of periodontal therapy [14]. So, it becomes essential to manage the post-operative hypersensitivity for the patient's benefit. In the present clinical study, a comparative evaluation was done between D/Sense® and Gluma® in preventing post-operative root sensitivity after non-surgical periodontal therapy.

According to the most accepted hydrodynamic theory, rapid flow of the fluid in the dentinal tubules distorts the pulp tissue at the pulp Dentin border. Any stimulus that causes fluid movement in the dentinal tubules gives rise to activation of the pulpal fibres, based on the above fact it can be explained that why chemical, mechanical or thermal stimulus produces only a painful response [15]. Scanning electron microscopic examination revealed wide open dentinal tubules in case of hypersensitive Dentin, and the count for open tubules was eight times higher in sensitive Dentin as compared to non-sensitive Dentin [16]. Also, the diameter of the dentinal tubules was found to be twice insensitive as compared to non-sensitive Dentin [17].

The treatment of dentinal sensitivity is made generally by sealing dentinal tubules through chemical or physical agents. However, other agents can block the nerve conductivity in the dental pulp by reducing the excitability of the nerves [18]. In some patients, more invasive treatment such as restoration, pulp extirpation, periodontal grafts and even extraction of the offending tooth may be the treatment of choice. Soft tissue grafts and guided tissue regeneration (GTR) procedures have also been advocated with the predictable outcome for the management of with Dentin hypersensitivity in gingival recession cases [19].

The present clinical study design is a randomised, double-blind and split-mouth design. This type of study design is measured as a standard for evaluating the hypotheses of no differences among management procedures. In the present clinical study, D/Sense®, and Gluma® were used to treat the root sensitivity after non-surgical periodontal therapy. D/Sense® has a dual mechanism of action. First it acts by precipitating the insoluble salts which occlude the dentinal tubules mechanically, and second, the soluble potassium penetrates deep into the dentinal tubules and exhibits a depolarising action on the nerve fibres. In-vitro studies conducted by Kim in 1986 and Al-Tayeb 2008 revealed that active potassium ion could reach the nerve endings at the Dentin pulpal junction by passing through the dentinal tubules [11], [20]. In a recent in-vitro scanning electron microscopy (SEM) study, five different dentin desensitisers were evaluated for dentinal tubule occlusion and dentin permeability. The result showed that D/Sense® crystal was significantly effective in reducing dentin permeability and tubule occlusion [21].

The result of the present study, regarding reduction in root sensitivity, is by the previous studies conducted by Al-Tayeb in 2008 and Kishore et al., in 2002, where D/Sense had resulted in a significant reduction in root sensitivity after non-surgical periodontal therapy [11], [22]. Similar results were reported by Crispin in his clinical study and concluded that D/Sense Crystal is effective in the management of the dentinal hypersensitivity [23].

The use of resin for the treatment of dentin hypersensitivity was proposed by Dayton et al., [24] and later, its efficacy was evaluated and confirmed in several clinical trials. Glutaraldehyde is an active desensitising compound present in Gluma®, which reacts and coagulates the serum albumin in the dentin fluid. The result of the present study showed that Gluma® was effective in reducing the root sensitivity after scaling and root planing through dentinal tubule occlusion. The mechanism of action for Gluma® was confirmed by the results of in-vitro SEM studies conducted by Yilmaz et al., [24] and Pereira et al., [25]. They discovered form the SEM analysis that, the active ingredients of Gluma® were effective in occluding the dentinal tubules. In the recent past, various clinical studies have confirmed the efficacy of Gluma® in reducing the root sensitivity after dental procedures [26], [27], which is by the result of the present study. De Assis et al., 2006 in their clinical study evaluated the efficacy of Gluma® desensitizer on dentin hypersensitivity in periodontally treated patients, and they concluded that Gluma® had no effect on reducing the sensitivity of teeth in periodontally treated (scaling and root planning) patients, which is in disagreement with the result of the present study [28].

Intergroup comparison showed a significant drop in sensitivity score at 1, 2 and 4 week in both the groups. The patients treated with Gluma® Showed a statistically significant reduction in VAS scores as compared to D/Sense® Group at 1, 2, and 4 weeks. At the end of 6 weeks, patients with Gluma® showed a slightly higher drop in VAS score, albeit not significant. The result of the present study is by the study conducted by Schupbach et al., [26] reported Gluma® has a long-term effect on the sensitivity induced by tooth preparation. In another clinical study, investigator compared the effectiveness of desensitiser products, and they found that Gluma® group showed a significant reduction in VAS scores at

post-treatment evaluation [29]. A study conducted by Jalalian et al., [30] concluded that Gluma® was less effective in reducing post crown preparation sensitivity as compared to potassium nitrate, which was in contrast to the results of the present study. Similarly, de Assis Cde et al. concluded from their clinical study that Gluma® did not affect the management of root hypersensitive in patients treated by non-surgical periodontal therapy for a period up to 4 weeks [28].

Investigators have described various other possibilities through which patients can get relief in clinical studies apart from the desensitising agents; may be due to the placebo effect or due to self-healing capacity of the dentin by the formation of secondary and reparative dentin. The relief consists of a mixture of physiological and psychological interactions, depending considerably on the doctor-patient relationship [31].

In conclusion, post-operative root sensitivity is one of the most frequent complications after non-surgical periodontal therapy. There are multiple products available in the market for the treatment of root sensitivity. The outcomes of the current clinical study verified a meaningful reduction in root sensitivity by both desensitising agents after non-surgical periodontal therapy. No adverse effects were reported in both groups throughout the study period. Gluma® showed a better reduction in VAS score as compared to the D/Sense® during the initial follow-up period. Whereas, almost similar VAS scores were observed between both the groups at the sixth-week follow-up. Both desensitising agents are equally effective in the reduction of post-operative root sensitivity in long term follow-up.

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Retraction

Retraction: Singhal R, Rai B. sTNF-R Levels: Apical Periodontitis Linked to Coronary Heart Disease. Open Access Maced J Med Sci. 2017; 5(1):68-1 and Rai B, Kaur J, Jain R. Salivary and serum 8-hydroxydeoxyguanosine level in simulated microgravity. Maced J Med Sci. 2010; 3(4):364-7

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Abstract

Editor-in-Chief has retracted the articles "sTNF-R Levels: Apical Periodontitis Linked to Coronary Heart Disease." published by Singhal R & Rai B and "Salivary and serum 8-hydroxydeoxyguanosine level in simulated microgravity." published by Rai B, Kaur J, Jain R. An internal investigation has raised sufficient evidence of the inconsistent results and high similarity index. As such, we retract these two articles from the literature and by guidelines and best editorial practices from the Committee on Publication Ethics. We apologise to our audience about this unfortunate situation.

Editor-in-Chief has retracted the articles "sTNF-R Levels: Apical Periodontitis Linked to Coronary Heart Disease." published by Singhal R & Rai B [1] and "Salivary and serum 8-hydroxydeoxyguanosine level in simulated microgravity." published by Rai B, Kaur J, Jain R [2].

One of the papers published by Rai B is already retracted [3], and for several other papers, there are concerns about the plagiarism or inconsistency of the results.

An internal investigation has raised sufficient evidence of the inconsistent results and high similarity index [4], [5]. As such, we retract these two articles from the literature and by guidelines and best editorial practices from the Committee on Publication Ethics [6]. We apologise to our audience about this unfortunate situation.

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Public Health

Prevalence, Profile, and Response to Work-Related Musculoskeletal Disorders among Egyptian Physiotherapists

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Keywords: Work-Related Musculoskeletal Disorders; Physiotherapists; Egypt; Prevalence

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Abstract

BACKGROUND: Despite that physiotherapists (PTs) are supposed to have adequate knowledge of musculoskeletal disorders and the different prevention strategies, they are at high risk of developing work-related musculoskeletal disorders (WRMDs).

AIM: This study aimed to investigate the prevalence, profile, predictors, and response to WRMDs among Egyptian PTs.

METHODS: A self-administrated questionnaire was distributed either manually or via e-mail to 564 PTs with at least two years of working experience. Questions elicited information about the personal and occupational history of the respondents in addition to the experience of WRMDs in the past two years.

RESULTS: Four hundred and fourteen respondents (82.6%) reported WRMDs within the last two years, with the lower back as the most common area affected (68.8%). More than half the PTs (54.8%) who sustained a WRMD reported that their injury took place in a private setting. Significant predictors for WRMDs were age (AOR = 0.78; 95% CI = 0.66, 0.91) and number of years of experience in physiotherapy practice (AOR = 1.26; 95% CI = 1.07, 1.49). In response to the injury, about 73.9% of the respondents stated that they did not officially report their injury and 55.8% of them reported losing a half day or more from their work.

CONCLUSION: The prevalence of WRMDs among Egyptian physiotherapists is high. Despite socioeconomic and cultural differences between Egypt and other countries, our study findings were consistent with the published reports. Further studies are needed to explore the cultural and psychosocial risk factors of WRMDs.

Introduction

According to the World Health Organization, work-related musculoskeletal disorders (WRMDs) are defined as injuries that include a wide range of inflammatory or degenerative diseases and disorders resulting in pain or functional impairment [1], [2]. They originate from muscles, joints, ligaments, tendons, and bones and last more than three days due to work-related events and conditions [3], [4]. Consequently, WRMDs lead to lost work time, work restrictions, loss of consciousness, career shift or even death [5], [6]. Moreover, these disorders are associated with economic and social burdens, that ultimately impact the quality of life [7].

It is well documented that health care workers are at high risk of musculoskeletal disorders [3]. Despite that nurses have reported the highest rates of WRMDs in different work settings, yet Physiotherapists (PTs) are exposed to the same risk factors [8], [9]. Repetitive tasks, continuous bending, awkward sustained postures, lifting and transferring patients are considered main risk factors, making healthcare providers vulnerable to musculoskeletal injuries [4], [5], [6], [7], [8].

Adding to the complexity, although PTs have adequate knowledge of musculoskeletal injuries and the different prevention strategies, this does not grant them protection from developing WRMDs [5], [9]. Moreover, evidence indicates that PTs tend to work while in pain or with a musculoskeletal injury even while exacerbating their condition. They are also less likely to report their injuries or seek care relying on self-treatment based on their clinical expertise [9].

Previous studies revealed that WRMDs are more common among fresh graduates and young PTs, mostly within the first five years of practice [3], [6]. On the other hand, old aged PTs are less liable to injury as they are more involved in documentary work than manual practice [10]. Most of the previous research revealed that the lower back is the most common area of injury reported among PTs [4], [5], [6], [7], [8].

The prevalence and profile of WRMDs among PTs vary according to the studied population and are influenced by various factors as age, sex, type of clinical setting and practice, years of experience and contact hours with patients. To date, there is lack of information regarding the prevalence of WRMDs among PTs in Egypt. Only two recent studies investigated this problem, one among Egyptian pediatric PTs and the other was limited by a small sample size [3], [11]. Therefore, this study was conducted to determine the prevalence, profile, and predictors of WRMDs among Egyptian PTs in addition to their response to such type of injuries aiming to contribute to the development of effective prevention and control strategies.

Methods

Study design and Participants

A cross-sectional design was used for the current study. Potential participants included all licensed Egyptian PTs working in different practice settings with at least two years of working experience. PTs who were retired or did not practice within the two years before the survey were excluded.

Sample size and technique

Epi Info software, version 7 was used to calculate the sample size. Based on the findings of the study conducted by Al-Eisa et al., [3], the prevalence of WRMDs was assumed to be 77%, with a precision of ± 5%, employing 80% power, 99% confidence interval, and with a design effect of 1.0, the minimal sample size required was 470 participants. Moreover, 20% was added to compensate for possible non- response; the final sample size was estimated to be 564 participants. A systematic random sampling technique was used to select participants from the membership list of the Egyptian Physical Therapy Association after approval of the ethical committee of Faculty of Physical Therapy, Cairo University.

Instrument

The survey instrument was a self-administrated questionnaire based on a previously published instrument by Holder et al., [5]. The questionnaire included two parts composed of predominately closed-ended questions. The first portion elicited information about the personal and occupational history of the participants including questions about sex, age, weight, height, setting, speciality, years of experience and hours of patient contact. The second portion inquired if the participant had experienced any WRMDs in the past two years or not. Respondents who reported WRMDs were asked about the type of injury, specific activities that caused the injury, the body part affected, the type of work setting in which the injury took place and the exacerbating activities. Additionally, they were asked questions related to the immediate and long-term responses to the injury.

Procedures

During the period between April and June 2018, five hundred and sixty-four copies of the questionnaire were distributed either manually at hospitals, clinics, and centres for PTs residing in Cairo and nearby governorates or via email for those living in remote governorates. A cover letter was attached to the questionnaire to explain the purpose of the study and to assure confidentiality. Also, a contact number and an email were provided in case of further inquiries. Participants were asked to complete and return the questionnaire within two weeks. Two reminders were sent to the participants who did not submit their questionnaires after two weeks. The study procedures were carried out by the ethics requirements of the Declaration of Helsinki.

Data analysis

Data were coded, entered and statistically analysed using the Statistical Package for Social Sciences (SPSS) version 21. Qualitative data were expressed as numbers and percentages. Quantitative data were presented as the mean ± Standard Deviation (SD). As the main outcome measures were binary variables namely WRMDs (1 variable), WRMDs according to most frequent body areas affected (6 variables), immediate responses to WRMDs (4 variables) and long term responses to WRMDs (3 variables), 14 multivariate logistic regression models were built to define the significant predictors among the tested independent variables by using binary

logistic regression test. The tested independent variables entered in each regression model were sex, age, BMI, physiotherapy practice area, years of experience and contact hours with patients/week. Results were reported as Adjusted Odds Ratio (AOR) and 95% Confidence Interval (CI). A p value less than 0.05 was considered to be statistically significant.

Results

A total of 501 out of 564 questionnaires were completed and returned with a response rate of 88.9%. The mean age of the respondents was 29.9 ± 6.4 years, with a range of 24-63 years, with nearly equal distribution regarding their sex. Estimation of the BMI of the respondents revealed that about two-thirds of the respondents were overweight and obese (63.9%), one third were normal weight, and only 0.6% were underweight. The background characteristics of the respondents are summarised in Table 1.

Table 1: Background characteristics of the respondents (Total = 501)

Variables	Number	Per cent
Sex		
Female	234	47.7
Male	267	53.3
Age in years (Mean ± SD)*	29.9 ± 6.4	
Weight in Kg (Mean ± SD)*	77 ± 17.2	
Height in cm (Mean ± SD)*	168.8 ± 9.7	
BMI** (Mean ± SD)*	26.9 ± 5	
Physiotherapy practice area		
General	261	52.1
Specialized	240	47.9
Years of Experience (Mean ± SD)*	8.4 ± 6	
Contact hours with patients/week (Mean ± SD)*	30.7 ± 20	

*Quantitative variables are presented as Mean ± SD; **BMI = Body Mass Index.

Prevalence and Profile of WRMDs

Four hundred and fourteen respondents (82.6%) reported WRMDs within the last two years. Two hundred seventy-seven (66.9%) reported sustaining more than one WRMD.

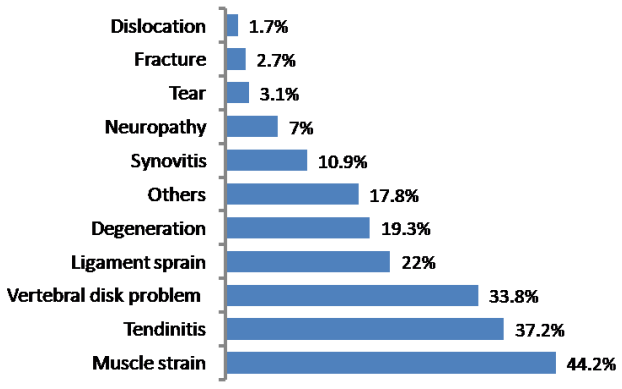

Figure 1: Percent distribution of WRMDs according to the type of injury (Total = 414); *Multiple responses were allowed*

The respondents reported the highest levels of WRMDs in the lower back (68.8 %), shoulder (40.8%), neck (36.7%), upper back (30.2%), wrist (29%) and knee (27.1%). While the elbow, ankle, and hip were the least reported areas (8%, 7% and 4.6% respectively).

