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ORIGINAL PAPER

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Low-level laser therapy on the rat's gastrocnemius morphometry submitted to a rheumatoid arthritis model

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ABSTRACT

Introduction. Rheumatoid arthritis (RA) is a chronic, systemic, autoimmune inflammatory disease of unknown origin, mainly affecting synovial joints and related structures, including the adjacent musculature, generating great disability and reduction in quality of life.

Aim. This study was designed to investigate the effect of low-level laser therapy (LLLT) on gastrocnemius of Wistar rats subjected to an experimental model of RA.

Material and methods. Forty male Wistar rats were used, separated into: acute and chronic, being subdivided into Control Group (CG): without intervention, Lesion Group (LG): submitted to lesion, Laser Control Group (LCG): without lesion and with treatment, and Laser Lesion Group (LLG): submitted to lesion and LLLT. The treatment with LLLT occurred in four points of the right knee, wavelength of 660 nm, energy density of 5 J/cm^2, energy per point of 0.003 J. Morphometric analysis was performed using a 40x magnification photomicrograph and analyzed using the Image-Pro-Plus 6.0 program.

Results. As result of the acute group there was a difference only for muscle mass, being higher in CG. For the chronic group there was significant difference for cross-sectional area, larger and smaller diameter, again with the control group obtaining higher values than the others, for the number of nuclei LG was lower than CG and LCG, but LLG was not different from any of them.

Conclusion. It is concluded that treatment with LLLT was not very effective in reversing the harmful effects of RA on the gastrocnemius muscle.

Keywords. photobiomodulation therapy, rheumatic diseases, skeletal muscle

Introduction

Rheumatoid arthritis (RA) is a chronic, systemic, autoimmune inflammatory disease of unknown origin, mainly affecting synovial joints and related structures, including the adjacent musculature, generating great disability and reduction in quality of life.[1-4] The prevalence of RA in the United States from 2004 to 2014 ranged from 0.41 to 0.54% of the population, affecting 1.28 to 1.36 million

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

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people in 2014, at a rate ranging from 0.29-0.31% among men to 0.73-0.78 among women.[5]

There are several therapies for the RA, however, they have many side effects, so new therapies are developed to overcome these limitations, such as immunotherapy and gene therapy.[1] Since the main therapeutic objectives are: reduction of pain and inflammatory activity, prevention of tissue degradation, increase function and improve quality of life, non-pharmacological resources have their relevance, such as thermotherapy, electrotherapy, whole body vibration, orthoses and low-level laser therapy (LLLT).[6,7]

LLLT has been used in the treatment of RA and has its mechanism of action described through the absorption of red and infrared radiation by the chromophores, which can increase enzymatic activity, production of adenosine triphosphate (ATP), protein synthesis and cell proliferation, resulting in analgesic effects.[8] In addition, it can assist in the joint protection process, reducing pain and stiffness.[9] Another important action of LLLT is the inhibition of chemotactic factors and prostaglandin synthesis in the early stages of inflammation.[10]

The anti-inflammatory, analgesic and healing effects of radiation emitted by LLLT are dependent on the characteristics of the laser, including wavelength, the mode of application, dose, duration and location.[11]

Aim

Given the existence of research gaps that evaluate the repercussion of RA treatment with LLLT on possible histological changes observed in skeletal muscle tissue, the objective of the present study was to analyze the effects of treatment with LLLT on the gastrocnemius muscle of Wistar rats submitted to an experimental model of complete Freund adjuvant (CFA) induced RA.

Material and methods

This research is characterized as quantitative, experimental, nonblind and randomized. It was approved by the Ethics Committee on Animal Use of the Universidade Estadual do Oeste do Paraná (Unioeste). The group consisted of 40 male Wistar rats, kept in plastic polypropylene boxes, with access to water and feed at will, controlled temperature at 21±1°C, light/dark photoperiod of 12 hours.

The animals were randomly separated into: acute, with a 7-day period of inflammation, and chronic with 28 days of inflammation, totaling 20 animals each. Then, for each period, the animals were again separated into four groups (n=5), for each period: Control Group (CG) – animals that were not submitted to injury with CFA, nor treated with LLLT; Lesion Group (LG) – animals submitted to RA induction, without treatment; Laser Control Group (LCG) – animals that were not submitted to injury but were treated with LLLT; Laser Lesion Group (LLG) – animals injured and submitted to treatment with LLLT.

Rheumatoid arthritis was induced by two injections of CFA (Mycobacterium butyricum, 0.5 mg/ml; 50 µl). The first injection was administered at the base of the animal's tail, intradermally. For this, the area of administration was trichotomized for subsequent asepsis with iodinated alcohol (1%). For the injection a 1 mL syringe and a 13x4.5mm needles were used, inserted approximately 1cm into the base of the tail in a subcutaneous manner, this being the first inflammatory stimulus. After seven days, the second injection was administered intra-articularly to the right tibiofemoral joint of the animals. For this application, the anterior area of the knee was trichotomized and the animals were manually contained for asepsis with iodinated alcohol (1%) and injection (1mL syringe and 13x4.5mm needle). The animals belonging to CG and LCG underwent the same protocol but received an injection with saline solution (0.9% sodium chloride).

Treatment with LLLT (Ibramed®) was performed punctually at four points on the right knee: anterior to the patella, medial side at the tibiofemoral joint, lateral side at the tibiofibular joint, and posterior in the popliteal region. Following the parameters: wavelength of 660 nm, energy density of 5 J/cm², power of 30 mW, spot area: 0.06 cm², irradiated energy per point of 0.003 J, irradiation time per point of 10 s. The treatment was performed on interspersed days during one week in the acute group, totaling four days of treatment. For the chronic group, 8 applications were performed, totaling 14 days of treatment. The animals belonging to the CG and LG of both groups were submitted to pen contact, but without the emission of the beam.

At the end of the experiment, the animals of both the acute and chronic groups were euthanized with an overdose of the association of anesthetic (ketamine - 240 mg/Kg) and a muscle relaxant (xylazine - 45 mg/kg). The gastrocnemius muscle of the right pelvic limb of the animals was collected, weighed, measured and fixed in Methacarn (70% Methanol + 20% chloroform + 10% glacial acetic acid), for 24 hours and stored in 70% alcohol. Subsequently, the muscles were included in histological paraffin, cut transversely in 7 µm of thickness (microtome Olympus CUT 4055), and the slides stained in hematoxylin and eosin to perform morphometric analysis.

For morphometric analysis, photomicrographs with 40x magnification were performed under the Olympus® DP71 microscope (USA) and analyzed using the Image-Pro-Plus 6.0 program (USA), which is calibrated to measure the cross-sectional area (µm²), the largest and smallest diameter of muscle fibers (µm), number of nuclei, number of fibers and ratio of nuclei per muscle fiber (nuclei ÷ fiber number).

The data were analyzed with the SPSS 20.0 program and presented with means followed by their respective

95% confidence intervals. After confirming the normality of the data, to perform the comparison of the different groups, it was used Generalized Linear Models, followed by the Sidak post-test, according to the different variables evaluated. In the area variables, the largest and smallest diameter, the distribution used was Gamma, and for the others the Normal distribution was used. The accepted level of significance was 5%.

Results

As a result for the gastrocnemius muscle mass, a significant difference was observed between the control animals for animals that had acute RA induction [Wald $X^2(3)$= 50.691; p<0.01], in which CG and LCG presented higher mass when compared to LG (p<0.01) and LLG (p<0.01) (Table 1). On the other hand, the chronic treatment animals did not present significant differences in the weight of the gastrocnemius muscle [Wald $X^2(3)$= 4.584; p=0.205] (Table 1).

Table 1. Mean and their respective 95% confidence intervals of the gastrocnemius muscle mass and length of Wistar rats

	Groups	Acute	Chronic
Mass	CG	1.48 [1.3-1.5]^a	1.54 [1.4-1.6]^a
	LCG	1.43 [1.3-1.5]^a	1.57 [1.4-1.6]^a
	LG	1.05 [0.9-1.1]^b	1.44 [1.3-1.5]^a
	LLG	1.13 [1.0-1.2]^b	1.49 [1.4-1.5]^a
Length	CG	24.85 [22.9-26.7]^a	25.55 [24.4-26.6]^a
	LCG	22.88 [20.9-24.8]^a	25.657 [24.5-26.7]^a
	LG	21.29 [19.3-23.2]^a	25.55 [24.4-26.6]^a
	LLG	22.28 [20.3-24.2]^a	25.29 [24.1-26.4]^a

Legend: CG – Control Group; LCG – Laser Control Group; LG – Injury Group; LLG – Injury Group + Laser. Different letters mean statistically different values (p≤0.05)

Regarding muscle length, both for chronic and acute treatment, no significant differences were observed between the experimental groups [Wald $X^2(3)$= 6.983; p=0.072], [Wald $X^2(3)$= 0.215; p<0.975] (Table 1).