Figure 1 illustrates that the most common types of WRMDs reported by the respondents were muscle strain (44.2%), tendinitis (37.2%) and vertebral disk problem (33.8%). Regarding the activities that caused the WRMDs, the top 3 prevalent activities reported were performing manual therapy techniques (32.6%), maintaining a position for a prolonged period (24.9%) and working when physically fatigued (20.8%) (Table 2).

Table 2: Frequency of WRMD according to the specific activity that caused the WRMDs (Total = 414)*

Activity that caused injury	Number	Per cent
Performing manual therapy techniques	135	32.6
Maintaining a position for a prolonged period	103	24.9
Working when physically fatigued	86	20.8
Performing repetitive tasks	78	18.8
Applying modalities	72	17.4
Bending/ twisting	72	17.4
Lifting heavy equipment or patients	48	11.6
Working in an Awkward/ Cramped Position	37	8.9
Transferring a Patient	32	7.7
Responding to an unanticipated/ Sudden movement by a patient	24	5.8
Slipping/ tripping/ falling	6	1.4

*Multiple responses were allowed.

More than half the PTs (54.8%) who sustained a WRMD reported that their injury occurred in a private setting (Figure 2). Seventy-five per cent of the respondents with WRMDs experienced recurrence of symptoms.

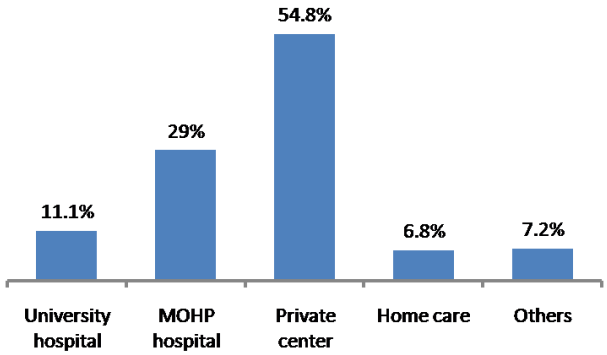

*Figure 2: Percent distribution of WRMDs by the type of setting in which the injury occurred (Total = 414); *Multiple responses were allowed*

Performing manual therapy techniques (36.5%), maintaining a position for a prolonged period (35.6%) and performing repetitive tasks (21.5%) were the most common activities that caused exacerbation of their symptoms during clinical practice (Table 3).

Predictors of WRMDs

Multivariate logistic regression analysis was performed to single out risk factors associated with WRMDs among PTs as shown in Table 4.

Table 3: Exacerbating activity as reported by respondents who experienced a recurrence of symptoms of WRMDs (Total = 312)*

Exacerbating activity	Number	Per cent
Performing manual therapy techniques	114	36.5
Maintaining a position for a prolonged period	111	35.6
Performing repetitive tasks	67	21.5
Bending/ twisting	47	15.1
Lifting	41	13.1
Working in an awkward/ cramped Position	38	12.2
Transferring a patient	27	8.7
Squatting	22	7.1
Performing overhead activities	18	5.8
Walking	13	4.2
Climbing stairs	12	3.8
Reaching	6	1.9

*Multiple responses were allowed

Significant predictors for WRMDs were age (AOR = 0.78; 95% CI = 0.66, 0.91) and number of years of experience in physiotherapy practice (AOR = 1.26; 95% CI = 1.07, 1.49).

Table 4: Multivariate logistic regression model for factors associated with WRMDs

Variables	WRMDs		P value*	AOR* (95% CI)
	Yes N = 414	No N = 87		
Sex, n (%)			0.218	1.38 (0.83 - 2.3)
Female	196 (83.8)	38 (16.2)		
Male	218 (81.6)	49 (18.4)		
Age in years, mean ± SD	29.7 ± 6.2	31 ± 7.2	0.002	0.78 (0.67 - 0.91)
BMI**, mean ± SD	27 ± 5.1	26.5 ± 4.7	0.155	1.04 (0.96 - 1.1)
Physiotherapy practice area, n (%)			0.722	0.92 (0.57 - 1.49)
General	213 (81.6)	48 (18.4)		
Specialized	201 (83.8)	39 (16.2)		
Years of Experience, mean ± SD	8.3 ± 5.9	9 ± 6.5	0.006	1.26 (1.07 - 1.48)
Contact hours with patients/week, mean ± SD	31.2 ± 20.3	28.6 ± 18.8	0.102	1.01 (0.99 – 1.02)

*P value, AOR (Adjusted Odds Ratio) and 95% CI (Confidence Interval) obtained by Binary logistic regression test; **BMI = Body Mass Index.

Table 5 summarises the results of six multivariate logistic regression models to define risk factors associated with WRMDs according to the most frequent body areas affected.

Table 5: Multivariate logistic regression models for factors associated with WRMDs according to most frequent body areas affected

Variables	Lower back		Shoulder		Neck		Upper back		Knee		Wrist	
	P value	AOR 95% CI	P value	AOR 95% CI	P value	AOR 95% CI	P value	AOR 95% CI	P value	AOR 95% CI	P value	AOR 95% CI
Sex (Female/ Male)	0.416	0.85 (0.57 - 1.26)	0.004	1.83 (1.21 - 2.78)	0.058	1.5 (0.99 - 2.28)	0.168	1.37 (0.88 - 2.14)	0.688	0.91 (0.57 - 1.45)	0.005	1.93 (1.22 - 3.07)
Age in years	0.001	0.81 (0.72 - 0.92)	0.009	0.84 (0.74 - 0.96)	0.118	0.9 (0.79 - 1.03)	0.148	0.9 (0.79 - 1.04)	0.246	0.92 (0.79 - 1.06)	0.452	0.95 (0.82 - 1.09)
BMI**	0.472	1.01 (0.98 - 1.06)	0.051	1.04 (1 - 1.08)	0.561	1.01 (0.97 - 1.06)	0.552	0.99 (0.94 - 1.03)	0.069	1.04 (0.99 - 1.09)	0.538	1.01 (0.97 - 1.06)
Physiotherapy practice area (General/ Specialized)	0.556	1.12 (0.77 - 1.62)	0.708	1.08 (0.73 - 1.58)	0.350	0.83 (0.56-1.23)	0.715	1.08 (0.71 - 1.64)	0.775	0.94 (0.61 - 1.45)	0.978	1.01 (0.66 - 1.54)
Years of Experience	0.007	1.2 (1.05 - 1.37)	0.016	1.19 (1.03 - 1.36)	0.170	1.1 (0.96 - 1.27)	0.170	1.11 (0.96 - 1.29)	0.344	1.08 (0.92 - 1.26)	0.668	1.03 (0.89 - 1.2)
Contact hours with patients/week	0.03	1.01 (1 – 1.02)	0.364	1.01 (0.99 - 1.02)	0.654	0.99 (0.99 – 1)	0.392	1.01 (0.99 – 1.02)	0.181	1.01 (0.99 – 1.02)	0.009	1.02 (1 – 1.03)

*P value, AOR (Adjusted Odds Ratio) and 95% CI (Confidence Interval) obtained by binary logistic regression test; **BMI = Body Mass Index.

These prediction models revealed that female PTs had an increased risk of WRMDs in the shoulder (AOR = 1.83; 95% CI = 1.21, 2.78), and wrist (AOR = 1.93; 95% CI= 1.22, 3.07) than their male counterparts. Older ages were associated with a decreased risk of WRMDs in the lower back (AOR = 0.81; 95% CI = 0.72, 0.92) and shoulder (AOR = 0.84; 95% CI = 0.74, 0.96), meanwhile, the number of experience years was associated with increased risk of WRMDs in the lower back (AOR = 1.2; 95% CI = 1.05, 1.37) and shoulder (AOR = 1.19; 95% CI = 1.03, 1.36). The number of hours in contact with patients per week was associated with increased risk of WRMDs in the lower back (AOR = 1.01; 95% CI = 1, 1.02) and wrist (AOR = 1.02; 95% CI = 1, 1.03).

Immediate and long-term response to WRMDs

As an immediate response to the injury, most of the respondents (73.9%) stated that they did not officially report their injury, and only about one third (38.6%) consulted a physician for their injury. Meanwhile, the vast majority of the PTs with WRMDs (95.4%) received treatment and more than half the respondents (55.8%) reported losing half day or more from their work as a result of injury (Table 6).

Regarding the long-term response to WRMDs, more than two-thirds of the respondents (72.5 %) indicated that they had altered their working habits as a response to their injury (Table 6). A variety of coping strategies were used by PTs who altered their working habits, changing working position frequently, using improved body mechanics and taking more breaks during the workday were the most common stated coping strategies (55%, 35%, and 31.7% respectively). While nearly half the respondents indicated that they had not limited their contact time with the patients nor considered changing their job in response to the WRMDs. (41.1% and 39.4% respectively) (Table 6).

Table 6: Immediate and long term responses to the injury as reported by the respondents who sustained a WRMD (Total = 414)*

Type of response	Response	Number	Per cent
Immediate response	Officially reported the injury	108	26.1
	Consulted a physician	160	38.6
	Lost half day or more of work as an immediate effect of the injury	231	55.8
	Received treatment	395	95.4
Long term response	Alteration of working habits	300	72.5
	Limitation of contact time with patients	170	41.1
	Considering changing their job	163	39.4

*Multiple responses were allowed.

Multivariate logistic regression analysis was performed to identify the significant predictors for each response (4 immediate and three long terms) as the dependent variable. Results from these prediction models revealed that PTs working in general practice were less likely to officially report their injury and consult physician than specialists (AOR = 0.58; 95% CI = 0.37, 0.92 and AOR = 0.55; 95% CI = 0.36, 0.84 respectively). In terms of gender, female PTs were more likely to consult a physician and alter their working habits in response to WRMDs (AOR = 1.83; 95% CI = 1.17, 2.87 and AOR = 1.62; 95% CI = 1, 2.6 respectively) compared to male PTs. Additionally,

increased BMI was independently associated with the likelihood of consulting a physician for the injury (AOR = 1.05; 95% CI = 1, 1.1).

Discussion

The purpose of this study was to investigate the prevalence and profile of WRMDs among physiotherapists in Egypt and to identify their causes and risk factors. What makes our study unique is the large sample size and the high response rate. Out of 564 questionnaires, 501 were returned with a response rate of 88.9%. In previous studies, however, the response rate was 53% in Australia [12], 58% in Nigeria [13], 59% in Turkey [6], and 75% in the United Kingdom [14]. Compared to the previous studies, the achieved high response rate may be explained by the increased perception and awareness among physiotherapists in Egypt about the value of participating in research activities.

The findings of this study indicate that Egyptian physiotherapists are at high risk of developing WRMDs in the workplace. On average, 82.6% of respondents reported at least one WRMD within the last two years, and 66.9% reported sustaining more than one WRMD (Table 1). By looking at the published rates of WRMDs and compare them to our findings, it is valid to say that WRMDs among physiotherapists in Egypt is high and needs further attention. It has been documented that WRMDs among physiotherapists have many burdens, including pain and distress, cost, decreased working hours, and potential disability [14], [15], [16]. Notably, muscle strain, tendinitis, and vertebral disk problem were the most repeatedly mentioned WRMDs types among our respondents (44.2%, 37.2%, 33.8%, respectively) (Table 1).

Consisting with the literature, our respondents reported the highest levels of WRMDs in the lower back (68.8 %), followed by shoulders (40.8%), neck (36.7%), and knees (27.1%). Although lower back pain (LBP) is the most common complaint among physiotherapists in the workplace in many countries [12], [17], [18], the high prevalence found in our study (68.8%) is alarming because it is among the highest in the published data. For instance, in Canada Mierzejewski and Kumar (1997) reported a prevalence of (49%) of LBP among physiotherapists [19]. Similar rates have been reported at (45%) in the USA [8], 35% in Australia [12], 38% in Greece [20], and 26% in Turkey [6]. In the Middle East, although there is a dearth of such research, the literature review identified three relevant studies [10], [21], [22]. In Shehab et al., (2003) study, 70% of physiotherapists who practice physiotherapy in the State of Kuwait reported LBP [21]. However, a recent study in the State of Kuwait documented a dramatic decrease in the prevalence of LBP from 70% in 2003 to 32% in 2010 [10], yet still similar to the international records. In Saudi Arabia, a recent study conducted an online survey targeted members of the Saudi Physical Therapy Association. They reported that 89% of participants had a work-related LBP [22]. In our study, however, nearly 70% of the participants reported LBP, which is among the highest published rates up-to-date. By combining our findings with past research, it is valid to conclude that LBP among physiotherapists in Egypt poses a significant population health problem. Despite a tremendous burden of LBP on individuals, it has been linked to psychological distress [23], and physical disability [24]. Consequently, LBP may negatively affect the productivity of physiotherapists and the overall efficiency of physiotherapy practice in Egypt.

Apart from LBP, this study, as in other studies, revealed that other parts of the body could be influenced by WRMDs [6], [25]. In this study, more than 40% of respondents reported shoulder pain, 36.7% reported neck pain, and 27.1% suffered from knee problems. Although the past research was focused on LBP and the evidence of WRMDs in other areas of the body is limited, our findings seem to be in line with the published rates [15], [26]. Interestingly, nearly one-third (27.1%) of the respondents reported knee problems. This is a striking finding of our study because it is much higher than the published rates in previous studies. In Salik and Özcan's (2004) study, about 8% of surveyed physiotherapists reported knee problems [6]. Similarly, Anyfantis and Biska (2018) reported that 6% [20], and Cromie et al., (2000) reported that 11.2% of participants had knee problems [18]. The high prevalence of knee problems reported in our study may be explained, in part, by the fact that almost two-thirds (63.9%) of the respondents were overweight and obese. Obesity was documented by many authors as a risk factor for many musculoskeletal problems, including LBP, hip, and knee problems [27], [28], [29].

In our study, the mean age of the respondents was (29.9 ± 6.4). This is an important finding because it confirms the link between work-experience and vulnerability to WRMDs. This finding goes by other studies stating that WRMDs typically occur in physiotherapists between the ages of 20 to 40 years [18], [30]. One explanation of this prevalence in young age may be due to lack of experience, lack of professional training, or limited prophylactic and/or coping strategies. It can also be referred to other cultural or socioeconomic factors in the early career years. This increased prevalence of WRMDs among the younger physiotherapists is problematic because such burdens can increase with age [31]. Effective interventions that prevent WRMDs, such as educational programs, improving the infrastructure, and seeking assistance from other healthcare staff in physically-demanding tasks (e.g., patient transfer) are suggested strategies to subsidise WRMDs [17].

In this study, we also collected data on the

most common activities that caused WRMDs in clinical settings (Table 3). Similar to the previously published data, our analyses showed that (32.6%) of the participants developed their injury during performing manual therapy techniques, followed by (24.9%) due to maintaining a position for a prolonged period, and (20.8%) while working when physically fatigued. Past studies have shown consistent correlations between biomechanical factors, such as awkward posture, manual therapy practices, repeated movement and lifting heavy objects, and the development of WRMDs [5], [32]. Campo et al., (2008) conducted a 1-year prospective study with physiotherapists to investigate the risk factors of WRMDs in the USA. They found that patient repositioning and transfers were the main risk factors for WRMDs [17]. Salik and Özcan (2004) identified the main causes of WRMDs as patient positioning and transfer, repeated movements, lifting heavy objects, and working when fatigued [6]. Two studies highlighted the risk of staying in the same position for a long time, manual therapy practices, and repeating the same movement on the musculoskeletal system of physiotherapists [8], [12]. In our study, the top exacerbating activity was performing manual therapy technique. This can be explained by the fact that manual therapy is common in Egypt, and the number of days treated patients usually exceeds the number of physiotherapists in a given clinical facility. Thus, most of the physiotherapists work when they are exhausted, and this adds extra loads on their body and exacerbate their musculoskeletal pain.

Equally important, research shows that physiotherapists are reluctant to talk about their pain due to WRMDs [9]. Interestingly, more than half the surveyed physiotherapists (54.8%) who sustained a WRMD reported that their injury occurred in a private setting, and 20% of them were working when physically fatigued. Furthermore, the majority of respondents (73.9%) stated that they did not report their injury, and only about one third (38.6%) consulted a physician for treatment (Table 2). This finding is important because it reflects the cultural and socioeconomic dimensions of WRMDs. The clinical culture of healthcare facilities, as well as the culture of physiotherapists themselves, may play an essential role in admitting any pain caused by patient care. As documented in previous research, more than half of the respondents did not report their WRMDs to their employers [9]. It is a common sense that physiotherapists in Egypt work in both the public and private sectors. Therefore, they usually experience heavy workloads [33]. Our survey revealed that more than half the respondents (55.8%) reported losing half a day or more from their work as a result of injury (Table 6). Because some of the hidden pains are disabling in the long run, this claim deserves further exploration because it may potentially lead to loss of experienced physiotherapists.

Regarding the long-term response to WRMDs, our analyses indicated that the majority of the respondents (72.5%) stated that they had changed their working habits as a response to their injury (Table 6). Several coping strategies were used by physiotherapists who modified or changed their working habits. For example, about 55% of respondents reported changing working position frequently during treatment sessions, 35% used improved body mechanics, and 31.7% had more breaks during the workday. Noteworthy, nearly 40% of surveyed physiotherapists considered leaving the profession due to WRMDs, which is higher to what has been found in the literature. Although Campo et al., (2008) reported the lowest rate of leaving the profession at less than 1%, others reported similar to our findings [17]. For example, Cromie et al., (2000) documented that one in six physiotherapists changed or left the profession due to WRMDs [18]. Anyfantis and Biska (2018) reported that 32% of the recruited physiotherapists considered changing the profession [20]. Contrarily, Alrowayeh et al., (2010) reported no changes in work habits nor desire to leave the profession among the surveyed physiotherapists in their study. This inconsistency among studies is perhaps due to cultural and socioeconomic factors [10]. In this study, we performed multivariate logistic regression analyses to see if there are associations between risk factors, such as age, gender, working hours and years of work experience, and WRMDs as shown in Table 4. Age and the number of years of experience in physiotherapy practice were identified as significant predictors for WRMDs. Our analyses also revealed that female physiotherapists are at an increased risk of WRMD in the upper limbs (mainly in the shoulder and wrists) than their male colleagues. The latter was proven true by many authors [6], [26], [34].

Limitations: This study has some limitations. As all cross-sectional designs, the findings can only recognise risk factors. Second, the self-administered questionnaire could have some recall bias. Participants may forget to mention all incidents of WRMDs. Also, since the physiotherapy job market in Egypt is extremely competitive, it is possible that some respondents underestimated or even hide their injury to show that they are fit and competence for the job. Finally, since we did not ask about the physical activity level of our participants, it is possible that athletes vs non-athletes physiotherapists experience WRMDs at different levels and frequencies.

In conclusion, this study reveals that the WRMDs among Egyptian physiotherapists is high and similar to their counterparts elsewhere. Despite socioeconomic and cultural differences between Egypt and other countries, our study showed relatively consistent findings to the published reports. Further research that focuses on cultural and psychosocial dimension may provide valuable insight into other issues and risk factors of WRMDs among physiotherapists in Egypt.

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Public Health

Knowledge and Attitudes about Breast Cancer among Women: A Wake-Up Call in Nigeria

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Abstract

BACKGROUND: Preventable deaths resulting from the scourge of breast cancer has become alarming and worrisome in many societies in developing countries, including Nigeria. Of much concern is the fact that breast cancer has continued to claim the precious lives of young, middle-aged, old, educated and non-educated women irrespective of their religion, socio-economic background and socio-demographic characteristics.

AIM: This study attempts to ascertain the knowledge and attitudes of women to breast cancer in Ogun State, Nigeria.

METHODS: The study adopts both primary and secondary data sources to examine the level of knowledge and attitude of women towards breast cancer with the view of suggesting probable solutions and recommendations for policy.

RESULTS: The result indicates that the awareness about breast cancer is overwhelming but only few women know about mammography; women in older age are 0.193 times less likely to attend breast cancer screening (p=0.000). Older women with secondary education that are either self-employed outside the home or full-time housewives are unfavourably disposed to breast cancer screening.

CONCLUSION: The authors recommend that concerned stakeholders in the health sector and policy decision makers should intensify action on cancer programmes and campaigns that could target older women especially housewives and women in middle level education.

Introduction

Breast cancer is the most common cancer affecting 25.2% of women and is also the second leading cause of cancer-related deaths among women [1]. Almost half of breast cancer cases and 60% of breast cancer-related deaths are estimated to occur in middle-and-low-income countries [2]. Globally, the devastating effects on women diagnosed with breast cancer are appalling [3]. Global cancer statistics show increased global cases of breast cancer and the rise is occurring at a faster rate in populations of the middle-and-low-income countries which may be due to increase in population growth and ageing [1], [2].

Breast cancer is an aggressive disease affecting women, irrespective of their age category. Women are particularly vulnerable and susceptible to breast cancer and their risks increases with advanced age [4]. The origin of breast cancer has not been fully unravelled but is attributable to some inter-related factors of genetics, hormones, the environment, socio-biology and physiological factors [3], [5]. Alatise and Schrauzer, for instance, have suggested that associated widespread pollution of the soil and water supply by a substance called “lead” may be a major contributory cause.

In a report by Siegel et al., [6] it was indicated that deaths as a result of breast cancer in Nigeria reached 13,264 or 0.70% and the age adjusted Death Rate is 28.11 per 100,000 population, ranking Nigeria 4th in the world. Adebamowo and Ajayi (1999) also stated that breast cancer is the most common cancer in Nigeria. In 2005, breast cancer was found to be the most common in Nigeria [7].

In the North-West geopolitical zone of Nigeria, cancer of the breast is second to cancer of the cervix, while the cancer registry at the University College Hospital (UCH), Ibadan revealed that it is the leading malignancy among women [3], [8], [9]. Also, in the North-central, breast cancer constitutes 22.41% of new cancer cases registered in 5 years and accounts for 35.41% of all cancers in women [10].

Breast cancer is undoubtedly the most dreaded cancer with lots of psychological impacts and one of the most popular malignancies that affect about one in every nine women [11]. It is a disease in which the malignant cells are developing in the tissue of the breast. Breast cancer is of two types, Lobular cancer which begins in many small sacks in the breast that produce milk and ductal cancer which develops in the tubes that carry milk from the lobules to the nipple [12]. It is also the type of cancer having the highest prevalence (45.7%) among the females in Nigeria and border Countries [13]. Common signs and symptoms of breast cancer include a change in a way the breast or nipple feels, change in how the breast or nipple seems and discharge of the nipple [11].

It is interesting to know that as debilitating as breast cancer disease is, majority of Nigerian women have little or no knowledge of the disease and even in situations where they are aware of the disease, their attitudes towards seeking healthcare is negative causing their untimely or preventable death. It has been observed that certain socio-cultural, religious, genetic and economic factors are responsible for this negative attitude.

Literature Review

It has been argued that a lack of basic knowledge and quality information delivery system for breast cancer is a great impediment to the life and well-being of women [14]. Breast cancer has been a major cause of death subtly killing women – especially those with little or no education. This is compounded by the lack of timely information about breast cancer and poor diagnostics screening methods for early detection [15], [14].

As important as knowledge of breast cancer is, it is not sufficient unless socio-cultural factors are taken into consideration by the health professional providing direct health-care [16]. Insufficient information concerning breast cancer has also been observed among the rural and urban dwellers in Nigeria; it is responsible for the poor perception of the ability to cure cancer earlier detected and the efficacy of screening tests [4].

Furthermore, the lack of awareness on the issue of vulnerability and susceptibility associated with breast cancer discourage many women from seeking intervention early or associate the symptoms they are experiencing with other health conditions [14].

Level of awareness regarding how to perform simple life-saving diagnostic breast checks such as breast self-examination (BSE) further compounds the problem of late detection. Empowerment of women with information on BSE is of paramount importance, especially in countries without modern technologies for breast cancer screening [17]. Most of the Nigerian rural communities lacked the required technological resources [18], but BSE can contribute greatly if women are informed about this technique, and regular practice would reduce late presentation [14].

Mammography screening may also be done to detect breast cancer in asymptomatic women. In spite of its limitations in LMCs due to the challenge of poor infrastructure, poverty, and inadequate human resources, it has been seen as the method of choice for screening and diagnosis which can significantly reduce breast cancer morbidity and mortality [19], [20], [21].

Certain socio-cultural factors also contribute to breast cancer prevalence in Nigeria. As opined by Akhigbe and Akhigbe, 2012, health beliefs vary across culture, and the fatalistic consequence of cancer may discourage many from participating in health-promoting behaviours. This isbecause illnesses or catastrophic events in this part of the world are attributed to a higher power (such as God), or they are meant to happen and cannot be avoided; as a result, fatalism becomes part of the person's world-view.

Chronic conditions in many African societies are often associated with witchcraft and evil spirits. Cultural values and ethnic diversity have an impact on health beliefs, which may influence how rural women interact with the Western medication, especially conditions such as breast cancer. Some women delay seeking treatment because of fear or stigma concerning their daughters as it is believed that they also might be affected by breast cancer and might not be considered for a good marriage. Furthermore, it is believed that cancer is a death sentence from God [16]. All these have continued to be crucial factors that may account for breast cancer prevalence in Nigeria and other Sub-saharan African countries.

This study seeks to ascertain the knowledge and attitude of women towards breast cancer in Ogun state. It also attempts to examine the factors responsible for breast cancer prevalence, thereby suggesting probable solutions and recommendations for policy decisions.

Methods

Study Design

This study utilized the structured questionnaire to elicit useful information on behavioural risk factors for breast cancer among women.

The inclusion criteria for this study involved a focus on all women of reproductive age irrespective of their cancer experience.

Study Location

This study was located in Ogun state. Nigeria. The state was randomly selected from the South-west out of the six geo-political zones in Nigeria.

Study Population

The study population was selected through a random-route walk within the wards that were selected in a local government area in Ogun state.

The research instrument covered history of the participant's life style in terms of occupation, sexual relationship, knowledge of cancer, participation in breast screening exercises, and history of breast cancer.

Ethical Considerations

Approval was sought from Covenant University Health Research Ethics Committee (CUHREC), and formal permission was also duly sought and obtained from head of households.

Due community reconnaissance procedure such as seeking express permission from community leaders was also done and each participant's consent was solicited. Dual consent was obtained in situations where the husband or the male partner is accessible. Each respondent was assured of confidentiality of their responses. Notwithstanding, all participants were encouraged to participate but were given the option to withdraw from the interview or refuse to answer any question they are not comfortable with.

Data analysis

A total of 764 women were interviewed in a randomly selected local government area in Ogun State. The extracted quantitative data were analysed using a three-level technique of analysis: Univariate, bivariate and multivariate techniques.

Frequency distribution was used to summarise the profile of respondents, cross tabulations were conducted simultaneously between two variables of interest and a binary logistic regression (BLR) was adopted to test the responsiveness of selected co-variates (such as age, educational attainment, working status, total life sexual partners and others) on women's participation in breast screening.

Results

Socio-demographic Profile of Respondents

A total of 764 women were sampled in Ogun state. Majority of them were between the age of 30-39 years (36.5%), with only about 0.7% aged 60 years & above. They were mostly married (64.8%), and 69.8% were Christian. Their highest educational attainment is secondary level education (44.6%), with majority claiming to be self-employed (58.6%). The most common source of income was trading (42.8%) (Table 1).

Table 1: Percentage Distribution of Respondents by Socio-Demographic Characteristics

	Freq.	%
Sample (N)	764	100.0
Age		
Less than 20	61	8.0
20-29 years	194	25.4
30-39 years	279	36.5
40-49 years	167	21.9
50-59 years	58	7.6
60 years & above	5	.7
Total	764	100
Marital Status		
Single/Never Married	190	24.9
Married/LWP	495	64.8
Separated/Divorced	46	6.0
Widowed	25	3.3
Cohabiting	8	1.0
Total	764	100
Religious Affiliation		
Christianity	533	69.8
Islam	206	27.0
Others	25	3.3
Total	764	100
Educational Attainment		
No Schooling	53	6.9
Primary Education	126	16.5
Secondary Education	341	44.6
Tertiary Education	244	31.9
Total	764	100
Working Status		
Employee	160	20.9
Self-Employed	448	58.6
Unemployed	29	3.8
Full-Time House-wife	38	5.0
Still Schooling	89	11.6
Total	764	100
Occupation		
Manufacturing	12	1.6
Trading/Distribution	327	42.8
Farming	5	.7
Education	76	9.9
Services	157	20.5
others	187	24.5
Total	764	100

Source: Computed from 2015 Breast Cancer Survey.

Respondents' Breast Cancer Knowledge

The frequency distribution of respondent's knowledge of breast cancer revealed that 92.3% have heard about breast cancer, 70.5% knew that it is preventable while only a fragment knew about

mammogram: 17.7% (Table 2).

This revelation is a pointer to the fact that, respondents in the study area have merely heard about breast cancer and probably might have also merely heard that it is preventable, but only a few knew about the procedure for its early detection.

Table 2. Percentage Distribution of Respondents by Breast Cancer Knowledge

Freq.	%
Ever Heard about Breast Cancer	
Yes	705	92.3
No	59	7.7
Total	764	100.0
Know Breast Cancer is Preventable	
Yes	539	70.5
No	219	28.7
No Response	6	.8
Total	764	100.0
Know Mammogram	
Yes	135	17.7
No	614	80.4
No Response	15	2.0
Total	764	100.0

Source: Computed from 2015 Breast Cancer Survey.

Respondents' Knowledge and Attitude towards Breast Cancer Screening

Table 3 showed the relationship between respondents' knowledge of mammography and their attitude towards breast cancer screening. It can be seen that 90.2% of respondents that are familiar with mammography are favourably disposed to breast cancer screening, while 9.8% are not. However, 86.9% of those not familiar with mammography are favourably disposed to breast cancer screening, while 13.1% are not.

This shows that many women in the study area have a favourable attitude towards breast cancer screening even though some of them claimed not to be familiar with mammography, as shown in Table 3.

Table 3: Relationship between Respondent's Knowledge about Mammograms and their Attitude towards Breast Cancer Screening

		Attitude towards Screening		Total
		Favourable	Not Favorable	
Know	Yes	120 (90.2%)	13 (9.8%)	133 (100.0%)
Mammogram	No	485 (86.9%)	73 (13.1%)	558 (100.0%)
Total		605 (87.6%)	86 (12.4%)	691 (100.0%)

Source: Computed from 2015 Breast Cancer Survey.

Co-variates of Women's Favourable Support for Breast Cancer Screening

Table 4 presents the Binary Logistic Regression (BLR) of the inter-relationship between selected co-variates and women's support for breast cancer screening.

The selected dependent variable is "attitude towards breast cancer screening" measured as "Yes" or "No" binary format.

Yes =1 (favourable support for Breast screening)

No = 0 (unfavourable support for Breast screening)

The selected independent variables measuring "socio-demographic characteristics" are age, residence, education, religion, marital status, total life sexual partners (TLSP) and working status.

The hypothesis estimated the log of likelihood $log\left(\frac{p}{1-P}\right)$ on the independent variable.

$$log\left(\frac{p}{1-P}\right) = \alpha + X_1\beta_1 + X_2\beta_2 + X_3\beta_3 \ldots X_n\beta_n$$

β = Coefficient, SE = Standard Error, Wald = interpreted by its magnitude, Sig. = P value/significance level, Exp (β) = Odd ratio indicating the likelihood of the occurrence of the independent variable, while RC = Reference Category.

Table 4: Binary Logistic Regression Illustrating Co-variates of Women's Favourable Support for Breast Cancer Screening

Selected variables	B	S.E.	Wald	Sig.	Exp(B)
Age group					
≤ 29 years	RC				
30-49 years	-1.643	.349	22.136	.000	.193
50 & above	-2.214	.716	9.569	.002	.109
Residence: Rural (RC)					
Urban	-.079	.283	.078	.779	.924
Education					
No Schooling	RC				
Primary Education	.133	.758	.031	.861	1.142
Secondary Education	-.031	.712	.002	.965	.970
Tertiary Education	.069	.741	.009	.926	1.071
Religious affiliation					
Christianity	RC				
Islam	-.471	.343	1.885	.170	.624
Others	.545	.632	.745	.388	1.725
Marital Status					
Single/Never Married	RC				
Married/LWP	1.452	.582	6.228	.013	4.272
Separated/Divorced	2.288	.762	9.008	.003	9.857
Widowed	2.564	.865	8.781	.003	12.987
Cohabitation	1.231	1.013	1.478	.224	3.426
TLSP: Only One (RC)					
2-3 Partners	.317	.316	1.006	.316	1.374
4 & above	.737	.499	2.182	.140	2.089
Working status: Employee	RC				
Self-Employed	-.695	.370	3.522	.061	.499
Unemployed	.600	.711	.712	.399	1.823
Full-Time Housewife	-.070	.666	.011	.917	.933
Constant	-2.315	.964	5.771	.016	.099
-2 Log likelihood = 358.190		Cox & Snell R Square = 0.078		Nagelkerke R Square = 0.149	

Source: Computed from 2015 Breast cancer Survey.