In the animals of the acute treatment group, all the variables analyzed showed no difference between the groups: cross section [Wald $X^2(3)$=2, 061; p=0.560], larger diameter [Wald $X^2(3)$= 7.306; p=0.063] and smaller muscle fiber diameter [Wald $X^2(3)$= 1.629; p=0.653], as well as number of nuclei [Wald $X^2(3)$= 6.324; p=0.097], number of fibers [Wald $X^2(3)$= 7.067; p=0.070], and nucleus ratio per fiber [Wald $X^2(3)$= 4.022; p=0.259] (Table 2).

Regarding the morphometric analysis of the chronic RA animals, it was observed a significant reduction in the cross-sectional area of the gastrocnemius muscle [Wald $X^2(3)$= 50.74; p<0.01], where CG differed from LCG (p<0.01), LG (p<0.01) and LLG (p<0.01), presenting a larger area when compared to other groups. The same result was obtained for the largest [Wald $X^2(3)$= 61.445; p<0.01] and smallest muscle fiber diameter [Wald $X^2(3)$= 83.476; p<0.01] (Table 3).

In respect to the number of muscle fibers of these animals, there was no significant difference between the groups [Wald $X^2(3)$= 6.565; p=0.087]. For the number of nuclei [Wald $X^2(3)$= 12.863; p=0.05] LG presented a lower number of nuclei when compared to CG (p<0.05) and LCG (p<0.05), an effect that was reversed by the effect of LBP (LLG p=0.01). Regarding the core/fiber ratio there was no significant difference [Wald $X^2(3)$= 0.803; p=0.849] (Table 3).

Discussion

In the present study it was observed that the model used to trigger RA was able to produce acute changes in muscle mass, and chronically there were changes in diameters and cross-sectional area, in addition to a reduction in the number of myonuclei, and only in this variable did LLLT produce an effect.

The CFA induced RA model has been widely used, being able to efficiently mimic the symptoms of human RA, as well as the effects triggered by this type of inflammation. The changes generated by the RA start in synovia and progress to the joint and muscle structures, generating a systemic effect.[12–14]

Table 2. Mean and their respective 95% confidence intervals of the cross-sectional area, largest and smallest diameter, number of fibers and nuclei, besides the nucleus/fiber ratio of the muscle fibers of the gastrocnemius muscle of the acute group

	Cross-sectional area (μm²)	Largest diameter (μm)	Smallest diameter (μm)	Number of fibers	Number of nuclei	Nucleus/fiber ratio
CG	159.21 [128.4-08.9]^a	16.03 [14.5-19.1]^a	9.55 [8.11-11.9]^a	29.84 [18.3-37.1]^a	57.3 [36.6-64.8]^a	1.96 [1.7-2.2]^a
LCG	174.49 [139.4-161.4]^a	16.53 [16.0-17.2]^a	10.97 [10.5-11.5]^a	22.2 [19.7-26.5]^a	39.24 [33.9-49]^a	2.10 [1.5-2.1]^a
LG	154.94 [134.8-180.1]^a	15.45 [14.2-17.7]^a	9.51 [9.0-10.3]^a	27.26 [19-30.9]^a	59.78 [34.6-76.7]^a	2.18 [1.7-2.5]^a
LLG	169.74 [150.3-201.2]^a	16.43 [14.0-19.5]^a	9.87 [8.7-12.0]^a	24.94 [16.7-31]^a	36.1 [16-77.4]^a	2.14 [1.2-2.9]^a

Legend: CG – Control Group; LCG – Laser Control Group; LG – Injury Group; LLG – Injury Group+Laser.
Different letters mean statistically different values (p≤0.05)

Table 3. Mean and their respective 95% confidence intervals of the cross-sectional area, largest diameter, smallest diameter, in μ, number of fibers and nuclei, besides the nucleus/fiber ratio of the muscle fibers of the gastrocnemius muscle of the chronic group

	Cross-sectional area (μm²)	Largest diameter (μm)	Smallest diameter (μm)	Number of fibers	Number of nuclei	Nucleus/fiber ratio
CG	120.69 [97.3-149.6]^{a}	14.77 [13.4-16.2]^{a}	9.46 [8.7-10.1]^{a}	35.30 [26.3-44.2]^{a}	94.60 [77.4-115.6]^{a}	2.88 [2.5-3.2]^{a}
LCG	55.21 [44.5-68.4]^{b}	9.93 [9-10.8]^{b}	6.21 [5.5-6.8]^{b}	32 [23-40.9]^{a}	94.50 [77.3-115.4]^{a}	3.08 [2.6-3.5]^{a}
LG	55.65 [44.8-69.0]^{b}	9.99 [9.1-10.9]^{b}	6.14 [5.4-6.8]^{b}	23.80[14.8-32.7]^{a}	67.10 [54.9-82]^{b}	2.91 [2.5-3.3]^{a}
LLG	42.47 [34.2-52.6]^{b}	9.22 [8.4-10.1]^{b}	5.51 [4.8-6.1]^{b}	39.80 [30.8-48.7]^{a}	110.9 [90.7-135.5]^{a}	2.85 [2.4-3.2]^{a}

Legend: CG – Control Group; LCG – Laser Control Group; LG – Injury Group; LLG – Injury Group + Laser. Different letters mean statistically different values ($p \leq 0.05$)

In clinical findings of RA, muscle atrophy is observed related to the affected region, with several factors that may be related to its appearance, including sedentariness, advanced age, inflammatory, infectious, autoimmune diseases and malnutrition. Such conditions also modify the capacity of the muscle to regenerate.[15]

In the present study, a significant loss of muscle mass was observed in animals submitted to the lesion already in its acute form, emphasizing that joint inflammation induced by CFA produces a pro-atrophic deleterious effect. Corroborating these findings, Silva et al., demonstrated a reduction of muscle mass in 20% of patients with RA as a result of an intense inflammatory process, which can lead to muscle fatigue, weakness and functional deficit, in addition to changes in the quality of life of patients with this type of arthritis.[16] Similar findings were reported by Ancuta et al., showing physiological changes in the deltoid muscle, including muscle fiber atrophy, increase in the number of mitochondria and nuclei, in addition to the presence of inflammatory cells invading the muscle fibers.[17] This was different from the present study in which the lesion group presented a lower number of nuclei and was reverted by LLLT.

In the present study, the experimental model did not affect muscle changes in the other variables in animals induced to acute RA. The harmful effects of RA are linked to its progressive character, which is caused by chronic inflammation in tissues.[18] Muscular involvement appears in the form of myalgia, weakness and muscular atrophy, which end up producing sarcopenia, being an element of rheumatoid cachexia, a frequent event in the RA.[16,19] This research corroborates this fact, since the morphological findings for the chronic lesion group, since a decrease of the cross-sectional area was noted, and consequently of its larger and smaller diameter.

Regarding the number of muscle fibers between the groups in this study, there was no significant difference, contradicting studies that present the decrease in the number of fibers caused by the loss of motor units due to muscle atrophies caused by rheumatoid sarcopenia, in addition to direct degenerative changes of muscle fibers.[18] This fact explains not only the direct alteration in muscle fibers, but also the loss of muscle mass observed in the acute phase, in the chronic phase the loss of mass was not observed, however, it may have been masked by a fat infiltration in muscle tissue, which was not evaluated, which is one of the limitations of the present study.[20]

In conditions of skeletal muscle loss, intracellular signaling cascades cause cell death, decrease of satellite cells and protein deterioration, thus generating a decrease of nuclei in the muscle when affected by the RA, explaining the result found in the decrease in the number of nuclei in the group affected by the RA compared to the other groups, which was reverted by laser radiation, given its protective action on muscle tissue.[21,22]

However, in the present study, LLLT was not competent to revert muscle changes from the joint inflammatory process in the other variables analyzed. This fact may be related to insufficient irradiation, due to the small energy delivered[23] or even the time of exposure to radiation.[24] Thus, new studies with dosimetry variations are suggested, seeking an effective design to act directly on inflammatory cells, producing minimal side effects and contributing to the quality of life of patients with this chronic rheumatic disease.

Conclusion

Thus, it is concluded that the RA model, especially in the long term, resulted in deleterious effects on muscle morphometry, and changes were observed in the measurement of the cross section, greater diameter and smaller diameter of the muscle fiber. Also, LLLT was effective in reversing only the change in the amount of myonuclei.

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ORIGINAL PAPER

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A novel method of immunomodulation of endothelial cells using *Streptococcus pyogenes* and its lysate

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ABSTRACT

Introduction. Coronary artery diseases and autoimmune disorders are common in clinical practice.