This model measured women's attitude towards breast cancer screening about their age, residence, education, religion, marital status, total life sexual partners (TLSP) and working status. As depicted in table 4, women that are older are unfavourably disposed to breast cancer screening and have a negative attitude towards it (r = -1.643 and -2.214), and they will be 0.193 and 0.109 times less likely to attend screening when compared to younger women. P-value = 0.000 and 0.002 respectively.

This analysis revealed a high statistical significance denoting some level of resistance to screening among older women about the younger ones. Additionally, the analysis also showed that women with a tertiary education together with those with primary education are favourably disposed towards breast screening (r = 0.069 and 0.133) and will be 1.071 and 1.142 times more likely to attend screening compared to women with no education while women with secondary education have a negative attitude towards screening (r = -0.031) and will be 0.970 times less likely to attend screening

when compared to women with no education. All the categories of education are however, not statistically significant, p-value > 0.05.

In the same vein, married women together with those that are separated/divorced including widows and those involved in co-habitation all showed a favourable attitude towards breast screening (r = 1.452, 2.288, 2.564 and 1.231). They will also be 4.27, 9.85, 12.9 and 3.4 times more likely to attend screening compared to women who are single and have never been married at p-value < 0.05 except women co-habiting at p-value ≥ 0.05. Lastly, working status is not significantly related to breast screening (p-value > 0.05) with only the unemployed showing a favourable attitude towards screening compared to the employees, the self-employed and the full-time house-wives.

Overall, the Cox & Snell R Square = 0.078 and the Nagelkerke R Square = 0.149 implying that only 7.8% and 14.9% of the change in attitude to breast cancer screening could be explained by all the independent variables.

Discussion

This study examined the knowledge and attitude of women about breast cancer. It also looked at the relationship between socio-demographic characteristics and attitude of women towards breast cancer screening.

The study found that almost all respondents in the study area have heard about breast cancer, and more than half knew that it is preventable with just only a fraction knowing of mammography. Even with the majority of the women not knowing what mammography is, an appreciable number of them have a favourable attitude towards it. The multivariate analysis was done to see the relationship between respondents' socio-demographic characteristics and their attitude towards breast screening. This was aimed at examining whether factors such as age, education, marital status and working status had a significant influence on women's attitude towards breast screening.

The binary logistic regression (BLR) showed that older women, with secondary education, with outside employment, self-employment, or full-time house-wives, have an unfavourable attitude towards breast screening. Women with primary education, tertiary education, married, divorced, widowed, co-habiting, and unemployed showed a favourable attitude towards breast cancer screening.

The relationship between breast screening and socio-demographic characteristics have been investigated in various studies across the world [25], [26].

Donato *et al.* for example compared attendees of breast cancer screening programs with non-attenders concerning demographic and socio-economic factors and found that response was higher amongst less educated, married, or widowed women than amongst the more educated, single, divorced, and immigrant women. Reasons for non-participation among others include lack of interest, fear and anxiety about breast cancer. In another study aimed at examining breast cancer awareness, attitude and screening practices in the six geopolitical zones in Nigeria [27], it was found that there was an unfavourable attitude towards breast screening even among those who were aware of the screening methods [28]. In other similar studies, no association was found between breast screening, age, educational attainment, profession and marital status [29], [30], [31].

Going by the findings from this study, it can be concluded that the majority of women in the study area have heard about breast cancer. They are, however, not familiar with mammography screening as one of the breast cancer screening method. Additionally, women that are older in age, women with secondary education, and women that are either employees, self-employed or full-time house-wives are unfavourably disposed to breast screening, while those with primary and tertiary education, that are either married, divorced, widowed, co-habiting, and unemployed showed a favourable attitude towards breast screening.

It is recommended that older women may be targeted for education on breast screening, and breast screening centres should be welcoming to older women. There is also a need for intensified campaigns and enlightenment programs to encourage all women irrespective of their educational background to participate in breast cancer screening. Finally, awareness campaigns and programs about breast screening should be taken to offices, market places, households and other places where we have women to encourage women who are busy with their employment or house chores to participate in breast screening activities.

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Public Health

Physicians' Perspective on Diabetes Mellitus Management within the Context of Personalized Medicine Era in Tabuk Governorate, Saudi Arabia

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Abstract

BACKGROUND: Minimizing the number of therapy failures and decreasing the diabetic complications can be achieved by the application of personalising diabetes therapy, based on patient`s genetics, however, currently, personalised Medicine (PM) in diabetes mellitus management is not extensively applied.

AIM: To assess the knowledge, attitudes, and willingness of physicians in practising of PM in diabetes management.

METHODS: A cross-sectional analytical study was implemented among 126 physicians from six different governmental hospitals and 12 primary care centres selected by the stratified random sampling technique in the Tabuk region of Saudi Arabia. A structured self-administered questionnaire was utilised for data collection. A simple scoring system (scale of 5 points) was utilised to assess knowledge and willingness. Likert scale was applied to evaluate the attitudes towards practising PM in DM management by the fixed choice response formats.

RESULTS: The majority of the participants (97.62%) claimed not receiving any PM and/or genomic medicine training. Most of them (82.54%) expressed unsatisfactory knowledge concerning personalised DM, whereas the medium level of attitudes was reported among 57.14% of them and a good level of willingness had been observed among 76.98% of the physicians.

CONCLUSION: Emphasizing on essential personalised DM management knowledge aspects should be given a considerable priority. Fortunately, positive attitudes and goodwill of physicians towards PM are encouraging and should be supported by policymakers.

Introduction

Diabetes mellitus (DM) is considered one of the biggest worldwide health problems of the 21st century as well as it is a considerable contributor to the morbidity and mortality all over the world caused by the non-communicable diseases. Currently, there are 415 million adults living with diabetes mellitus in different areas of the world, and this is expected to increase by more than 50% over the coming 20 years. In addition to that number, there are 318 million adults with impaired glucose tolerance, which at higher risk of developing diabetes in the future [1], [2]. Saudi Arabia has one of the highest diabetic prevalence (14.4%) country in the world [3]. Also, Saudi Arabia has one of the highest annual incidence rates of type 1 diabetes in children aged 0-14 years, with 31.4 new cases per 100,000 population [4]. Despite the availability of many effective oral therapies to treat diabetes, a considerable percentage of patients do not achieve the required glucose-control rate or may be subjected to adverse effects, from these drugs [5]. Therefore, it is suggested that the response of patients to anti-diabetic therapies is subjected to inter-individual variability, possibly due to genetic factors

regarding drug absorption, distribution, and metabolism. Therefore, exploring genetic markers associated with drug reaction can assist physicians with the decisions of drug selection, dose modification, therapy duration, and escaping adverse drug effects [6].

Most of type 2 diabetic patients have polygenetic forms of the disease in which each gene locus shares only a little risk [7]. For example, the association of the E23K variant in KCNJ11 with an increased risk for secondary failure of sulfonylurea in type 2 DM patients explains how a gene can influence the response to a drug [8]. Also, patients with OCT1 polymorphisms (organic cation transporter 1), which is the main port of entry for metformin into hepatocytes and enterocytes have a decreased response to metformin [9]. Fortunately, nowadays, there are many treatment options for diabetes patients that can improve outcomes for many of them. However, there is still a need to define the appropriate treatment for each, since a good number of monotherapy treatments fail within three years and diabetes related-complication and death continue [10], [11]. Therefore, personalising diabetes therapy based on the genetics of patients can reduce the number of treatment failures and decreasing the diabetes-associated complications but, there is a limited implementation of personalised medicine (PM) in treatment of DM [12].

Personalised medicine (PM) involves identifying specific information about a specific patient and then prescribing a drug that is particular for that patient [13]. The National Academy of Sciences (NAS) defined PM as "the use of genomic, epigenomic, exposure and other data to define individual patterns of disease, potentially leading to better individual treatment" [14]. Its goal is to utilise specific information about the patient to specify the appropriate intervention [15]. The intervention could range from a common drug given to several patients to a drug specific for the patient's genetic profile [16]. Evidence-based support exists for benefits of applying PM for diabetes management in patients with certain monogenic forms of diabetes, and it is expected that applying this strategy for the more common forms of DM will occur with expanding knowledge of polygenic determinants of diabetes [17].

The application of PM in the clinical practice is expected to occur. However, physicians need to be aware of the correct test at the correct time and interpret the test result in the right way and alter their prescription behaviour accordingly. However, we are at an early stage of its application, and a dramatic shift will be needed in the main fields of medical research for this new area of medicine to be fully implemented [18]. Also, numerous challenges in health research exits as there is a need for more understand of the diseases 'mechanisms and translate research results into practice [19].

The study aimed to assess knowledge, attitudes, and willingness of physicians to practice PM in diabetes mellitus management in Tabuk, Saudi Arabia.

Material and Methods

A cross-sectional analytical design has been adopted in this study, which has been conducted in six different governmental hospitals and 12 primary care centres in Tabuk province. The province has an area of 108,000 km² and a population of 791,535 in 2015 [20]; its capital is Tabuk city and composed of six sub-directorates (Tabuk city, Taimaa, Haqel, Duba, Alwajh and Umlujj).

All physicians specialised in internal medicine and/or its diabetic related sub-specialities, namely, endocrinology, paediatrics, community medicine and geriatrics, were included. Physicians who are not providing direct patient care, e.g. administrators and not practising clinical medicine or diabetic related sub-specialities were excluded.

The researchers have followed 3 steps to develop a standardised self-administered questionnaire: The first step was a literature survey of relevant PM studies to identify the pertinent variables and formulating a preliminary questionnaire by the researchers. The second step was taken the opinion of 4 family medicine, and 2 research methodology professors and consultants on the proposed questionnaire in which the nominal group technique has been utilised and then modifications and adjustments have been done. The third step was testing the validity and reliability of the approved questions by the studied experts. Therefore, the studied physicians were asked to respond to a structured 35-items self-administrated questionnaire, designed to evaluate the diabetes management practice variables as related to PM. The independent variables including age, gender, health facility, highest qualification, source of PM awareness, duration of work experience in years and training in PM and/or genomic medicine and dependent variables (knowledge, attitudes and willingness) were selected and revised by five medical research experts. Approximately 20 minutes were needed by the participants to complete it. Ten Knowledge questions with correct or incorrect responses were applied. Assessment of attitude has been based on the Likert scaling method [21] using 10 statements scored as follows: 3 for agreement, 2 for undecided and 1 for disagreement. The total score has been computed by summing the individual scores. Forced-choice response scale (yes or no) was applied to assess willingness degree.

Multi-stage random sampling technique was applied to allocate the physicians. At the first stage,

three sub-directorates from Tabuk province (Tabuk city, Taimaa, and Haqel) have been randomly selected by simple random sampling technique. At the second stage, two governmental hospitals and four primary health care centres were selected by the stratified random sampling technique from each sub-directorate. At the third stage, physicians have been selected by systematic random sampling technique. Only, the eligible subjects have been recruited (126 physicians).

Pre-test study has been conducted throughout the first two months in the preparatory phase of the study to: formulate the research problem for more precise investigation, refine the study variables, and test the validity and reliability of the study tools and instruments.

Ethical approval for carrying out the study was obtained from the regional research committee of Tabuk region. Furthermore, all of the study participants were briefly informed that all their personal information would be kept confidential. Refusal to answer any question or withdraw from the study at any time was granted to all participants. They were informed that there was no "correct" or "incorrect" answer, and they were requested to express their opinions and beliefs freely. Their informed consents were taken.

Data entry and analysis were done using the Statistical Package for Social Sciences version 22 (SPSS Inc., Chicago, IL). Arithmetic means and standard deviations were applied to describe continuous data, whereas frequency and percentages were applied for categorical data. Chi-squared tests were utilised to assess the associations between categorical data and unpaired t-test to test for the significant difference between two continuous variables. A p-value of less than 0.05 was considered for statistical significance.

Results

The study included 148 physicians, with a response rate of 85.06%. Physicians who were not in direct contact with patients (n = 6) and/or not practising diabetic management (n = 16) were excluded. Thus, a total of 126 physicians participated in the study. Of the participants, 53.17% and 46.83% were males and females, respectively, as shown in Table (1) with average age and experience years of 37 ± 3.68 and 13 ± 6.42, respectively. Only 9.52% of them have obtained a doctorate or Saudi Board degrees, and surprisingly, 2.38% of them received PM and/or Genomic training. The main source of their PM knowledge (57.14%) was the internet.

Table 1: General Characteristics of the Studied Physicians

Character	Health Facility				Total (N = 126)	
	Hospitals (N = 68)		Primary Health Care Centers (N = 58)			
	No.	%	No.	%	No.	%
Age (years): Mean± SD	39.46 ± 4.54		34.12 ± 2.68		37.00 ± 3.68	
			t = 8.17, P< 0.05			
Sex:						
Males	41	60.29	26	44.83	67	53.17
Females	27	39.71	32	55.17	59	46.83
			χ^2 = 0.34, P>0.05			
Highest Qualification:						
MBBS	15	22.06	38	65.52	53	42.06
Diploma	14	20.59	5	8.62	19	15.08
Master	28	41.17	14	24.14	42	33.34
Doctorate or Saudi Board	11	16.18	1	1.72	12	9.52
			χ^2 = 26.62, P< 0.05			
Experience years: Mean ± SD	16.16 ± 8.84		9.27 ± 3.57		13 ± 6.42	
			t = 5.89, P > 0.05			
PM and/or Genomic Training:						
Received	3	4.41	-	0.00	3	2.38
Did not receive	65	95.59	58	100.00	123	97.62
Source of PM Knowledge:						
Internet	39	57.35	33	56.90	72	57.14
Text books	10	14.71	12	20.69	22	17.46
Medical journals	11	16.18	4	6.89	15	11.91
In-service training	5	7.35	7	12.07	12	9.52
Others	3	4.41	2	3.45	5	3.97
			χ^2 = 0.45, P > 0.05			

It's obvious from Table 2 and Figure 1 that only 11.1% of participants have a good level of knowledge regarding important PM elements in DM management. Also, the fair and poor levels of knowledge among them were 6.4% and 82.5%, respectively.

Table 2: Personalized DM Medical Knowledge Assessment of the Studied Physicians

Personalised DM Medical Aspect	Studied Physicians' Knowledge (N. 126)			
	Correct Answer		Incorrect Answer	
	No.	%	No.	%
Definition	7	5.56	119	94.44
Rationale	10	7.94	116	92.06
Vision	8	6.35	118	93.65
Objectives	9	7.14	117	92.86
Components	9	7.14	117	92.86
Clinical Diagnostic Methods	26	20.63	100	79.37
Required laboratory investigations	22	17.46	104	82.54
Recent PM drugs	16	12.70	110	87.30
Most important defective genes	7	5.56	119	94.44
Recent Management Strategies	12	9.52	114	90.48
Overall Assessment:	No.		%	
Good	14		11.1	
Fair	8		6.4	
Poor	104		82.5	

It's clear from Table 3 and Figure 2 that nearly more than half (57.1%) of participants have positive attitudes towards important PM aspects in DM management.

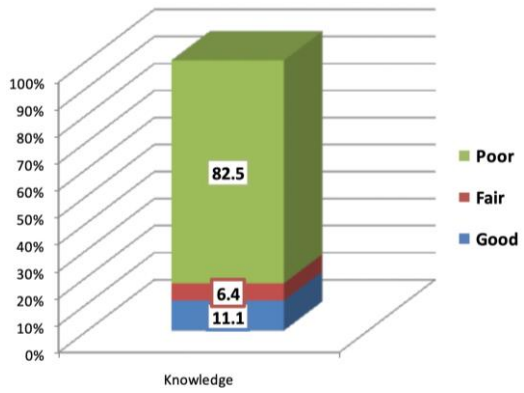

Figure 1: Overall Assessment of knowledge of Physicians Regarding Personalized Medicine in Diabetes Miletus Management

Also, one may notice that 64.29%, 29.37%, 27.78%, 22.22%, and 19.05% of them have an unfavourable attitude towards medical curricula sufficiency, widespread applicability, easy patients' accessibility, current achievements and current high costs of practising PM in DM management, respectively.