Aim. In this study, *Streptococcus pyogenes* and its lysate were studied to modify the endothelial function.

Material and methods. HUVEC cells were seeded in the cell culture, and *Streptococcus pyogenes* were added to the cell culture, and the supernatant was studied for the secreted proteins. In the second phase, the bacterial lysate was synthesized, and the lysate was added to cell culture and studied.

Results. When *S. pyogenes* alone was added to culture, E Cadherin, Angiostatin, EpCAM and PDGF-AB were some of the biomarkers elevated significantly. HCC1, IGFBP2 and TIMP were some of the biomarkers which showed a reduction. When the lysate was added, the cell-culture was maintained for a longer time, and it showed the synthesis of immune regulatory cytokines. Heatmap analysis showed a significant number of proteins/cytokines concerning the immune/pathways, and toll-like receptors superfamily were modified. BLC, IL 17, BMP 7, PARC, Contactin2, IL 10 Rb, NAP 2 (CXCL 7), Eotaxin 2 were maximally increased. By principal component analysis, the results observed were significant.

Conclusion. There is potential for a novel method of immunomodulation of the endothelial cells, which have pleiotropic functions, using *S. pyogenes* and its lysates.

Keywords. biomarkers, endothelial cells, immune-regulation, *Streptococcus pyogenes*

Introduction

Immune-related disorders are common in clinical practice. Rheumatic heart diseases are common in the general population in the Asian countries with a prevalence of about 7.7 to 51/1000 community based on echocardiography screening in school in India.[1] *Streptococcus pyogenes* infections are associated with sore-throats, and they are also associated with rheumatic heart diseases. Rheumatic fever is associated with migratory joint pains and occasionally pan-carditis. In patients with rheumatic heart diseases, the incidence of coronary artery diseases is low.[2-5] This was observed in some stud-

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* The study was presented as an abstract in Synthetic Biology UK 2019

Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

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ies in India and neighboring countries. The incidence of rheumatic heart disease is showing a decreasing trend, and the incidence of coronary artery disease is rising in the recent days. The incidence in our center and other centers as well show a low association of coronary artery disease with rheumatic valvular heart diseases, irrespective of age and metabolic characteristics.[2,3] The incidence of mixed procedure i.e., combined valve surgeries and bypass surgeries, is <5%. And further analysis looking for rheumatic valvular etiology in these combined procedures would be much lesser, even in high volume centers like TUMS.[4]

S. pyogenes has anti-tumour activity which was studied in the early 1990's by Coley. *S. pyogenes* are known to produce a unique enzyme that is useful in cleaving immunoglobulin G in the blood (Ides).[6-8] Its usefulness therapeutically has been tested to cleave IgG antibodies.[9,10] *S. pyogenes* secrete serum optical factor, which shows increased uptake of HDL, and thereby it can reduce atherosclerosis.[11,12]

In this study, *S. pyogenes* was used to infect the endothelial cells, and the endothelial response was evaluated. Also, in the later part of the study the *S. pyogenes*' lysate was used on the endothelial cells, and the results were evaluated. Microorganisms can prevent autoimmune disorders though this phenomenon is not well studied.[13] Identifying the immunogenic potentials can identify potential uses of *S. pyogenes* to prevent cardiovascular, autoimmune, and tumor problems. The incidence of autoimmune disorders is common in the migrant populations especially of Asian ethnicity.[14,15] This is commonly attributed as the hygiene hypothesis, and the exact mechanism is speculative and not decisively studied.

In our center out of 650 consecutive valve replacement surgeries six cases underwent concomitant coronary artery bypass surgery. Out of these 6 cases a clear etiology of rheumatic heart disease was not established on any of the cases, and the primary etiologies were ischemia MR and degenerative sclerotic calcific aortic valve. The study was performed in search of novel applications of *S. pyogenes* in regulating immune functions, and its related effects on cardiovascular and its pleiotropic functions.

Aim

Bacterial infections can modulate immune functions though this phenomenon is poorly studied. In this study, the role of *S. pyogenes* and its lysate was evaluated for possible beneficial effects on the endothelial cells.

Material and methods

S. pyogenes were obtained from the laboratory (*Streptococcus pyogenes* Rosenbach – ATCC,19615TM Lancefield Group A). The bacteria were cultured by seeding, and the colonies were inoculated with endothelial cells. Serial observations about the endothelial cells were made at regular intervals by microscopy. The supernatant was collected, and various inflammatory markers were studied at serial time intervals.

Colony forming unit (CFU) assay

S. pyogenes was grown on of BD Bacto-Brain Heart Infusion agar plates with 5% CO_2 at 37 ^{0}C overnight. Streptococcus pyogenes was collected into 5 ml of Brain Heart Infusion broth. 10-fold serial dilutions with Brain Heart Infusion broth was then run. For each dilution, plate 100ul of cells on Brain Heart Infusion agar plates was added. Incubation of plates was performed with 5% CO_2 at 37^{0}C overnight. The calculation was made- CFU: 2 x 10^9 CFU/ml.

Cell treatment

Trypsin digested HUVEC cells was mixed with fresh complete medium in 50 ml tubes and aliquot the cells in two 6-well plates. The cells were grown to 80% confluence (9 cm^2 surface area/well, 2 ml culture medium/well). The cells were washed with 2 ml PBS/well, twice. *S. pyogenes* stock dilution: the Streptococcus stock was 2 x 10^9 CFU/ml. 100-fold dilution was made using 2 x 10^7 CFU/ml and sterile PBS, and 10ul *Streptococcus* stock, and 990ul sterile PBS. The infection dose was 100,000 CFU/well. The Streptococcus inoculation amount was 100,000 CFU / 2 x 10^7 CFU/ml (100-diluted) = 5 x 10^{-3} ml = 5µl per well. 30µl of 100-fold diluted *Streptococcus* stock was added into 24 ml new complete medium without any antibiotics and mixed well. 2 ml/well mixture was distributed into each well. The samples were collected at following timepoints (Table 1). At each time point, the cell culture supernatants were collected and centrifuged at 500 g for 10 min at 4^{0}C. The supernatants were transferred (~ 1.9 ml) to new tubes. The samples were stored at -80^{0}C till use. Each time point, the untreated controls were also collected. Total 10 samples were collected.

Table 1. Study plan

Time Point	Starting	Ending	*Streptococcus pyogenes* infection	
			Treated	Untreated
0-hr	8:00 AM	8:00 AM	100,000 CFU + **PBS**	**PBS**
2-hrs	8:00 AM	10:00 AM	100,000 CFU + **PBS**	**PBS**
6-hrs	8:00 AM	2:00 PM	100,000 CFU + **PBS**	**PBS**
10-hrs	8:00 AM	6:00 PM	100,000 CFU + **PBS**	**PBS**
14-hrs	8:00 AM	10:00 PM	100,000 CFU + **PBS**	**PBS**

In the second phase of the study, the *S. pyogenes*' lysate was prepared from the same bacteria. The lysate

Table 2. Elevation in MIF, lymphotactin, LIF, OPN, MSP, IL-9, Lymphotactin, IL18 BPa and TECK

LOD	MAX	(pg/ml)	0 hour treated	2 hour treated	6 hour treated	10 hour treated	14 hour treated	(pg/ml)	14 hour untreated
14.2	40,000.0	6Ckine	1.9	1.4	6.2	0.3	0.0	6Ckine	2.0
10.9	4,000.0	Axl	0.0	0.0	0.0	16.1	14.8	Axl	11.8
17.8	20,000.0	BTC	0.0	0.0	0.0	0.0	0.0	BTC	0.0
26.2	40,000.0	CCL28	0.0	0.0	0.0	0.0	0.0	CCL28	0.0
26.7	50,000.0	CTACK	0.0	0.0	0.0	0.0	0.0	CTACK	0.0
11.7	20,000.0	CXCL16	0.0	6.6	0.4	6.4	19.2	CXCL16	36.4
13.7	10,000.0	ENA-78	2.1	0.0	0.0	0.0	9.0	ENA-78	2.0
62.4	20,000.0	Eotaxin-3	0.0	0.0	0.0	0.0	0.0	Eotaxin-3	0.0
4.2	10,000.0	GCP-2	0.0	0.0	0.0	0.0	0.0	GCP-2	2.8
2.9	1,000.0	GRO	1.4	3.2	4.1	6.9	8.4	GRO	25.0
6.7	4,000.0	HCC-1	0.0	7.4	44.9	69.6	90.9	HCC-1	213.1
4.0	3,333.3	HCC-4	0.0	0.1	0.0	0.4	0.4	HCC-4	0.2
800.0	200,000.0	IL-9	0.0	206.2	28.9	0.0	109.6	IL-9	0.0
49.6	100,000.0	IL-17F	0.1	0.1	0.3	0.1	0.4	IL-17F	0.0
52.0	60,000.0	IL-18 BPa	4.0	3.4	0.0	0.0	33.3	IL-18 BPa	0.0
7.7	10,000.0	IL-28A	0.0	0.0	0.5	0.0	0.0	IL-28A	0.0
302.0	100,000.0	IL-29	0.0	30.5	0.0	0.0	0.0	IL-29	0.0
97.8	40,000.0	IL-31	0.0	0.0	0.0	0.0	7.8	IL-31	0.0
13.7	10,000.0	IP-10	0.5	0.0	1.8	0.0	0.9	IP-10	0.1
16.8	10,000.0	I-TAC	0.0	2.4	0.0	0.0	5.9	I-TAC	9.7
28.4	13,000.0	LIF	0.0	16.4	18.8	0.2	47.9	LIF	0.0
15.6	10,000.0	LIGHT	0.0	0.0	0.0	0.0	0.9	LIGHT	0.0
47.5	100,000.0	Lymphotactin	31.3	43.7	45.7	0.0	78.8	Lymphotactin	49.3
5.7	2,000.0	MCP-2	0.9	0.0	0.7	0.0	1.7	MCP-2	1.7
4.2	4,000.0	MCP-3	0.0	0.0	0.0	0.0	0.0	MCP-3	0.0
4.6	1,111.1	MCP-4	0.0	0.1	0.4	0.0	0.1	MCP-4	0.5
4.9	1,111.1	MDC	0.0	0.0	0.0	0.0	0.3	MDC	0.2
13.6	4,000.0	MIF	72.4	70.9	323.2	709.2	1,417.9	MIF	74.6
4.5	4,000.0	MIP-3a	0.0	0.5	0.7	0.5	2.5	MIP-3a	0.8
17.8	20,000.0	MIP-3b	0.0	0.0	0.0	0.0	1.6	MIP-3b	0.0
15.0	10,000.0	MPIF-1	0.0	0.0	1.7	2.0	0.1	MPIF-1	0.7
213.2	100,000.0	MSP	0.0	2.4	39.5	17.7	61.1	MSP	21.1
1.9	444.4	NAP-2	0.0	0.0	0.3	0.0	0.1	NAP-2	0.1
28.6	100,000.0	OPN	37.1	52.9	36.9	1.8	36.8	OPN	14.2
4.9	4,000.0	PARC	0.0	0.2	0.4	0.6	0.5	PARC	1.2
219.7	100,000.0	PF4	0.0	0.0	0.0	0.0	0.0	PF4	0.4
2.8	10,000.0	SDF-1a	0.1	0.2	0.3	0.1	2.8	SDF-1a	1.4
6.9	10,000.0	TARC	0.0	0.0	0.0	0.0	0.0	TARC	0.9
85.2	100,000.0	TECK	0.0	0.0	0.0	0.0	38.0	TECK	0.0
7.3	10,000.0	TSLP	0.0	0.0	0.4	0.0	2.9	TSLP	10.2

was added to the endothelial cells, and the endothelial cell response was studied at serial intervals -0, 36, 138, 336, 672 hours, and control samples were studied. The biomarkers secreted were studied, and the results were achieved.

HUVEC Cells and Lysate preparation

Normally subculture of cells is performed, when the culture has reached approximately 80% confluence, new flasks are seeded at a density of 2,500 cells per cm^2. Cells are typically ready to passage after 4 to 7 days in culture, when inoculated with 2,500 cells/cm^2.

Culture medium was prepared and bacteria was grown at CO_2 incubator. Fresh cells were collected and cell lysate was prepared by lysozyme digestion and DNAase digestion. Then centrifugation was performed to obtain the clarified supernatant. The lysate was passed through 0.22 μm filter. The cell lysate protein concentration was then measured. Aliquot lysate protein was in small size and it was stored at -80°C till use.

HUVEC stimulation

HUVEC cells were thawed and grown in 6-well plate. 2 wells (A, B well): Well A is negative control without adding cell lysate. Well B is test well with adding cell lysate 40μg/ml. The cells were passed twice a week. With every cell pass, the same amount of cell lysate 40μg/ml was added to cell well. Each time one vial was used and left over lysate was discarded. The cell culture supernatants was collected (~1.5 ml) at following time points. Totally 10 samples will be collected (A1-5, B1-5). For array test, 5 samples were used - i.T0 ii. T36hrs (1.5 days) iii.168hrs (7 days) iv. 336hrs (14 days) v. 672hrs (28 days). Centrifugation of the supernatants at 500 g for 10 min to remove any cell debris was performed, and the samples were stored at -80°C immediately.

Statistical analysis - data filtration

The biomarkers demonstrating no variation among all the samples (zero variance) were excluded from the data profile analysis since they do not contribute regarding distinguishing samples from each other.

Table 3. Reduction in BMP-7, FGF-7, IGFBP 1-2 and 6, OPG, PDFG AA, PIGF and GDF15

LOD	MAX	(pg/ml)	0 hour treated	2 hour treated	6 hour treated	10 hour treated	14 hour treated	(pg/ml)	14 hour untreated
39.7	10,000.0	AR	15.3	14.2	1.9	8.6	0.6	AR	0.0
2.8	2,000.0	BDNF	0.6	0.7	1.7	1.3	0.8	BDNF	1.8
21.7	6,666.7	bFGF	14.4	19.3	15.4	15.2	6.7	bFGF	12.0
103.6	33,333.3	BMP-4	0.4	1.0	0.6	0.0	0.0	BMP-4	0.9
551.9	100,000.0	BMP-5	189.0	0.0	3.5	0.0	0.0	BMP-5	0.0
151.5	40,000.0	BMP-7	93.5	30.6	78.1	8.0	11.9	BMP-7	56.3
4.3	10,000.0	b-NGF	0.1	0.0	0.0	0.0	0.0	b-NGF	0.0
0.2	200.0	EGF	42.2	35.8	38.5	37.8	36.6	EGF	38.9
6.5	10,000.0	EGF R	0.9	0.0	1.1	0.6	0.1	EGF R	3.0
26.0	10,000.0	EG-VEGF	0.0	0.0	0.0	0.0	0.0	EG-VEGF	0.0
184.2	33,333.3	FGF-4	8.9	35.4	0.0	0.0	0.0	FGF-4	0.0
28.2	10,000.0	FGF-7	35.0	0.0	0.0	54.9	18.1	FGF-7	41.9
1.2	2,000.0	GDF-15	0.5	0.8	9.0	16.0	37.0	GDF-15	47.9
10.2	4,000.0	GDNF	0.0	0.0	0.0	0.2	0.0	GDNF	1.0
23.1	10,000.0	GH	0.0	10.7	0.0	5.4	0.0	GH	1.9
10.3	10,000.0	HB-EGF	1.3	0.0	0.0	10.2	2.5	HB-EGF	0.1
10.9	4,000.0	HGF	0.0	2.6	0.0	4.7	0.0	HGF	2.5
6.5	5,000.0	IGFBP-1	11.6	5.1	3.8	8.3	1.2	IGFBP-1	12.7
46.8	20,000.0	IGFBP-2	4.0	67.3	305.9	442.8	443.7	IGFBP-2	1,074.7
485.5	200,000.0	IGFBP-3	131.5	288.2	0.0	313.8	0.0	IGFBP-3	18.5
719.3	200,000.0	IGFBP-4	648.4	259.4	166.1	99.8	0.0	IGFBP-4	40.8
138.8	100,000.0	IGFBP-6	122.6	28.3	99.1	123.4	75.7	IGFBP-6	132.7
65.6	20,000.0	IGF-1	48.4	15.9	0.0	23.5	0.0	IGF-1	12.5
78.3	20,000.0	Insulin	5,894.2	4,512.6	4,837.5	4,683.9	4,757.0	Insulin	4,939.6
30.3	40,000.0	MCSF R	6.1	0.0	0.0	0.0	0.0	MCSF R	0.0
18.5	10,000.0	NGF R	0.0	0.0	0.0	0.0	0.0	NGF R	0.0
63.0	40,000.0	NT-3	0.0	0.0	0.0	0.0	0.0	NT-3	0.0
13.2	10,000.0	NT-4	0.0	0.0	0.0	1.0	0.0	NT-4	0.0
9.0	4,000.0	OPG	0.0	2.8	10.9	16.0	9.3	OPG	35.5
11.5	10,000.0	PDGF-AA	2.9	1.2	35.8	44.1	48.9	PDGF-AA	91.6
3.1	4,000.0	PIGF	0.9	3.6	10.5	9.9	12.6	PIGF	24.9
8.8	10,000.0	SCF	6.8	0.9	4.7	1.9	0.0	SCF	3.7
28.1	20,000.0	SCF R	2.2	3.7	2.0	5.5	0.0	SCF R	0.7
5.2	1,111.1	TGFa	0.0	0.0	0.0	0.0	0.0	TGFa	0.0
814.4	100,000.0	TGFb1	0.0	0.0	0.0	0.0	0.0	TGFb1	0.0
70.0	40,000.0	TGFb3	16.1	7.8	22.2	6.8	0.0	TGFb3	5.6
13.6	3,333.3	VEGF	0.2	0.6	0.0	0.0	0.0	VEGF	0.0
28.2	10,000.0	VEGF R2	4.8	3.9	0.0	0.0	0.0	VEGF R2	0.2
61.2	40,000.0	VEGF R3	6.5	0.0	4.3	0.0	0.0	VEGF R3	6.0
12.5	20,000.0	VEGF-D	0.0	0.0	0.0	0.0	0.0	VEGF-D	0.0

Heatmap

The biomarker values were standardized (centering and scaling) by subtracting the average and then dividing by the standard deviation. The standardized data were plotted in a heatmap with hierarchical clustering by Euclidean distance.