Table 3: Attitudes of the Studied Physicians towards Important Personalized DM Medical Aspects

Personalised DM Medical Aspect	Studied Physicians' Attitudes (N. 126)					
	Favourable		Undecided		Unfavourable	
	No.	%	No.	%	No.	%
Importance in Medical Progress	79	62.70	41	32.54	6	4.76
Physicians' Acceptance	102	80.95	20	15.87	4	3.18
Easy Patients' Accessibility	42	33.33	49	38.89	35	27.78
Current Achievements	48	38.10	50	39.68	28	22.22
Ethical Considerations	82	65.08	27	21.43	17	13.49
Wide Spread Applicability	38	30.15	51	40.48	37	29.37
Medical Curricula Sufficiency	9	7.14	36	28.57	81	64.29
Needed Research	100	79.37	15	11.90	11	8.73
Current High Costs	73	57.93	29	23.02	24	19.05
Future Mapping of Medicine	105	83.33	19	15.08	2	1.59
Overall Attitudes Assessment:		No.			%	
Favourable		72			57.1	
Un-decided		34			27.0	
Un-favorable		20			15.9	

Finally, the results indicate that 77% of participants have a good willingness degree regarding PM practising in DM management, with 90.48%, 82.54%, and 80.95% of them reporting that they agree to practice PM in DM management, looking for training on personalized diabetic management, and intersected in personalized diabetic management practicing, respectively, Table 4 and Figure 3.

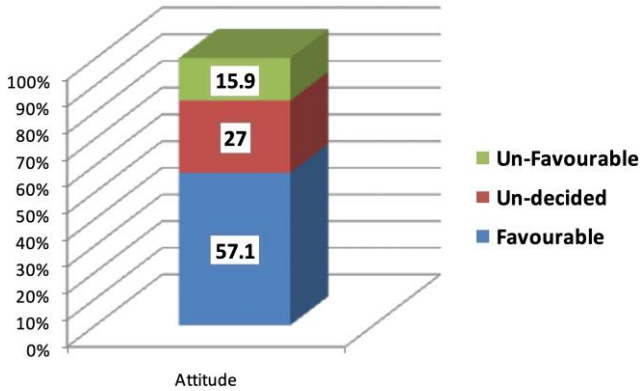

Figure 2: Overall Assessment of Attitudes of Physicians towards Personalized Medicine in Diabetes Miletus Management

Discussion

Because of the multifactorial and complex nature of diabetes mellitus, variability has been reported in the response of patients to most currently available oral hypoglycemics. Therefore, different guidelines recommended using a patient-specific approach and choosing management strategies that provide the patient with the most benefit and the least harm [22].

Table 4: Willingness Assessment of the Studied Physicians Regarding Personalized DM Medical Practice

Personalised DM Medical Aspect	Studied Physicians' Willingness (N. 126)			
	YES		NO	
	No.	%	No.	%
Intersected in PM Practicing	102	80.95	24	19.05
Agree to Practice PM	114	90.48	12	9.52
Ready to Learn PM Practicing Principles	92	73.02	34	26.98
Keen to have PM Practicing Degree	90	71.43	36	28.57
Looking for training on PM	104	82.54	22	17.46
Will search for recent PM topics-as needed	87	69.05	39	30.95
Disseminate PM materials on colleagues	71	56.35	55	43.65
Try to organize PM workshop(s)	48	38.10	78	61.90
Try to attend PM conference(s)	62	49.21	64	50.79
Eager to Practice PM	97	76.98	29	23.02
Overall Enthusiasms' Assessment:	No.		%	
Good	97		77.0	
Fair	22		17.4	
Poor	7		5.6	

Despite the existence of several studies investigating diabetes, there is a lack of evidence regarding knowledge, attitudes and practices of physicians and their influence on diabetes and its risk in the community [23].

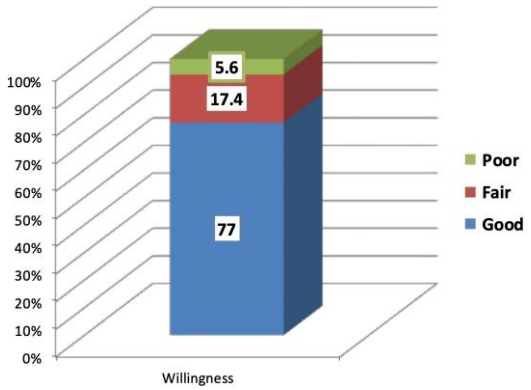

Figure 3: Overall Assessment of Physicians' Willingness to Practice Personalized Medicine in Diabetes Miletus Management

The World Health Organization (WHO) in 2016 recommended that countries with high diabetic prevalence, as the case in Saudi Arabia have to fight the rise in diabetes prevalence, to minimise diabetes-related premature deaths and to improve access to main diabetes therapies and basic technologies. Such commitment should address the key gaps in the diabetes knowledge base and conduct evaluation studies of innovative programs intended to change attitudes and behaviour [24]. Therefore this study was conducted to explore the knowledge, the extent of positive attitudes, and willingness of physicians to practice PM in diabetes management to provide sound and practical data for diabetic decision makers' program development.

Personalised treatment of diabetes based on patient genomic can reduce the number of therapy failures and reducing the rate of diabetic-related complications and consequently improve the patients` care [12]. However, in the present study, only 2.38% of surveyed physicians received PM and/or Genomic training.

With many advances in PM on the horizon, the researcher has expected that the PM knowledge of the studied physicians would be satisfactory and

increased exponentially. However, the current results showed a considerable very low gap in physicians' knowledge regarding the basic principles of personalised medicine as only 11.1% of physicians had a good knowledge regarding important personalised diabetic management elements. Also, insufficient knowledge was observed in another study carried among oncologists [25]. However, a better level of knowledge was reported in another study conducted by Chow-White P et al. among oncologists [26].

The practice of PM for diabetes (PMFD) includes 4 processes [27]. First, is the specification of genes and biomarkers for diabetes; Second, is the allocation of resources to early detect or prevent diabetes; Third, is the selection of personalised treatments for affected patients; Fourth, is the measurement of circulating biomarkers of diabetes to evaluate the response to prevention and/or therapy. It is important to notice that the detection of efficiency in anti-diabetic drug development is possible if genetically [28] or nutritionally [29] drug determinants are identified in patients with diabetes. Also, patients who are at high risk for diabetes, as evidenced by genetic testing can be subjected to preventive measures, such as alteration in lifestyle or therapies, to prevent or at least delay the diabetes development [30]. Thus, PM allows prescribing personalised drugs with less time lost with an inappropriate response or with side effects [31]. Unfortunately, in the present study, definition, rationale, vision, objectives, components, recent drugs, most important defective genes and recent management strategies were identified correctly by a minority of physicians.

Despite the lack of knowledge about personalised diabetic management reported among physicians in the present study, they expressed a favourable attitude towards important personalised diabetic management aspects as most of them had a favourable attitude towards physician`s acceptance. However, a considerable proportion of them had a favourable concern regarding high cost and ethical consideration. The results also indicated good willingness degree among physicians regarding personalised diabetic management practising, with the majority of them reported that they agree to practice personalised diabetic management, looking for training on personalised diabetic management, and intersected in personalised diabetic management practising. Recent studies also reported that physicians in the USA perceived their lack the knowledge and skills to incorporate genomic medicine in their practices; despite their positive attitude towards it [32], [33].

The present study has some important limitations. The cross-sectional design was merely descriptive without investigating the factors that could be associated with physicians` knowledge and willingness. Due to the limitations of international studies in that area, we could not sufficiently compare our findings with others. Also, the results were based on a self-administered structured questionnaire (quick, easy, participants answer at their convenience, and cost-efficient but may lack conscientious responses, differences in understanding and interpretation by participants and lack of personalisation). Therefore, carrying out the study only in Tabuk city could impact the generalizability of results. However, the transferability model of generalizability [34] can be adopted and applied in the Tabuk region and the northern parts of Saudi Arabia, given the proximal similarity of such geographical and demographic parts. Finally, we have to realise that both individual- and system-level factors likely contribute to differences by race and ethnicity in use and responses to PM practice as stressed by Kaphingst, and Goodman (2016) [35]. Thus, given the demographic and predominant consanguineous marriage pattern in KSA, it is of great importance to conduct national genomic studies and investigations to determine the needed PM areas for the Saudi population properly.

In conclusion, a high priority should be given to continuing medical education for physicians regarding essential personalised diabetic management knowledge. Additionally, discovering the positive attitudes and favourable willingness of physicians towards the application of PM in patient's care are promising and should be encouraged by decision makers.

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Modified Delphi Consensus on Developing Home Care Service Quality Indicator for Stroke Survivor in Yogyakarta, Indonesia

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Keywords: Home care; Modified Delphi; Indicator development; Quality service; Quality of care

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Abstract

BACKGROUND: Assessing the quality of health services provided at home (home care) is a challenge. The formulation of indicators requires open-minded people, who able to formulate several purposes objectively, and play an active role in decision making.

AIM: To test the face validity of the home care quality indicator in stroke patients with the modified Delphi method.

METHODS: Eighty-one indicators generated from previous studies were assessed using 3 processes to get the final results: 1) conducted modified Delphi in two rounds, namely rating or scoring by experts (using median scores); 2) reviewing qualitative suggestions from experts during the Delphi process (using comments from both Delphi rounds); 3) sorting out and correcting the grammar of the appropriate indicator (based on the median score > 7, and no disagreement).

RESULTS: Eighty-seven experts were involved in the first round Delphi and 34 experts in the second round. The experts were home care team selected from health care institutions in Yogyakarta with various professional backgrounds. Delphi process resulted in 67 indicators from 81 indicators which were divided into 10 domains: 1) Personal (2 indicators), 2) Documents (13 indicators), 3) Professionalism development (3 indicators), 4) Supporting facilities (8 indicators), 5) Administrative activities (4 indicators), 6) Health workers interaction with patients and families (15 indicators), 7) Physical conditions (2 indicators), 8) Self-actualization (1 indicator), 9) Psychological condition (5 indicators), 10) Family independent and coping (14 indicators). Selected indicators got to score more than 7 and no disagreement at all.

CONCLUSION: Sixty-seven indicators of the quality of home care, which were generated from modified Delphi consensus, were face validated. Further research could be conducted particularly on the trial process of these indicators at the actual home dwelling service setting.

Introduction

Efforts to assess the quality of health services and indicators that represent the quality assessment are still an extensive discussion until now. The formulation of indicators requires open-minded people, who able to formulate purposes objectively, play an active role in decision making, highly committed to achieving the highest standards of performance and willing to accept the suggestion, to create new ideas and methods [1].

Assessing the quality of health services provided at home (home care) is a challenge because of the many influencing environmental factors. In previous studies, the author has explored the expectations of stroke patients with home care, as a candidate indicator of home-based service outcomes (patient and family centred care) (unpublished articles). Although some previous publications have compiled indicators for home care services, the validity and reliability of the methods used are still low. So in this paper, the author begins the preparation of indicators with the involvement of patients and families besides the literature study, then the list of indicators obtained is requested for assessment by experts with the modified Delphi method.

The first home care quality indicator set (HCQIs) was issued by Inter-RAI, an international research consortium specialised in the development and application of standardised assessment instruments in 1913 [2]. Second generation HCQIs was developed in 2013, introducing several improvement indicators, including a more acceptable risk adjustment strategy and the addition of indicator domains [3]. This instrument proved to be applicable in 30 countries in America and Europe, but no one has mentioned its application, especially in Southeast Asia. It is necessary to develop indicators using recognised methods by minimising bias and taking from valid sources [4].

The main objective of the study was to identify and develop indicators to assess the quality of home care services with stroke home care quality indicators (SHCQI) through the consensus of experts who were able to contribute to the assessment of the quality of home care for stroke patients.

Methods

Eighty-one indicators produced from previous studies were assessed using 3 processes to get the final results: 1) conducted modified Delphi in two rounds, namely rating or scoring by experts (using median scores); 2) reviewing qualitative suggestions from experts during the Delphi process (using comments from both Delphi rounds); 3) sorting out and correcting the grammar of the appropriate indicator (based on the median score > 7, no disagreement). This study has received an ethical clearance letter from the Ethics Committee of the Faculty of Medicine, Public Health and Nursing, Gadjah Mada University.

Results

For Delphi Phase I, the author provided an instrument that contained indicators of the quality of home care services for stroke patients to experts involved in-home care services. The instruments contain 81 indicators. The instruments were filled independently by experts, starting in mid-February 2018 until the end of March 2018. The experts were asked to give a score on the indicator, from numbers 1 to 9 as well as comments on each item. A value of 1-3 means that the indicator had a role and significance that was not/less important to assess the quality of home care services, a value of 4-6 means that the indicator has an important role and significance to assess the quality of home care services, and a value of 7-9 means its indicator has a very important role and significance to assess the quality of home care services. The experts were all health workers at one hospital and two health centers, Yogyakarta, Indonesia as many as 70 experts.

A total of 81 indicators, along with scores given by 70 experts, were included in the excel program, as well as input/suggestions provided by experts. The scores were then analysed by the SPSS program to obtain the median value of each indicator. Only indicators with a median value of 7 to 9 were taken and will be used as potential indicators for Delphi Phase II (appropriate indicators).

For Delphi Phase II, the second version of the indicator list (the result of improvements from Delphi I) was taken to the discussion forum, which was attended by experts once again. The experts were asked to give scores, and comments on indicators with score criteria like in Delphi Phase I. Delphi Phase II emphasised the discussion process between experts so that all agreed on a particular score. If disagreements in giving scores or no agreement were found, then voting or taking the most votes was applied. The total experts involved in Delphi Phase II were 34 experts, from hospitals and health centres in Bantul Regency, Yogyakarta. This expert panel activity is carried out 4 times. These experts represent all health workers, consisting of specialist doctors, general practitioners, nurses, nutritionists, physiotherapists, and others. The expert characteristics of Delphi Phase I and Phase II are presented in Table 1.

Table 1: The expert characteristics of Delphi Phase I (N = 70) and Delphi Phase II (N = 34)

Profession and educational degree	Delphi I		Delphi II	
	n	F (%)	n	F (%)
Midwifery (Diploma 3)	3	4.3	2	5.9
Doctor				
Medical Specialist	3	4.3	1	2.9
General Practicioner	7	10	6	17.6
Postgraduate Master (Family Medicine)	1	1.4	1	2.9
Dentist (Undergraduate)	1	1.4		
Dietician				
Diploma 3	4	5.7	3	8.8
Undergraduate	1	1.4	1	2.9
Nurse				
Diploma 3	33	47.1	8	23.5
Diploma 4	1	1.4	1	2.9
Undergraduate	9	12.9	3	8.8
Health Promotion				
Undergraduate	1	1.4		
Postgraduate Master	2	2.8		
Public Health				
Undergraduate			1	2.9
Postgraduate			2	5.9
Dentist (Diploma 4)	3	4.3	1	2.9
Medical Analyst (Diploma 3)			2	5.9
Sanitarian (Diploma 3)			1	2.9
Psychologist (Postgraduate Master)	1	1.4	1	2.9
Gender				
Male	9	12.9	5	14.7
Female	61	87.1	29	85.3
Age				
Mean (SD)	36.8 (10.9)		37.7 (10.8)	
Median (min-max)	35 (21-60)		36 (23-60)	

Scores from 67 indicators of the second version and qualitative advice from experts were included in the Excel program and data were analysed through the SPSS program to find out the median of each indicator. Indicators with a median value of 7 to 9 (appropriate indicators) will be the final indicator of the quality of home care services for stroke patients. The indicator will be developed into a questionnaire

assessing the quality of home care services for stroke patients.

Most of the experts involved in-home care services were nurses, followed by doctors. Experts involved in Delphi Phase II were the same as experts in Delphi Phase I, but from 70 experts at the beginning only 34 experts were present at this Delphi Phase II, so the characteristics of experts in Delphi II were not much different from the Delphi I. The results of calculation of the median value of each indicator from Delphi I and Delphi II are presented in Table 2.