Principal Component analysis

The various expression levels of multiple biomarkers may come from a common underlying factor/mechanism. The principal component analysis (PCA) decompose the data set into different principal components (PCs) sorted by their contribution to total variance/variation in the dataset. These PCs are linear transformations/combinations of standardized biomarker values. By observing the location of a sample on the plot of the first 2 PCs explaining the most variation, we can tell the pattern of samples.

Software

All the analyses were conducted in the R programming language V3.6.0 (R Core Team 2017).

Results

Direct Streptococcus pyogenes' effects

Tables 2 to 6 summarize the effects of the bacteria on the cell culture of endothelial cells. There is a significant reduction in HCC1, IGFBP2, PDGFAA, and TIMP. Macrophage inhibiting factor and lymphocyte inhibition factors showed a decrease in the levels. There is a marked increase in E Cadherin, Angiostatin, DAN, Ep-CAM, CFG RIIBC, PDGF- AB, gp 130, TPO, Tie 2, and Angiogenin. ICAM-1, IL6, Endoglin, Trail 3, and PRE-CAM-1 showed an increasing trend.

Streptococcus pyogenes' Lysate Challenge

The results of the streptococcal lysate challenge on the endothelial cells are summarized in the heat map results (Figure 1). BLC, IL 17, BMP 7, PARC, Contactin2, IL 10 Rb, NAP 2 (CXCL 7), Eotaxin 2 were maximally increased. Other secreted markers that were induced – trappin2, SDF-1a, FGF7, CCL 28, 4-1BB, VCAM 1, VEGF R3, GITR, SIGLEC 5, IL13 R1, CD30, TGF B2 and GP 130. The markers which were unequivocally reduced were MIF, Fas, IL13R2, TIMP2, Follistatin,

Table 4. Increase in all the cytokines in the table listed (except Galactin7 and ST2)

LOD	MAX	(pg/ml)	0 hour treated	2 hour treated	6 hour treated	10 hour treated	14 hour treated	(pg/ml)	14 hour untreated
263.8	11,111.1	Activin A	346.3	251.0	343.9	281.7	472.9	Activin A	209.1
16.8	10,000.0	AgRP	30.3	16.5	45.4	18.9	184.3	AgRP	20.0
5.5	2,000.0	Angiogenin	37.9	29.9	43.4	29.8	144.1	Angiogenin	26.2
56.3	40,000.0	ANG-1	246.5	209.1	257.7	258.2	322.4	ANG-1	163.3
2,267.7	1,000,000.0	Angiostatin	4,942.9	3,576.9	8,953.9	3,369.3	40,517.2	Angiostatin	3,978.9
10.4	10,000.0	Cathepsin S	25.7	19.2	27.9	26.0	184.6	Cathepsin S	17.8
10.4	10,000.0	CD40	11.0	13.1	23.3	23.6	37.7	CD40	27.8
23.1	10,000.0	Cripto-1	5.2	0.0	20.4	0.0	35.2	Cripto-1	0.0
169.4	40,000.0	DAN	374.8	70.5	562.2	165.8	11,816.4	DAN	119.4
49.8	8,888.9	DKK-1	35.7	192.3	1,122.9	1,466.7	2,365.2	DKK-1	3,009.6
173.9	80,000.0	E-Cadherin	333.6	149.1	1,478.0	128.0	4,275.4	E-Cadherin	167.7
13.7	20,000.0	EpCAM	8.0	0.5	17.5	4.1	189.7	EpCAM	2.8
6.3	2,000.0	FAS L	20.3	4.6	19.0	5.0	40.8	FAS L	12.1
58.0	10,000.0	Fcg RIIBC	128.9	43.3	323.8	60.5	568.9	Fcg RIIBC	134.4
22.8	40,000.0	Follistatin	2.7	5.5	15.2	23.0	86.0	Follistatin	63.2
102.6	100,000.0	Galectin-7	25.0	48.2	58.6	21.9	56.5	Galectin-7	81.9
235.1	100,000.0	ICAM-2	433.2	203.9	3,766.3	3,949.3	9,610.2	ICAM-2	2,397.8
50.2	10,000.0	IL-13 R1	534.1	531.2	1,004.0	567.2	2,667.3	IL-13 R1	444.9
70.7	20,000.0	IL-13 R2	140.1	105.9	153.7	228.2	155.1	IL-13 R2	119.3
103.7	40,000.0	IL-17B	310.7	241.8	1,552.4	384.4	551.4	IL-17B	179.7
22.3	10,000.0	IL-2 Ra	98.5	57.8	108.3	88.1	207.3	IL-2 Ra	19.1
253.8	100,000.0	IL-2 Rb	199.8	134.6	170.4	265.7	191.3	IL-2 Rb	206.8
171.6	40,000.0	IL-23	0.0	4.7	122.3	26.1	59.0	IL-23	0.0
8.2	4,000.0	LAP(TGFb1)	34.5	66.5	188.4	175.7	267.7	LAP(TGFb1)	211.7
48.7	20,000.0	NrCAM	112.7	47.1	125.4	91.0	71.7	NrCAM	55.0
107.8	40,000.0	PAI-1	1,907.2	17,224.8	23,895.7	22,084.3	20,426.1	PAI-1	16,711.3
28.4	10,000.0	PDGF-AB	45.6	28.4	92.9	63.5	138.1	PDGF-AB	15.5
70.9	20,000.0	Resistin	49.0	4.1	56.6	26.7	49.8	Resistin	9.2
32.7	13,333.3	SDF-1b	14.9	6.3	52.0	5.0	10.3	SDF-1b	1.3
239.7	80,000.0	gp130	330.2	37.4	859.8	320.1	892.3	gp130	16.3
25.2	40,000.0	Shh-N	11.6	14.8	31.2	32.8	42.2	Shh-N	54.0
32.2	10,000.0	Siglec-5	91.6	10.7	344.8	45.1	95.8	Siglec-5	38.5
14.2	4,000.0	ST2	28.6	0.9	27.7	17.1	31.1	ST2	35.0
104.8	40,000.0	TGFb2	57.1	18.1	85.6	54.4	72.2	TGFb2	42.9
46.5	10,000.0	Tie-2	122.7	22.5	451.4	51.2	79.3	Tie-2	27.0
335.0	200,000.0	TPO	249.1	66.3	155.7	51.1	212.2	TPO	57.5
26.3	8,000.0	TRAIL R4	61.5	38.7	54.8	33.1	58.3	TRAIL R4	31.5
111.9	20,000.0	TREM-1	468.6	178.7	2,561.2	209.8	468.1	TREM-1	167.4
26.4	20,000.0	VEGF-C	57.8	1.9	37.9	35.5	37.3	VEGF-C	19.6
118.8	40,000.0	VEGF R1	106.8	593.6	2,745.2	3,514.9	4,425.7	VEGF R1	8,579.2

IGFBP-2, DKK1, TNF R2, Hb EGF. There are 18 biomarkers showing zero variance among all the samples, including BTC, IL-9, IL-29, MCP-4, CD40, DAN, E-Cadherin, b-NGF, EGF R, EG-VEGF, FGF-4, IGF-1, NT- 3, SCF, TGFb3, IL-5, BCMA, and E-Selectin. These biomarkers were excluded from the analysis. Details of the principal components and the results are shown in figure 1 and 2 (and supplement file). The results of the principal component analysis are significant.

Discussion

Direct Streptococcus pyogenes immune response – decreased biomarkers

Specific biomarkers like HCC1, IGFBP2, PDGF-AA, and TIMP decrease in the levels compared to controls. Hemofiltrate CC is a chemokine, which attracts and acts through CCR1 receptors.[16] It is widely secreted by various tissues. Insulin-like growth factor binding protein 2 reduces the risk of diabetes. IGFBP2 is implicated in the regulation of IGF in most tissues.[17] Blocking of IGF BP2 results in the reduction of tumor and metastasis.[18] Platelet-derived growth factor AA is involved in the migration of smooth muscle cells.[19-21] Reduction in the TIMP metallopeptidase inhibitor1 and also has anti-angiogenic activity is associated with a reduction in the adverse clinical events in acute kidney injuries.[22] MIF (macrophage migration inhibitory factor) and LIF (leukemia inhibitory factor) levels were reduced. MIF is a widely expressed pleiotropic cytokine, and it is involved in the stimulation of other inflammatory cytokines like TNF alpha, INF gamma, IL6, IL 12, CXCL8 etc.[23] LIF is involved in cell differentiation, and maturation and stimulation lead to JAK/STAT and MAPK cascades.[24]

Increased biomarkers with direct bacterial infection

Increased activity was found in E Cadherin is involved in cell-to-cell interactions, and they have tumor suppressor effects.[25] Angiostatin, DAN, is an inhibitor of BMP and TNF. Angiostatin is engaged in the reduction of angiogenesis.[26,27] There is a marked increase in EPCAM, which are complex proteins that promote transcription factor-mediated pluripotency reprogramming,[28] CFG RIIC and PDGF AB.[29] The Platelet-derived growth factor has active angiogenic potential and mitogenesis and

Table 5. Mild increase in GMCSF, IL6, IL13, TNFb and TNF RI and mild reduction in TIMP 2

LOD	MAX	(pg/ml)	0 hour treated	2 hour treated	6 hour treated	10 hour treated	14 hour treated	(pg/ml)	14 hour untreated
2.3	666.7	BLC	0.0	0.0	0.0	0.0	0.0	BLC	0.0
15.4	4,000.0	Eotaxin	0.0	0.0	0.0	0.0	0.0	Eotaxin	0.0
11.9	1,000.0	Eotaxin-2	0.0	0.0	0.0	0.0	0.3	Eotaxin-2	0.0
35.0	20,000.0	G-CSF	0.0	0.0	0.0	0.0	0.1	G-CSF	2.0
4.8	1,000.0	GM-CSF	0.0	0.0	0.0	3.4	6.1	GM-CSF	0.9
14.8	4,000.0	I-309	0.0	0.0	0.0	1.1	1.6	I-309	0.0
56.5	33,333.3	ICAM-1	23.2	54.7	69.6	76.2	159.3	ICAM-1	52.0
14.2	2,000.0	IFNg	0.0	0.0	0.0	0.0	7.0	IFNg	0.0
5.4	2,000.0	IL-1a	0.0	0.0	2.8	6.0	15.0	IL-1a	11.4
2.3	1,000.0	IL-1b	0.0	0.0	0.0	0.0	0.0	IL-1b	0.0
5.7	222.2	IL-1ra	0.0	0.0	0.0	0.0	0.0	IL-1ra	7.2
6.8	2,000.0	IL-2	0.8	0.0	0.0	0.0	0.0	IL-2	4.5
5.2	2,000.0	IL-4	0.0	0.0	0.0	0.0	0.0	IL-4	0.0
17.3	4,000.0	IL-5	0.0	2.7	0.0	0.0	0.0	IL-5	8.7
8.1	2,000.0	IL-6	0.0	9.2	22.7	77.4	136.3	IL-6	53.8
19.5	10,000.0	IL-6R	0.0	0.0	0.0	0.0	0.0	IL-6R	0.0
14.0	4,000.0	IL-7	0.0	3.3	0.0	0.0	0.0	IL-7	9.8
2.1	500.0	IL-8	4.6	16.5	7.0	4.9	17.2	IL-8	128.7
8.8	4,000.0	IL-10	0.0	0.8	0.0	0.0	0.1	IL-10	2.4
44.0	20,000.0	IL-11	0.0	0.0	0.0	0.0	3.8	IL-11	0.0
17.9	10,000.0	IL-12p40	0.0	0.0	0.0	0.0	0.0	IL-12p40	0.0
1.2	500.0	IL-12p70	0.3	0.5	0.2	0.0	1.0	IL-12p70	0.6
17.2	1,000.0	IL-13	4.2	9.1	23.0	23.5	69.3	IL-13	97.3
41.0	4,000.0	IL-15	0.0	0.0	0.0	4.5	27.9	IL-15	32.1
14.8	5,000.0	IL-16	0.0	0.0	0.0	0.0	0.0	IL-16	1.0
11.1	4,000.0	IL-17	0.0	0.0	0.0	0.0	0.6	IL-17	0.0
15.2	2,000.0	MCP-1	7.2	24.0	100.7	276.7	266.4	MCP-1	219.0
3.0	4,000.0	MCSF	0.0	0.0	0.0	0.0	0.0	MCSF	0.0
11.2	5,000.0	MIG	0.0	0.0	0.0	0.0	0.6	MIG	0.2
17.1	3,333.3	MIP-1a	0.0	0.0	0.0	0.0	0.0	MIP-1a	0.0
2.8	1,000.0	MIP-1b	0.0	0.0	0.0	0.0	0.0	MIP-1b	0.0
6.7	3,333.3	MIP-1d	0.0	0.0	0.0	0.0	0.0	MIP-1d	0.0
4.0	2,000.0	PDGF-BB	0.0	0.0	0.9	3.9	9.6	PDGF-BB	5.7
29.9	6,666.7	RANTES	0.0	0.0	0.0	0.0	0.0	RANTES	0.0
89.8	13,333.3	TIMP-1	0.0	1,286.7	2,866.2	2,781.3	3,291.9	TIMP-1	3,752.9
24.5	40,000.0	TIMP-2	0.0	53.1	359.7	444.6	566.4	TIMP-2	1,439.1
37.2	2,000.0	TNFa	0.0	44.1	18.3	49.4	41.8	TNFa	55.4
66.1	20,000.0	TNFb	0.0	6.2	0.0	13.5	19.9	TNFb	2.3
36.6	40,000.0	TNF RI	0.0	0.0	7.5	1.2	10.8	TNF RI	7.0
118.3	40,000.0	TNF RII	0.0	0.0	0.0	0.0	9.1	TNF RII	0.0

acts on various tissues. Angiogenin may maintain blood homeostasis and participates in anti-inflammatory activity and has antibacterial and antiviral properties.[30]

Streptococcus pyogenes' lysate effects and Heat map analysis

When the cells were treated with *S. pyogenes*' lysate, the levels of BLC -the B lymphocyte chemoattractant protein (CXCL13) was increased.[31] Contactin 2 is a neuronal membrane protein, and it acts as an active cell adhesion molecule.[32-34] IL 17 is an inflammatory protein, and it was induced after lysate treatment. IL17 induces the production of GCSF and chemokines like CXCL1 and 2.[35] IL17 is strongly associated with chronic inflammation associated with autoimmune disorders. PARC (parkin like ubiquitin ligase) is a cytoplasmic anchor protein to p53-associated protein complexes.[36-38]

CXCL7 is involved in neutrophil chemotaxis, adhesion to the endothelial cells, and trans-endothelial migration of the cells.[39-41] Chemokine CXCL 7 is engaged in neutrophil-platelet crosstalk, and also it is actively involved in the growth of renal cell carcinoma.[42] IL10 Rb is the receptor for IL10, and it actively participates in inflammatory signaling.[43, 44] IL 10 regulates memory T cell development in response to viral infections.[45] Eotaxin 2 is an eosinophilic chemoattractant protein, and it acts through CCR3, and it is actively involved in the recruitment of other inflammatory cells also.[46] BMP 7 acts on the BMP receptor, which is actively involved in the process of inflammation and atherogenesis. Exogenous administration of BMP7 improves left ventricular remodeling and function in acute myocardial infarction.[47]

Trappin 2 is a serine protease inhibitor, and it has anti-inflammatory actions on the mucosal surfaces.[48] It also has anti-retrovirus activities on the mucosal surfaces. SDF1 alpha and its chemokine receptor play a significant role in hematopoietic cell mobilization, cancer metastasis, and ischemic injury repair in myocardial infarction tissues.[49] FGF 7 (fibroblastic growth factor) has an active role in tissue repair.[50] CCL 28 is a mucosa-associated epithelial chemokine, and it is associated with the recruitment of the cells, and it helps in T and B cell accumulation in mucosal surfaces.[51] 4-1BB (CD137) signalosome promotes T cell prolifer-