Table 2: Median value and indicator

	Delphi Phase I			Delphi Phase II		
No	Indicators	Median score	Categorize	Naration of indicator modification	Median score	Categorize
1	Officers involved in the home care team:			1. Officers involved in the home care team:		
	a. Medical spesialist	6	*Uncertained*	a. General Practitioner	8.5	*Appropriate*
	b. General Practitioner	7	*Appropriate*	b. Primary Nurse (minimum education Diploma 3 degree)	9	*Appropriate*
	c. Primary Nurse	8	*Appropriate*	c. Physiotherapist	8	*Appropriate*
	d. Physiotherapist	7	*Appropriate*	d. Dietician	8	*Appropriate*
	e. Dietitian	7	*Appropriate*	e. Psychologist	9	*Appropriate*
	f. Psychologist	6.5	*Uncertained*			
	g. Laboratory staff	6	*Uncertained*			
	h. Clergyman	6	*Uncertained*			
2	The home-care team is available 24 hours a day, 7 days a week for consultation via mobile phone	6	*Uncertain*	2. The home-care team conducts home visits within 6 working days and working hours.	8	*Appropriate*
3	The home-care team is available 7 days a week for home visits	6.5	*Uncertain*			
4	Special medical records available for home care patients	7	*Appropriate*	3. Special medical records available for home care patients	9	*Appropriate*
	The form that must be available in medical records:			Form that must be available in medical records:		
5	Assessment form,			4. Assessment form,		
	a. The general condition of patients and families: physical, psychological, social and spirituality	8	*Appropriate*	a. The general condition of patients and families: physical, psychological, social, spirituality and level of knowledge	9	*Appropriate*
	b. Pain	7.5	*Appropriate*	b. Pain	9	*Appropriate*
	c. Decubitus risk	8	*Appropriate*	c. Decubitus risk	9	*Appropriate*
	d. Fall risk	8	*Appropriate*	d. Fall risk	9	*Appropriate*
	e. Caregiver stress level	8	*Appropriate*	e. Caregiver stress level	8.5	*Appropriate*
6	Data analysis form	7	*Appropriate*	5. Data analysis form	9	*Appropriate*
7	Procedure form	7.5	*Appropriate*	6. Procedure form	9	*Appropriate*
8	Form evaluation of patient and family conditions	7	*Appropriate*	7. Form evaluation of patient and family conditions	9	*Appropriate*
9	A summary form of the patient's condition if the patient dies	7	*Appropriate*	8. A summary form of the patient's condition if the patient dies	8.5	*Appropriate*
10	The adverse event reporting form of the treatment performed	7	*Appropriate*	9. The adverse event reporting form of the treatment performed	9	*Appropriate*
11	Available forms of patient and family satisfaction levels for home care services	7	*Appropriate*	10. Available forms of patient and family satisfaction levels for home care services	8.5	*Appropriate*
12	A complaint form for patient or family complaints	7	*Appropriate*	11. There is a complaint form for patient or family complaints	9	*Appropriate*
13	Professional development of home care officers:			12. Professional development of home care officers:		
	Early home care training when accepted as a home care officer	8	*Appropriate*	a. Early home care training when accepted as a home care officer	9	*Appropriate*
14	Scientific activities (seminars, conferences) relating to case management at home care	7	*Appropriate*	b. Scientific activities (seminars, conferences) relating to case management at home care	8.5	*Appropriate*
15	Conduct research for the development of home care programs	6	*Uncertain*			
16	A regular schedule of meetings between home-care members to discuss patient care plans	7	*Appropriate*	13. Regular schedule of meetings between home-care team members at least once a month, to discuss patient care plans	8.5	*Appropriate*
	Supporting facilities in home care			Supporting facilities in home care		
17	Availability of information (leaflets) about home care services	7	*Appropriate*	14. Availability of information (leaflets) about home care services	7.5	*Appropriate*
18	There is room for discussion between home care teams	7	*Appropriate*	15. There is room for discussion between home care teams	8	*Appropriate*
19	Availability of educational media	7	*Appropriate*	16. Availability of educational media/health education, for example, leaflets that are by the care needed by the patient	9	*Appropriate*
20	The minimum equipment that is brought on to the patient's home			17. The minimum equipment that is brought on to the patient's home		
	a. Sphygmomanometer and stethoscope	8	*Appropriate*	a. Sphygmomanometer and stethoscope	9	*Appropriate*
	b. Weight Scales	5	*Uncertain*	b. Penlight	9	*Appropriate*
	c. Pen light	7	*Appropriate*	c. Reflex Hammer	8	*Appropriate*
	Administrative activities for implementing home care:			Administrative activities for implementing home care:		
21	The home care team visits the patient's home according to the agreed schedule	8	*Appropriate*	18. The home care team visits the patient's home according to the agreed schedule	9	*Appropriate*
22	Clinical audits are part of a quality improvement program	7	*Appropriate*	19. Clinical audits are part of a quality improvement program	6	*Uncertain*
23	All adverse events are reported and documented in medical records	8	*Appropriate*	20. All adverse events are reported and documented in medical records	8.5	*Appropriate*
24	The process of managing patient or family complaints is documented	7	*Appropriate*	21. The process of managing patient or family complaints is documented	8.5	*Appropriate*
25	The officer fills out the medical record each home care visit	7	*Appropriate*	22. The officer fills out the medical record every time a home care visit	9	*Appropriate*
26	The clinical summary of the patient is filled in a medical record after the patient has quit the homecare program or dies	7	*Appropriate*	23. The clinical summary of the patient is filled in at RM after the patient has quit the homecare program or dies	8	*Appropriate*
	Officer interaction with patients and families:			Officer interaction with patients and families:		
27	Health workers ask complaints and desires of patients and families	8	*Appropriate*	24. Health workers ask complaints and desires of patients and families	8	*Appropriate*
28	Health workers check vital signs	8	*Appropriate*	25. Health workers check vital signs	9	*Appropriate*
29	Health workers review/evaluate patient pain	8	*Appropriate*	26. Health workers review/evaluate patient pain	9	*Appropriate*
30	Health workers assess/evaluate the risk of decubitus/pressure sores in patients	8	*Appropriate*	27. Health workers assess/evaluate the risk of decubitus/pressure sores in patients	9	*Appropriate*
31	Health workers assess/evaluate the risk of falling in patients	8	*Appropriate*	28. Health workers assess/evaluate the risk of falling in patients	9	*Appropriate*
32	Health workers check the physical condition of patients and families	8	*Appropriate*	29. Health workers check the physical condition of patients and families	9	*Appropriate*
33	Health workers review / evaluate the psychological condition of the patient	7	*Appropriate*	30. Health workers review / evaluate the psychological condition of the patient	9	*Appropriate*
34	Health workers review / evaluate the social conditions of patients and families	7	*Appropriate*	31. Health workers review / evaluate the social, economic and cultural conditions of patients and families	7.5	*Appropriate*
35	Health workers review / evaluate patient and family spirituality	7	*Appropriate*	32. Health workers review / evaluate patient and family spirituality	7	*Appropriate*
36	Doctors review the medication that patients receive regularly	8	*Appropriate*	33. Doctors review the medication that patients receive regularly	9	*Appropriate*
37	Health workers measure the patient's weight	6	*Uncertain*			
38	Health workers assess the independence of patients with the Barthel Index	7	*Appropriate*	34. Health workers assess the independence of patients with the Barthel Index	9	*Appropriate*
39	Health workers convey conditions and plans for nursing to families and patients clearly and language that is easy to understand and friendly	8	*Appropriate*	35. Health workers convey conditions and plans for nursing to families and patients clearly and language that is easy to understand and friendly	9	*Appropriate*
40	Health workers provide opportunities for patients and families to consult	8	*Appropriate*	36. Health workers provide opportunities for patients and families to consult	9	*Appropriate*
				37. Health workers give medical procedure according to a problem found (based on data analysis results)	9	*Appropriate*
	Fulfilling the needs of daily activities / ADL:					

41	The patient can carry out activities on the bed, such as moving from a lying position, tilting right and left, and positioning the body when in bed.	7.5	*Appropriate*	38. The patient's ability/independence to carry out daily activities / ADL increases	8.5	*Appropriate*
42	The patient can walk in a flat place; if they use a wheelchair, they are still used	7	*Appropriate*			
43	Patients can walk the stairs	6	*Uncertain*			
44	The patient can carry out activities in small rooms such as using a washroom or bedpan or urinal, walking to and from the bathroom, cleaning the bathroom after using/flushing the toilet, changing diapers and arranging all the equipment needed.	7	*Appropriate*			
45	The patient can wear and take off the clothes	7	*Appropriate*			
46	The patient can control micturition	7	*Appropriate*			
47	The patient can control defecation	7	*Appropriate*			
48	The patient can self-care, such as combing hair, brushing teeth, shaving facial hair, dressing up, washing hands and face	7	*Appropriate*			
49	The patient can bath and wash the whole body	7	*Appropriate*			
50	The patient can take a meal by his/her self; regardless of the eat technique including tube feeding	7	*Appropriate*			
51	The patient takes medicine according to the prescription by the Doctor	7.5	*Appropriate*			
52	The patient controls or follows up the medical condition according to the schedule	8	*Appropriate*			
53	The home-care patient does not acquire complications in the following:			39. The home-care patient does not acquire complications as follows:		
	a. Pneumonia	7	*Appropriate*	a. Pneumonia	6.5	*Uncertain*
	b. Urinary tract infection	7	*Appropriate*	b. Urinary tract infection	6.5	*Uncertain*
	c. Post-stroke pain	7	*Appropriate*	c. Post-stroke pain	7	*Appropriate*
	d. Deep vein thrombosis	7	*Appropriate*	d. Deep vein thrombosis e. Hemiparesis	6	*Uncertain*
	The home care-patient performs the following social activities according to his/her capability			The home care-patient performs the following social activities according to his/her capability	4	*Uncertain*
54	The patient can re-perform his/her most favourite hobby	7	*Appropriate*	40. The patient can re-perform his/her most favourite hobby	4	*Uncertain*
55	The patient can carry out the activity in the community	7	*Appropriate*	41. The patient can re-perform his/her most favourite hobby	5.5	*Uncertain*
56	The patient can gather and play with children or grandchildren	7	*Appropriate*	42. The patient can gather and play with children or grandchildren	7	*Appropriate*
57	The patient can visit relative's house	6.5	*Uncertain*	43. The patient can gather and play with children or grandchildren	5	*Uncertain*
58	The patient can perform praying	7	*Appropriate*	44. The patient can perform praying on the bed or in other places	9	*Appropriate*
	The psychological status of the home care-patient should be:			The psychological status of the home care-patient should be:		
59	The patient expresses happiness to live his/her life	7	*Appropriate*	45. The patient expresses happiness to live his/her life	7.5	*Appropriate*
60	The patient expresses expecting live long	7	*Appropriate*	46. The patient expresses expecting live long	7.5	*Appropriate*
61	The patient expresses a strong belief to heal	7	*Appropriate*	47. The patient expresses a strong belief to heal	7.5	*Appropriate*
62	The patient expresses having a harmonic relationship with the other family members	7	*Appropriate*	48. The patient expresses having a harmonic relationship with the other family members	7	*Appropriate*
63	The patient expresses accepting his/her medical condition	8	*Appropriate*	49. The patient expresses accepting his/her medical condition	8	*Appropriate*
64	The patient expresses no regret in his/her medical condition	7	*Appropriate*	50. The patient expresses no regret in his/her medical condition	7.5	*Appropriate*
65	The patient expresses no fear or worry in his/her medical condition	7	*Appropriate*	51. The patient expresses no fear or worry in his/her medical condition	7.5	*Appropriate*
66	The patient expresses the capability to hold anger	7	*Appropriate*	52. The patient expresses the capability to hold anger	7.5	*Appropriate*
67	The patient expresses committing no stress	7	*Appropriate*	53. The patient expresses committing no stress	7.5	*Appropriate*
68	The patient expresses committing no depression	7	*Appropriate*	54. The patient expresses committing no depression	7.5	*Appropriate*
69	The patient expresses being happier to outhouse activity than in-house activity	7	*Appropriate*	55. The patient expresses being happier to outhouse activity than in-house activity	8	*Appropriate*
70	The patient expresses no inferior feeling in his/her medical condition	7	*Appropriate*	56. The patient expresses no inferior feeling in his/her medical condition	8	*Appropriate*
71	The family asks/consult to the health worker about:			57. The family asks/consult to the health worker about:		
	a. The patient's diet	8	*Appropriate*	a. The patient's diet	9	*Appropriate*
	b. At home-training procedure	8	*Appropriate*	b. At home-training procedure	9	*Appropriate*
	c. The patient's medicines	8	*Appropriate*	c. The patient's medicines	9	*Appropriate*
	d. Follow up schedule	8	*Appropriate*	d. Follow up schedule	9	*Appropriate*
	e. The problems/burden carried out	8	*Appropriate*	e. The problems/burden carried out	7	*Appropriate*
	Role of the family in taking care of the patient at home			At-home role of the family in looking after the patient at home		
72	The family reminds the patient to take medicines	8	*Appropriate*	58. The family reminds the patient to take medicines	9	*Appropriate*
73	The family reminds the patient about follow up schedule	8	*Appropriate*	59. The family reminds the patient about follow up schedule	9	*Appropriate*
74	The family accompanies the patient during follow up	8	*Appropriate*	60. The family accompanies the patient during follow up	9	*Appropriate*
75	The family prepares the allowed food for the patient	8	*Appropriate*	61. The family prepares the allowed food for the patient	9	*Appropriate*
76	The family helps ROM training at home	8	*Appropriate*	62. The family helps ROM training at home	8.5	*Appropriate*
77	The family encourages the patient	8	*Appropriate*	63. The family encourages the patient	9	*Appropriate*
78	The family accompanies and listens to the patient's talk or complaint	8	*Appropriate*	64. The family accompanies and listens to the patient's talk or complaint	8	*Appropriate*
	To reduce the psychological burden, the family needs to do some of the following acts:			To reduce the psychological burden, the family needs to do some of the following acts:		
79	The family shares the feeling or problems to the other member, such as children, relatives	7	*Appropriate*	65. The family shares the feeling or problems to the other member, such as children, relatives	8	*Appropriate*
80	The family takes recreation	7	*Appropriate*	66. The family takes recreation	7	*Appropriate*
81	The family checks up to the medical condition to the health service	8	*Appropriate*	67. The family checks up to the medical condition to the health service	8.5	*Appropriate*

Based on Table 2, we can observe that there are 10 indicators determined by the professionals as uncertain (median < 7) as the instruments for assessing the quality of home care services. Therefore they were eliminated from the list. Based on the expert's suggestion on the appropriate indicators, we revised the order of the sentences, add items for the indicator, and merge several indicators into one indicator item which was considered more proper. The result of the indicators revision was presented in the column of the modified indicators sentences. The next processes were grammar improvement of the appropriate indicators, the addition of 2 new indicators, and merge of 12 indicators about daily living activities, based on the expert's suggestions or inputs. At the end of Delphi Phase I, we obtained 67 indicators. Then the expert in an expert panel discussed and reassessed these 67 items. The discussion resulted in 54 appropriate indicators for home care quality (Table 3).

Discussion

The achievement on an indicator implies the quality of service. According to the quality management theory of Donabedian, the quality of service required three aspects: structure, process, and output [5].

Table 3: List of the face validity indicators according to Delphi Phase II

Category	Domain	No	Face validity Indicators
Structure	Personal	1	The Health Officers included in a home care team:
			a. General Physician
			b. Nurse in charge of a patient with a minimum education of Diploma 3
			c. Medical rehabilitation staff
			d. Nutritionist
			e. Psychologist
		2	Home care team carries out home visit corresponding to the agreement between the team and the patient
	Documents	3	Availability of home care complementary forms inside the patient's medical record
			Home-care complementary forms inside the medical record
		4	Form of assessment,
			a. General condition of the patient: physical, psychological, social, spiritual, and knowledge level
			b. The general condition of the family: knowledge level and assets/resources map in the family
			c. Pain
			d. Risk of decubitus
			e. Risk of fall
			f. The stress level of the family and the family caregiver
		5	Form of data analysis
		6	Form of the treatment record
		7	Form of evaluation/development of the patient and the family condition
		8	Form of patient condition resume if the patient died
			The other complementary forms and separated from the medical record:
		9	Form of adverse events reporting
		10	Form of satisfaction level of the patient and the family toward the home care service
		11	Form of the patient or the family complaints
	Professionalism	12	Professional development for the home caregiver:
			a. Briefing/orientation about home care in the first days becoming home care officer
			b. Scientific activities (seminar, conference) related to the home care case management
		13	Regular inter-home care team member schedules and coordination forums to discuss the patient plan of care
	Facilities		Supporting facilities for home care:
		14	Availability of information (leaflet) about home care service
		15	Availability of discussion room for home care team member
		16	Availability of education media/health education, including leaflet suitable to the care needed by the patient
		17	Minimum instruments availability during a home visit
			a. Sphygmomanometer and stethoscope
			b. Measuring band
			c. Penlight
			d. Reflex hammer
			e. Minor surgery set
Process	Administration process		Administrative activities during home care implementation:
		18	All adverse events are reported and recorded in the medical record
		19	Documentation of the maintenance process of the patient and the family complaints
		20	The officer fills out the medical record each home care visit
		21	The patient's clinical resume fulfilled in the medical record after the patient discontinues the service or died
	Interaction process		Interaction between the officer and the patient and the family:
		22	The health officer asks the desires or complaints of the patient and the family
		23	The health officer examines the vital signs
		24	The health officer assesses/evaluates the patient pain
		25	The health officer assesses/evaluates the risk of decubitus/wounds in the patient
		26	The health officer assesses/evaluates the risk of fall in the patient
		27	The health officer examines the physical status of the patient
		28	The health officer assesses/evaluates the psychological status of the patient and the family
		29	The health officer assesses/evaluates the social, economic, cultural status of the patient and the family
		30	The health officer assesses/evaluates the spiritual status of the patient and the family
		31	The doctor regularly reevaluates the medicines received by the patient
		32	The health officer assesses the nutritional status of the patient
		33	The health officer assesses/evaluates the level of independence of the patient and the family
		34	The health office delivers the care status and plans to the family and the patient in clear, detail, hospitable, and understandable sentences
		35	The health officer opens a session for the patient and family to consult
		36	The health officer gives the care according to the factual problems (based on the data analysis result)
Output	Physical well-being	37	The capability/independence of the patient to perform a daily living activity is not declined
		38	The home care patient does not complicate the following condition:
			a. Post stroke pain
	Self-actualisation		Socially, the home care patient performs the following activities according to his/her capability:
		39	The patient is sociable with the children or grandchildren
	Psychological state	40	The patient can pray
			The psychological status of the home care patient includes the following condition:
		41	The patient expresses sincerely and patiently accepting his/her medical condition
		42	The patient has a real motivation in life
		43	The patient expresses the harmonic relationship between the patient and the family members
		44	The patient feels glad during outhouse activity and does not expect to be alone
	Family independent and coping	45	The family consults to the health officer about:
			a. The patient's diet
			b. The home training procedure
			c. The medicines are taken by the patient
			d. The follow-up schedule of the patient
			e. The problems/burdens acquired
			The role of the family at home:
		46	The family reminds the patient of the time to take medicine
		47	The family reminds and accompanies the patient to health check
		48	The family prepares the allowed foods for the patient
		49	The family helps the patient doing ROM (range of motion) training at home
		50	The family encourages the patient
		51	The family accompanies and listens to the patient's talk and complaint
			To reduce the mental burden, the family can do these following acts:
		52	The family shares the problems to the other members, such as children, relatives
		53	The family takes recreation
		54	The family checks up to the medical condition to the health service

An approach to the structure and process founded by Donebedian turned out to be one of the references mostly used to assess the service quality. It was proven by Kajonius's research which compared between a nursing home and home care. There were 35 indicators used in this survey. The indicators of structure used were the costs per elderly, the staffing, and the training; the indicators of the process which were studied included the respect, information, influence (allowing the autonomy). The number of elderlies who expressed respect was larger in the elderly acquiring home care than a nursing home. There was no component of structure correlated significantly to the satisfaction of the elderlies (correlation test showed 0 to weak correlation), while all components of process correlated significantly to the satisfaction of the elderlies (correlation test showed a moderate to strong correlation) [6].

The indicators establishment in this study utilised the modified Delphi consensus, which had been recognised as a valid method [7]. The modified Delphi method, also known as the RAND/UCLA Appropriateness Method (RAM), initially aimed to ensure the effectiveness of a health intervention given to patients and to be the main instrument in assessing the accuracy and inaccuracy of a medical or surgical procedure, but currently its use is broader for all health fields. RAM emphasises the determination of indicators based on the degree of benefits and losses that the patient will receive (appropriateness).

The other method conducted by Scaccabarozzi studied on the assessment of end of life service quality in a home palliative care using the method of Rasch analysis. This identified 5 indicators easy to use by the health care providers: "interview with the caregivers, sustainable training for the medical and nursing staffs, intervention by multidisciplinary specialists, psychological support to the patient and family, supply of medicines at home) and identified 3 problematic indicators (the availability of regulation on local network of palliative care as the reference, the needs on the care in most of the problematic patients who needed high-intensity care, and the percentage of cancer patient died at home) [8]. This method of analysis was able to reveal which indicators could be achieved and which indicators that needed extra efforts to be achieved. The analysed indicators in this study were mostly indicators of process. The patient's expectation to die at home was assumed as an unsuccessful indicator. It correlated to the operational and organisational aspect which correlated to the inability to develop a structure which can ensure comprehension between the governmental pathway and the care continuity.

The other method to assess the service quality was Outcome Assessment and Information Set (OASIS), which was used to measure the quality and plan of home care in the US. This instrument had a lower to moderate validity and reliability value, as well as the implementation in measuring outcome or outcome-based quality improvement was debatable [9].

First set of indicators of home care quality (HCQIs) was established by Inter-RAI (The Resident Assessment Instrument). The advantages of interRAI HCQIs use included more standardised items of assessment, a more comprehensive set of indicators, and a better capacity to provide group measuring from the different HCQI compared to individual measuring. These were useful to provide a complete evaluation of the service quality. HCQI second generation consisted of 23 indicators that included 8 functional indicators, 10 clinical indicators, 5 social and medication indicators [3].

The quality in the health service standards and indicators recommended in United States of America and Australia included effectiveness, efficiency, safety and risk, timeliness, equity, and person and family-centred care, which offered advantage and guideline to achieve optimal health status for elderly, as well as to optimize transitional care from hospital to home.

Allen studied the quality indicator of outcome in transitional care (post-discharge care) for older people and their caregivers transferring from hospital to home. Indicator of outcome included effectiveness (based on evidence and given to the right patient), efficiency (effective care, time, cost, and resource), timeline (on time), safety and risk (a care that carried out lower risk and no harm), equity (a fair care for everyone), person and family-centred care and experience (respecting expectation, value, objective of the patient and family, inviting the patient and family in decision making) [10].

A critical review on evidence needed expertise from the people who understood the matter of evidence-based medicine, in another hand an assessment on quality on stroke patient home care needed people who concerned in-home care service and neurology [11]. Therefore, we convincedly stated that indicators resulted from this process were appropriate and valid. The indicators could be a minimum criterion with consideration on evidence, synthesis and critical process.

In conclusion, the modified Delphi process enabled the elimination of an initial list of 81 candidate indicators to the final list of 54 candidate indicators. This process was involving 70 experts from different professional backgrounds. The final list of candidate indicators will be useful as a guide to identifying the quality service of stroke survivors at home dwelling care.

This research recommended further research to test the feasibility of the established criteria, including a test on content validity, construct validity, and instrument reliability. The outcome from the established indicators needed a high consistency. Hence the analysis of the correlation between

indicators scores obtained by the trial of indicators implementation could be able to strengthen the validity of the indicators.

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Review Article

Hepatitis E Infection in Nigeria: A Systematic Review

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Abstract

BACKGROUND: Research done globally on hepatitis E virus (HEV) infection is far fewer compared with other types of hepatitis virus infection. Little is known on the prevalence of HEV in Nigeria.

AIM: The present study presents the prevalence of HEV infection in Nigeria from a few available research papers on HEV. The detailed statistical analysis was used to analyse the prevalence of HEV in humans and animals.

MATERIAL AND METHODS: A literature search in Web of Science, Scopus and PubMed databases was done, and a final 7 articles were selected. Minitab 17.0 was used to perform the correlational and binary logistic analyses.

RESULTS: Serum and faecal analysis of blood and stool samples of 1178 humans and 210 pigs (animals) were done, and the presence of anti-HEV IgG or HEV RNA in the study samples were 127 and 138 respectively. Further analysis showed the prevalence of HEV are 10.8% and 65.7% in humans and animals, respectively. Weak positive non-significant association (r = 0.327, p-value = 0.474) was obtained between the target (humans and animal) and the HEV infection (positive) groups. The application of binary logistic regression yielded an equation that can be used to predict the target group from the HEV positive humans or animals. Generally, the logistic model was not statistically significant (p-value = 0.376), and the model was able to explain 9.3% of the deviation or variability of the model. The odds ratio is OR = 1.0344 with 0.9550, 1.204 95% Confidence Interval (CI). Thus, in Nigeria, the odds of prevalence of HEV in animals are 1.0344 higher than humans.

CONCLUSION: The risk factors obtained from the few available articles are consistent with the global epidemiology of HEV infection. Food and animal handlers and those that consume unsafe water are the key people at risk of HEV infection in Nigeria.

Introduction

Hepatitis E is an inflammatory liver disease caused by a non-enveloped positive-sense [1] and single-stranded ribonucleic (RNA) genome called the hepatitis E virus (HEV). The virus is in four different types identified as genotypes 1, 2, 3 and 4. Genotypes 1 and 2 have been isolated in humans while 3 and 4 are found in animals. HEV is transmitted mainly through faecal contamination of drinking water and uncooked foods. Generally, the risk factors of the disease include lack of access to cheap, safe and potable drinking water, unhygienic living conditions and indiscriminate disposal of human wastes, poverty, the insecurity that results to a strain on access to basic life amenities and limited access to quality health services [2]. Symptoms of the disease include mild fever, anorexia, nausea, jaundice, dark urine, pale stools and slight liver inflammation, although, rare cases have been reported to result to fulminant hepatitis in pregnant women [3], [4]. Diagnosis of HEV infection includes detection of the HEV in stool or serum samples and serological tests for identification of anti-HEV Ig G and Ig M [1]. Measures to be taken to eliminate the risk factors are consistent supply of

clean and safe water, improving personal hygiene and ensuring proper disposal of human wastes.

Hepatitis E is one of five known human hepatitis viruses. Others are A [5], B [6], [7], C [8], [9], [10] and D [11]. The prevalence of HEV is very low compared with the others, especially hepatitis B virus (HBV). Numerous articles that investigated different aspects of HBV infections in Nigeria [12], [13], [14] and few papers are available for HEV infection.

This paper aims to present a statistical analysis of a few articles published on the prevalence of HEV infection in Nigeria. The few papers are traceable to the low prevalence of the disease in the world of which the studied country is a particular case. A similar investigation has been done and can be found in [15], [16], [17], [18], [19], [20], [21].

Material and Methods

Study Design

The study design is Web of Science, Scopus and PubMed databases. The keywords "Hepatitis E" OR "HEV" AND “Nigeria” were queried in Scopus, PubMed and Web of Science databases. PubMed, Scopus and Web of Science (WOS) returned 301, 66 and 11 articles respectively. Further evaluation showed that the articles in WOS are also contained in Scopus and are littered with hepatitis B and studies on areas not related to Nigeria. A final seven (7) articles were sieved out after all the unrelated articles were removed.

Eligibility Criteria

The papers that contained the prevalence of HEV in their abstracts were included. Risk of bias was almost nonexistent since the sample size is small.

Ethics Statement

No ethical rules were violated. All the sources of information were duly acknowledged.

Statistical Analysis

The absence of numerous studies places undue restrictions on the use of different statistical techniques. The statistical techniques are needed to reveal hidden patterns as long as the sample size is appreciably high [22], [23], [24], [25], [26], [27], [28]. The 7 articles were analysed using Minitab version 17.0, binary logistic regression was used for the analysis, and this was as a result of splitting of the target groups into two distinct and non-overlapping classes. P-value of 0.05 was considered significant.

Results

The summary of the data presentation of the 7 articles [29], [30], [31], [32], [33], [34], [35] are presented in Table 1. The publication span 10 years. It can be seen that (6/7) of both the methodology and diagnostics (tool for analysing serum and faecal samples) used in the articles are cross-sectional studies and enzyme-linked immunosorbent assay (ELISA) technique respectively.

Table 1: Studies on hepatitis E virus infection in Nigeria

Author	Publication Year	Methodology	Target Group	Diagnostics	Sample size	HEV positive
[29]	2008	Observation	Hospitalized	Therapy	1	1
[30]	2013	Cross sectional	Non-hospitalized	ELISA	132	36
[31]	2014	Cross sectional	Pigs	ELISA	90	69
[32]	2014	Cross sectional	Non-hospitalized	ELISA	462	43
[33]	2015	Cross sectional	Non-hospitalized	ELISA	406	31
[34]	2018	Cross sectional	Pigs	ELISA	120	69
[35]	2018	Cross sectional	Non-hospitalized	ELISA	177	16

Prevalence of HEV in the samples

HEV positive means the presence of anti-HEV IgG or HEV RNA in the study samples. The prevalence for each case is obtained using the dataset of Table 1, by dividing the total number of HEV positive and the total number investigated and multiplying by 100. This is presented in Table 2.

Table 2: Prevalence of the two types of HEV infection in Nigeria

	Humans	Animals
Total investigated	1178	210
HEV positive	127	138
Prevalence	10.8%	65.7%

Correlation Analysis

Correlation coefficient was obtained between the target group (humans = 0, animal = 1) and the HEV positive and it was computed to be (r = 0.327, p = 0.474).

Binary Logistic Regression

Binary logistic regression was performed to determine the association between the target group y (humans = 0, animal = 1) and the HEV positive (x).

Table 3: Deviance table

Source	DF	Adj Dev	Adj Mean	Chi-Square	P-Value
Regression	1	0.7842	0.7842	0.78	0.376
Error	5	7.5915	1.5183		
Total	6	8.3758			

This is to determine if the prevalence of HEV can predict whether the target group is animal or human.

Table 4: Model Summary

Deviance Deviance R-Sq	R-Sq(adj)	AIC
9.36%	0.00%	11.59

The details are presented in Tables 3 to 7, and the logistic regression equation is Exp(y) = -2.33+0.338x.

Table 5: Coefficient of the model

Term	Coef	SE Coef	VIF
Constant	-2.33	2.04	
x	0.0338	0.0407	1.00

Discussion

The few available studies on HEV infection in Nigeria is due to the less severe nature of the disease when compared with Hepatitis B infection. The low research activities on HEV can also be as a result of the low incidence of the disease, self-medication, the use of traditional or alternative medicine and non-hospitalization. Hospital records about the HEV infection will encourage cross-sectional and retrospective studies.

Table 6: Odds Ratios for Continuous Predictors

	Odds Ratio	95% CI
x	1.0344	(0.9550, 1.1204)

The risk factors available from the few articles are consistent with the global epidemiology of HEV infection. The risk factors are faecal contamination of water and food, improper disposal of human and animal wastes, poverty, unhygienic environment and inadequate access to quality healthcare services.

Table 7: Goodness-of-Fit Tests

Test	DF	Chi-Square	P-Value
Deviance	5	7.59	0.180
Pearson	5	6.61	0.252
Hosmer-Lemeshow	4	4.60	0.330

Serum and faecal analysis of blood and stool samples of 1178 humans and 210 pigs (animals) were done, and the presence of anti-HEV IgG or HEV RNA in the study samples were 127 and 138 respectively. Further analysis showed the prevalence of HEV are 10.8% and 65.7% in human and animals, respectively. The high level of the prevalence in animals calls for urgent action to avoid infections to human via faecal oral transmission.

There is a weak positive non-significant association between the target groups (humans and animals) and the HEV infection (positive). This is the outcome of the prevalence that showed that the prevalence of both groups is different; that is, high for animals and low for humans.

The application of binary logistic regression yielded an equation that can be used to predict the target group from the HEV positive humans or animals. Generally, the logistic model was not statistically significant (p-value = 0.376), and the model was able to explain only 9.3% of the deviation or variability of the dependent variable. The odds ratio was gotten to be; OR = 1.0344 (0.9550, 1.204) 95% CI. Thus, in Nigeria, the odds of prevalence of HEV in animals are 1.0344 higher than humans.