Table 6. Mild increase in ALCAM, Endoglin, MICB, PECAM1, µPAR, VCAM 1 and Xedar

LOD	MAX	(pg/ml)	0 hour treated	2 hour treated	6 hour treated	10 hour treated	14 hour treated	(pg/ml)	14 hour untreated
16.2	10,000.0	4-1BB	0.0	0.0	0.0	0.0	2.1	4-1BB	0.0
14.9	10,000.0	ALCAM	0.0	0.0	3.5	1.3	16.8	ALCAM	6.9
19.7	10,000.0	B7-1	0.0	0.0	0.0	0.0	8.8	B7-1	0.0
37.0	20,000.0	BCMA	0.0	0.0	0.0	0.0	0.0	BCMA	0.0
17.3	10,000.0	CD14	0.0	0.0	0.0	0.0	2.8	CD14	0.0
27.2	10,000.0	CD30	0.0	0.0	0.0	0.0	0.0	CD30	0.0
26.3	10,000.0	CD40L	0.6	0.0	0.0	0.0	1.2	CD40L	0.0
24.1	10,000.0	CEACAM-1	0.0	0.0	0.0	0.0	3.1	CEACAM-1	1.5
5.1	4,000.0	DR6	0.4	0.0	0.9	1.9	2.5	DR6	5.1
23.1	20,000.0	Dtk	0.0	0.0	2.5	0.4	12.9	Dtk	0.0
10.5	4,000.0	Endoglin	0.3	8.1	17.9	36.4	51.4	Endoglin	9.6
57.8	20,000.0	ErbB3	0.0	0.0	0.0	0.0	0.0	ErbB3	6.0
39.3	13,333.3	E-Selectin	0.0	0.0	0.0	0.0	2.9	E-Selectin	0.0
5.0	2,000.0	Fas	0.8	0.5	0.2	0.7	1.1	Fas	2.4
3.0	2,000.0	Flt-3L	0.0	0.0	0.0	1.1	0.0	Flt-3L	0.5
23.1	10,000.0	GITR	0.0	0.0	0.0	1.6	0.0	GITR	7.5
34.6	40,000.0	HVEM	0.0	2.9	0.0	0.0	0.0	HVEM	0.7
80.3	100,000.0	ICAM-3	0.0	3.4	0.0	0.0	6.8	ICAM-3	3.5
192.9	100,000.0	Contactin-2	0.0	0.0	8.9	0.0	7.1	Contactin-2	38.5
12.1	4,000.0	IL-1 RI	0.0	2.3	0.0	1.2	0.0	IL-1 RI	1.4
38.8	10,000.0	IL-2 Rg	0.0	0.0	0.0	0.0	1.8	IL-2 Rg	0.0
9.8	4,000.0	IL-10 Rb	0.0	0.7	0.0	0.0	0.7	IL-10 Rb	0.0
23.6	10,000.0	IL-17R	7.9	26.3	9.6	3.9	0.6	IL-17R	8.2
55.4	20,000.0	IL-21R	0.0	3.7	9.7	0.0	1.7	IL-21R	0.0
8.9	4,000.0	LIMPII	0.0	0.0	1.2	0.0	4.9	LIMPII	3.4
1.5	1,000.0	Lipocalin-2	3.6	1.7	1.7	0.7	1.5	Lipocalin-2	2.1
162.9	100,000.0	L-Selectin	8.9	29.3	0.0	17.0	32.6	L-Selectin	53.5
5.2	2,000.0	LYVE-1	0.0	0.6	2.7	0.0	3.4	LYVE-1	0.0
15.2	10,000.0	MICA	15.0	12.2	5.6	1.9	13.9	MICA	12.9
40.0	15,000.0	MICB	33.5	59.2	1.8	1.7	51.9	MICB	22.9
9.3	5,000.0	NRG1-b1	0.0	0.0	0.0	0.0	0.7	NRG1-b1	0.0
293.9	100,000.0	PDGF Rb	0.0	10.7	0.0	0.0	65.0	PDGF Rb	5.1
39.7	20,000.0	PECAM-1	19.5	103.2	185.0	207.1	326.8	PECAM-1	127.5
8.3	3,333.3	RAGE	0.2	1.9	0.0	0.0	0.0	RAGE	0.0
24.7	10,000.0	TIM-1	0.0	11.1	0.0	3.2	0.0	TIM-1	0.0
7.9	5,000.0	TRAIL R3	0.0	2.2	13.8	35.0	74.4	TRAIL R3	17.6
9.0	10,000.0	Trappin-2	148.1	4.6	1.2	3.4	0.0	Trappin-2	0.1
21.1	40,000.0	uPAR	0.0	20.7	51.2	82.8	111.1	uPAR	75.0
320.9	200,000.0	VCAM-1	0.0	34.0	15.9	23.7	77.0	VCAM-1	0.0
34.6	10,000.0	XEDAR	0.0	0.0	15.8	12.4	22.7	XEDAR	0.0

ation and survival and results in increased T cell effector functions.[52,53] VCAM1 is an inflammatory protein involved in cell to cell adhesions, and it also effectively induces angiogenesis.[54,55]

Glucosteroid induced TNFR related protein (GITR) is expressed by T cells and its ligands, and it boosts T cell activity. GITR agonistic stimulation is emerging as a promising therapeutic concept.[56] SIGLEC 5 is a leucocyte receptor that recognizes sialic acid structures and helps in leucocyte recruitment.[57] VEGFR3 is a receptor for vascular endothelial growth factors C and D and it is involved in lymph-angiogenesis and to some extent in VEGF A induced angiogenesis as well.[58] IL13 overcomes insulin resistance by promoting anti-inflammatory macrophage differentiation in adipose tissue.[59] CD30 is expressed on the surfaces of the endothelial cells though it is primarily expressed by lymphoid tissues. They are expressed in non-lymphomatous tumors. CD30 signaling is involved in proliferation, differentiation and survival (anti-apoptosis).[60] TGF B2 is expressed in the endothelium, and it plays an essential role in angiogenesis.[61] GP130 is a glycoprotein that participates in IL6 mediated inflammation and vascular pathologies, and it also has a negative feedback control.[62]

Reduced biomarkers with lysate

MIF is a widely expressed pleiotropic cytokine, and it is involved in the stimulation of other inflammatory cytokines like TNF alpha, INF gamma, IL6, IL 12, CXCL8 etc.[23,63] MIF is elevated in type 1 and 2 diabetes. Fas activation is associated with autoimmune disorders, which can be modulated by downregulation.[64] TIMP 2 are matrix metalloproteinases which are involved in inflammation in the cancer cells.[65] IGFBP2 are inhibitory and stimulatory to some of the tumours.[66] IL13 R2 is involved in mediating inflammation leading to myocarditis. Hence a reduction in these receptors could reduce inflammatory changes.[67,68] Dickkopf 1 family proteins are active modulators of Wnt pathways, and mostly their effects are inhibitory.[69] TNF R2 has proinflammatory and some anti-inflammatory aspects as well. The stimulatory and inhibitory effects had attracted considerable interest in the treatment of autoimmune diseases and cancer.[70] Follistatin is actively involved in activin A-follistatin regulation of cardiac inflammation