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Review Article

Dietary Fibre Protective against Colorectal Cancer Patients in Asia: A Meta-Analysis

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Keywords: Asia; Colorectal cancer; Dietary fibres; Meta-analysis; Protective

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Abstract

BACKGROUND: The association between dietary fibre and colorectal cancer risk is controversial.

AIM: This systematic review and meta-analysis were performed to determine the dietary fibre protective against colorectal cancer patients in Asia.

METHODS: The authors conducted a meta-analysis of published research articles on dietary fibre protective against colorectal cancer patients in Asia published between January 2000 and March 2019 in the online article databases of PubMed, ProQuest and EBSCO. Pooled odds ratios (OR) were calculated with fixed and random-effect models. Publication bias was visually evaluated by using funnel plots and statistically assessed through Egger's and Begg's tests. Data were processed using Review Manager 5.3 (RevMan 5.3) and Stata version 14.2 (Stata Corporation).

RESULTS: This study reviewed 405 articles. There are 10 studies conducted a systematic review and continued with Meta-analysis of relevant data with several sample 49,964 patients. The results showed dietary fibre protective against colorectal cancer patients in Asia (OR = 0.66 [95% CI 0.56-0.77, p=0.008]). There was significant publication bias for studies included in dietary fibre protective against colorectal cancer patients in Asia.

CONCLUSION: This analysis confirmed dietary fibre protective against colorectal cancer patients in Asia.

Introduction

Colorectal cancer is cancer that starts in the colon or the rectum. Colorectal cancer is one of the most common malignancies present in the world, ranks the third most frequent malignant disease diagnosed in the United States and fourth in Asia [1], [2]. Based on data from the World Health Organization, colorectal cancer is increasing rapidly in Asian countries. In Southeast Asia, in 2008, there are an estimated 1.6 million new cases of colorectal cancer and 1.1 million deaths [3].

Based on colorectal cancer risk factors are differentiated into nonmodifiable and modifiable risk factors. Nonmodifiable risk factors: age, race, genetics, family history, history of tumours, and ulcerative colitis. Modifiable risk factors: lifestyles such as cigarette use, low physical activity, long-term alcohol consumption, and bad dietary patterns [1], [4], [5].

Diet is a food choice that is commonly eaten by a person or population. Diet estimated an effect 30%-50% incidence of colorectal cancer worldwide. One of the dietary factors that contribute to colorectal cancer is a low-fibre diet. Dietary fibre is inversely proportional to the risk of colorectal cancer; high dietary fibre is evident in the prevention of colorectal cancer [6], [7].

The low-fibre diet is associated with an

elevated risk of colorectal cancer [7]. However, due to wide geographical variation, the demography of colorectal cancer patients differs from those between developed and developing countries. This happens changes in dietary patterns and lifestyle, possibly due to globalisation and improving economic status, as well as the availability of screening programme may account for the risk of colorectal cancer.

Several previous studies have shown that dietary fibre is associated with a reduced risk of colorectal cancer [8], [9], [10]. But these results have no effect of dietary fibre for colorectal cancer; high dietary fibre intake was not associated with a reduced risk of colorectal cancer [11], [12]. However, the research results are not always consistent. Therefore, this study aims to determine the association of dietary fibre protective against colorectal cancer patients in Asia with some research through the Meta-analysis study so that the conclusion drawn have stronger strength. This leads to an increase in prevention, detection strategies in colorectal cancer patients.

Material and Methods

Study design and research sample

This research is quantitative research with meta-analysis study design. The meta-analysis followed the preferred reporting items for Systematic Reviews and Meta-Analysis (PRISMA) statement [13]. Meta-analysis was used to figure dietary fibre protective against colorectal cancer patients in Asia. The research samples were published research articles published between January 2000 and March 2019 in online article databases of PubMed, ProQuest and EBSCO.

Operational definitions

The variables of this study included independent variables consisted of dietary fibre (fruit, vegetables, cereals); and dependent variable: colorectal cancer.

Research procedure

This study was conducted by collecting data through the identification of published research articles on dietary fibre protective against colorectal cancer patients in Asia in online article databases of PubMed, ProQuest and EBSCO (Figure 1).

Identification of 405 articles, done by review 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) not case-control and cohort study (c) the information provided in the results were insufficient for data extraction.

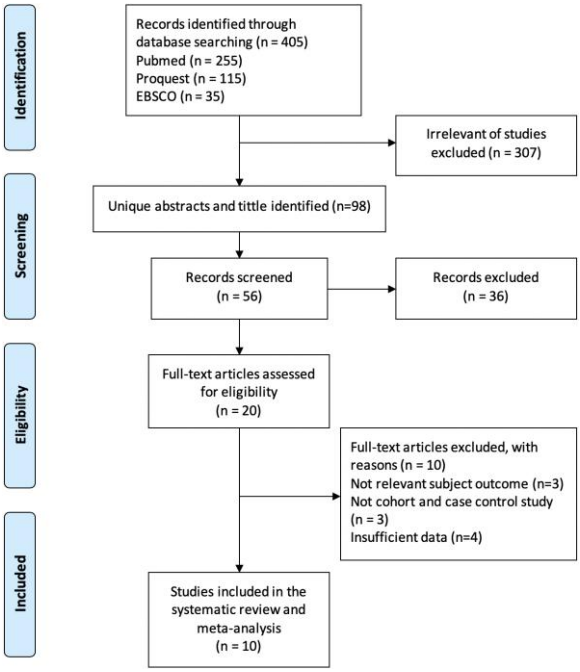

Figure 1: Flow diagram research procedure

Data collection technique

The data collection is done through an online search. The search was limited to English language articles. The article type was limited to journal articles. The research subject was limited to research with a human subject. The time of publication was limited from January 2000 to March 2019. The abstract of articles with potentially relevant titles was reviewed, while the irrelevant articles were excluded.

Furthermore, articles that have potentially relevant abstracts will be reviewed in full-text, while the irrelevant articles were excluded. The inclusion criteria of this study sample were researched on dietary fibre protective against colorectal cancer patients with case-control and cohort study. Exclusion criteria were: the research was not available in full-text form and when these criteria were not satisfied or if the provided information was insufficient for data extraction. The following data were obtained from each article: first author's name and year of publication, region, type of study, characteristics, duration of the study, number of samples, fibre intake (g/day), dietary assessment and effect size.

Two independent investigators carefully extracted information from all studies that satisfied the inclusion criteria by a standardized protocol. Disagreements were resolved by three other investigators. Quality assessment was conducted using Newcastle–Ottawa Quality Assessment Scale (NOS). The papers with a total score of 0-3, 4-6, and 7-9 points were specified as the poor, moderate, and high quality [14].

Table 1: Systematic review association of dietary fibre with colorectal in Asia

First author, year	Region	Type of study	Characteristic	Duration of study	Number of samples	Fibre intake (g/day)	Dietary assessment	Effect size	NOS
Chiu et al [15]	China	Case control	M, F 30-74 years	5 years	2,483	M: 7.0 vs 12.4 F: 6.0 vs 10.5	Food frequency questionnaire	OR (95% CI):0.6 (0.4-1.0)	8
Otani et al [16]	Japan	Case control	M 20-74 years	5 years	331	13.8 vs 14.9	Food frequency questionnaire	OR (95% CI):0.69 (0.45-1.90)	7
Shin et al [17]	China	Cohort	F 40-70 years	2 years	283	N/A	Food frequency questionnaire	RR (95%CI): 1.1(0.6–1.8)	7
Wakai et al [18]	Japan	Cohort	M, F 40-79 years	7.6 years	43,115	M: 6.7 vs 13.4 F: 7.4 vs 13.4	Food frequency questionnaire	RR (95%CI):0.73 (0.51-1.03)	8
Nashar et al [19]	Saudi arabia	Case control	M, F 30 years	1 years	100	11 vs 23	Food frequency questionnaire	OR (95% CI): 0.68 (0.41-1.01)	6
Nayak et al [20]	India	Case control	M, F 18-85 years	2 years	432	N/A	Food frequency questionnaire	OR (95% CI): 0.15 (0.05-0.46)	7
Uchida et al [21]	Japan	Case control	F 20-74 years	5 years	1,631	9.2 vs 19.8	Prosky-AOCA method	OR (95% CI): 0.76 (0.54–1.08)	8
Ramadas et al [22]	Malaysia	Case control	M, F ≥ 30 years	1 years	118	3.94 vs 7.07	Food frequency questionnaire	OR (95% CI): 0.14 (0.05–0.40)	6
Zhong et al [9]	China	Case control	M, F mean age cases 56.7 ± 10.6; age controls 56.4 ± 10.5	2 years	954	9.2 vs 10.5	Food frequency questionnaire	OR (95% CI): 0.68 (0.48–1.02)	7
Shong et al. [8]	China	Case-control	M, F 30-70 years	1 year	517	20.52 vs 22.98	Food frequency questionnaire	OR (95% CI): 0.40 (0.21–0.76)	7
Total of sample					49,964				

Abbreviation: M, Male; F, Female; NOS, Newcastle–Ottawa Quality Assessment Scale.

Data analysis

The analysis held to get the value of log odds ratio, which is the combined odds ratio value from the research. Results were pooled using the odds ratio with corresponding 95% confidence intervals (CIs). Significant heterogeneity was indicated by $I^2 > 50\%$ because these tests presented minimal statistical power in cases with few studies and small sample sizes. A random effect model was used when significant heterogeneity was observed; otherwise, a fixed effect model was utilised. A two-tailed *P*-value of < 0.05 was considered statistically significant.

Publication bias was visually evaluated by using funnel plots and statistically assessed through Egger's and Begg's tests. Meta-analysis was carried out in Stata version 14.2 (Stata Corporation).

Results

The selection of studies was conducted to obtain 10 studies with total of sample 49,964 patients related to dietary fibre protective against colorectal cancer patients in Asia (Table 1) [8], [9], [15], [16], [17], [18], [19], [20], [21], [22].

Dietary fibre protective against colorectal cancer patients in Asia is shown in Figure 2.

Figure 2 shows meta-analysis of dietary fibre protective against colorectal cancer patients in Asia (OR = 0.66 [95% CI 0.56-0.77, p = 0.008]). It's mean the result of the study revealed the protective effect of dietary fibre observed in meta-analysis study was consistent with some previous findings.

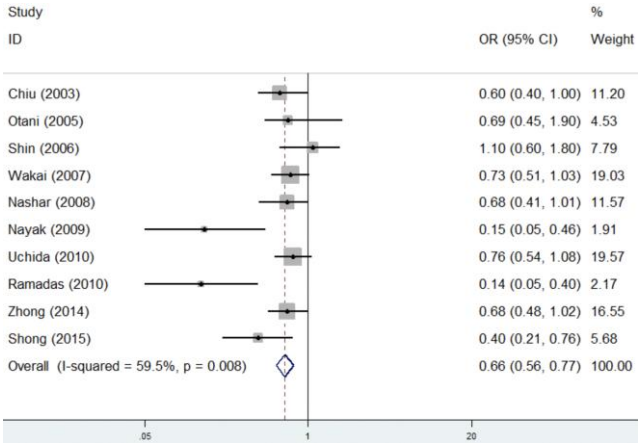

Figure 2: Dietary fibre protective against colorectal cancer patients in Asia

Heterogeneity among studies (I^2 = 59.5%) shows a variation of heterogeneity research for dietary fibre protective against colorectal cancer patients. Funnel plots was constructed to identify publication bias among studies dietary fibre protective against colorectal cancer patients in Asia (Figure 3).

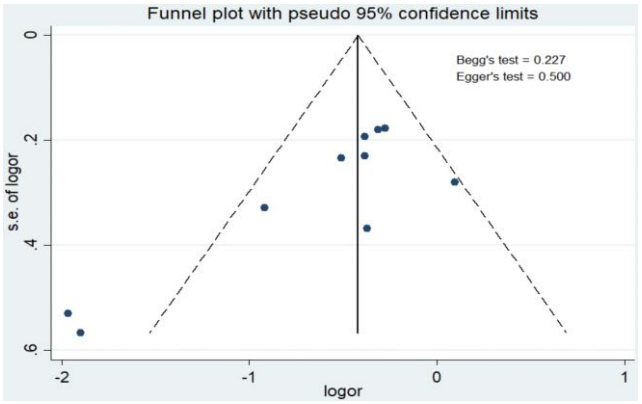

Figure 3: Funnel plots dietary fibre protective against colorectal cancer patients in Asia

There was no significant publication bias for studies included in dietary fibre protective against colorectal cancer patients in Asia, Egger's test (p = 0.500) and Begg's test (p = 0.227).

Discussion

The result of a meta-analysis of dietary fibre protective against colorectal cancer patients in Asia (OR = 0.66 [95% CI 0.56-0.77, p = 0.008]). Dietary fibre has a variation of heterogeneity between studies for the occurrence of colorectal cancer. Several studies have suggested the role of dietary fibre for protective colorectal cancer. High fibre intakes, particularly from cereals or grains and fruit, are associated with a reduced risk of incident colorectal adenoma and cancer [8], [12], [23]. Prospective study within a population-based screening trial suggests that individuals consuming the highest intakes of dietary fibre have reduced risks of incident colorectal adenoma and distal colon cancer and that this effect of dietary fibre, particularly from cereals and fruit, may begin early in colorectal carcinogenesis [11], [24].

Fibre source from fruit, vegetable, legume, cereal and grains. Intakes of carotenoids, light green vegetables, yellow-orange vegetables, broccoli, corn, carrots, bananas, garlic, and legumes (including soy products) were inversely associated with risk, even after adjustment for vegetable fibre. The data support a protective role of fibre from vegetables against colorectal cancer [25]. High intake of dietary fibre, in particular, cereal fibre and whole grains, was associated with a reduced risk of colorectal cancer [10].

A previous study in USA known that mean intake of dietary fibre (DF) is far below recommendations, with children and adolescents aged 2 to 19 years consuming an average of less than 14 g of DF per day. Adults 20+ years old consume, on average, about 17 g of DF per day, and men consume significantly more DF than women. Non-Hispanic black adults consume significantly less DF compared with other race/ethnic groups. Lower family income and living at less than 131% of poverty were associated with lower DF intakes among adults. Federal and local government policies should encourage consumption of all vegetables, including the white potato, as an important source of DF [26].

Another study in Africa known the total median [interquartile range (IQR)] values for total, insoluble, and soluble fiber consumed were 4.6 g [0.0-48.9], 0.0 g [0.0-18.0], and 0.0 g [0.0-15.0], respectively. Females had a higher median [IQR] intake of total (5.1 g [0.0-48.9] vs. 4.3 g [0.0-43.9]), insoluble (0.0 g [0.0-18.0] vs. 0.0 g [0.0-12.0]), and soluble fiber (0.0 g [0.0-14.9] vs. 0.0 g [0.-7.3]) than males, respectively [27]. Several studies are different from some aspects of others. For instance, the French cohort, who started in 1993, only adjusted for total energy intake.

On the other hand, the US cohort, which adjustment factors were too few, and the Italian cohort, which started in 1987, the range of the highest and lowest dietary fibre intake was relatively narrow (20.1 g/d vs 16.6 g/d, respectively) [28]. Mechanism of dietary fibre may decrease the risk of colorectal cancer by increasing stool bulk, diluting faecal carcinogens, and decreasing transit time, thus reducing the contact between carcinogens and the lining of the colorectum.

Also, bacterial fermentation of fibre results in the production of short chain fatty acids, which may have protective effects against colorectal cancer. Fruit, vegetable, legume, cereal and grains fibre may also protect against colorectal cancer, including antioxidants, vitamins, trace minerals, phytate, phenolic acids, lignans, and phytoestrogens [29], [30], [31].

There were a few limitations in this meta-analysis. First, three studies seemed potentially eligible to be included in this meta-analysis, but the full texts were not accessible. This issue may raise the possibility of selection bias. Second, there were a relatively large number of exclusions due to missing data to be included to meta-analysis, and not analysis for the proportion of participants for the different sex group. Third, validation data were not available for dietary questionnaires in several studies.

Conclusion

This analysis confirmed dietary fibre protective against colorectal cancer patients in Asia. The results of this study recommend the need to maintain the dietary fibre. Dietary fibre may be an effective measure for colorectal prevention. This study suggests the need for education and counselling about eating habits and the importance of avoiding foods with high fibre content.

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