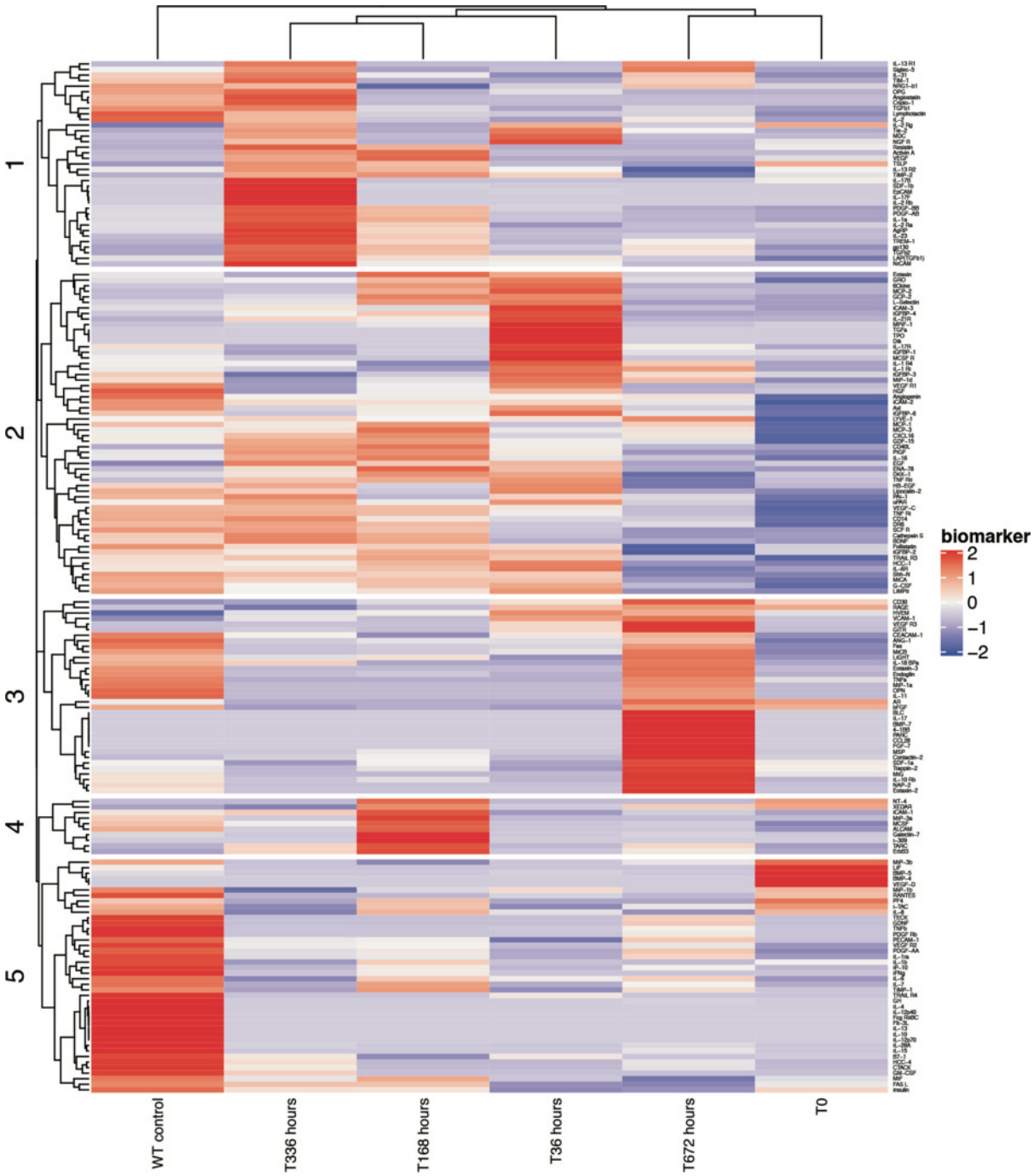

Fig. 1A. Heat map and the classification of the principal components (1-5) for analysis of the biomarkers after S. pyogenes lysate treatment on the endothelial cells.

and fibrosis.[71] Heparin-Binding Epidermal Growth Factor-Like Growth Factor inhibits cytokine-induced NF-κB Activation.[72] The heat map analysis shows a significant change in more proteins, and thereby it is possible to infer that *S. pyogenes* has a role in immune regulation.

The above observations indicate that the immune system undergoes various modifications by the *S. pyogenes*' direct challenge. Certain cytokines/parameters are increased, and some are decreased. In the long term, the immune memory and its regulation are complex, and it is also subjected to many positive and negative feedback regulations. Also, when the lysate is added, modulations are seen. Hence, *S. pyogenes* is associated with changes in the immune system, which can influence potential regulations in the immune homeostasis of the individuals. The negative impact causing rheumatic heart diseases by Streptococcus pyogenes can have a positive influence in modifying the immune-related and metabolic functions.

Chaos signaling and decoding

It is indeed difficult to predict the immune response in the future to a certain extent. It can be inferred that the

Ord	Biomarker	Ord	Biomarker	Ord	Biomarker	Ord	Biomarker	Ord	Biomarker
Cluster1									
1	IL-13 R1	9	TGFb1	17	Activin A	25	IL-17F	33	TREM-1
2	Siglec-5	10	Lymphotactin	18	VEGF	26	IL-2 Rb	34	gp130
3	IL-31	11	IL-2	19	TSLP	27	PDGF-BB	35	TGFb2
4	TIM-1	12	IL-2 Rg	20	IL-13 R2	28	PDGF-AB	36	LAP(TGFb1)
5	NRG1-b1	13	Tie-2	21	TIMP-2	29	IL-1a	37	NrCAM
6	OPG	14	MDC	22	IL-17B	30	IL-2 Ra		
7	Angiostatin	15	NGF R	23	SDF-1b	31	AgRP		
8	Cripto-1	16	Resistin	24	EpCAM	32	IL-23		
Cluster2									
1	Eotaxin	13	Dtk	25	Axl	37	DKK-1	49	BDNF
2	GRO	14	IL-17R	26	IGFBP-6	38	TNF RII	50	Follistatin
3	6Ckine	15	IGFBP-1	27	LYVE-1	39	HB-EGF	51	IGFBP-2
4	MCP-2	16	MCSF R	28	MCP-1	40	Lipocalin-2	52	TRAIL R3
5	GCP-2	17	IL-1 R4	29	MCP-3	41	PAI-1	53	HCC-1
6	L-Selectin	18	IL-1 RI	30	CXCL16	42	uPAR	54	IL-6R
7	ICAM-3	19	IGFBP-3	31	GDF-15	43	VEGF-C	55	Shh-N
8	IGFBP-4	20	MIP-1d	32	CD40L	44	TNF RI	56	MICA
9	IL-21R	21	VEGF R1	33	PIGF	45	CD14	57	G-CSF
10	MPIF-1	22	HGF	34	IL-16	46	DR6	58	LIMPII
11	TGFa	23	Angiogenin	35	EGF	47	SCF R		
12	TPO	24	ICAM-2	36	ENA-78	48	Cathepsin S		
Cluster3									
1	CD30	8	ANG-1	15	TNFa	22	IL-17	29	Contactin-2
2	RAGE	9	Fas	16	MIP-1a	23	BMP-7	30	SDF-1a
3	HVEM	10	MICB	17	OPN	24	4-1BB	31	Trappin-2
4	VCAM-1	11	LIGHT	18	IL-11	25	PARC	32	MIG
5	VEGF R3	12	IL-18 BPa	19	AR	26	CCL28	33	IL-10 Rb
6	GITR	13	Eotaxin-3	20	bFGF	27	FGF-7	34	NAP-2
7	CEACAM-1	14	Endoglin	21	BLC	28	MSP	35	Eotaxin-2
Cluster4									
1	NT-4	3	ICAM-1	5	MCSF	7	Galectin-7	9	TARC
2	XEDAR	4	MIP-3a	6	ALCAM	8	I-309	10	ErbB3
Cluster5									
1	MIP-3b	10	IL-8	19	IL-1b	28	IL-12p40	37	HCC-4
2	LIF	11	TECK	20	IP-10	29	Fcg RIIBC	38	CTACK
3	BMP-5	12	GDNF	21	IFNg	30	Flt-3L	39	GM-CSF
4	BMP-4	13	TNFb	22	IL-6	31	IL-13	40	MIF
5	VEGF-D	14	PDGF Rb	23	IL-7	32	IL-10	41	FAS L
6	MIP-1b	15	PECAM-1	24	TIMP-1	33	IL-12p70	42	Insulin
7	RANTES	16	VEGF R2	25	TRAIL R4	34	IL-28A		
8	PF4	17	PDGF-AA	26	GH	35	IL-15		
9	I-TAC	18	IL-1ra	27	IL-4	36	B7-1		

Fig. 1B. Legends for Figure 1

endothelial response could be significant and probably chaotic, which determine transcription and gene regulation.[73] Chaos in the immune system could be a natural method of selection to strengthen immune functions. Optical laser chaos signals, which are high speed when studied are found to synchronize and it has many features.[74,75] Similarly, decoding these chaotic signals in immune system would potentially lead to our better understanding of immune system.

Streptococcus and viral infections – both together forever

In the recent times coronavirus infections (Covid-19) pandemic is rampant, and the infection selectively affects various countries, and the mortality statistics were varied in different countries. The Southeast Asian countries, India and neighboring countries, Africa and eastern European countries are relatively less affected so far, at his time of writing. The *S. pyogenes*', tropical bacterial infections and other viral are common in these areas, and they could provide cross-immunity to the Covid19 infections.[76] Bacteria can synthesize restriction enzymes like nucleases and inhibit viruses.[77] It has been shown that bacterial presence can reduce the intensity of viral infections.[78] Our study also reflects the immune regulation changes due to *S. pyogenes* as well as by its lysate.

Environmental modifications

In the theory of natural selection, the environment or the Nature in various forms could offer protection by its selective mechanisms in various geographic locations.[79] These could not necessarily be simple mechanisms but also as chaotic or cross-immunity methods. The commonly available Streptococcus bacteria and the common viruses could be the mechanism of choice in the form of natural selection by 'unhygienic' means.[80]

Metabolic protection and autoimmunity regulation

The rarity of diabetes and rheumatic heart disease was observed by legendary physicians like Joslin and

Steinchron in the early 1920s and 1930's respectively.[81,82] Our study also suggests various metabolic modulators being stimulated and some inhibited. Also, Joseph Barach made similar observations in that period and attributed the views to immune regulation changes. Our observations, such as increased IL13, decreasing Fas, and Dickkopf proteins, also indicate possible metabolic protection. Antibodies seen in rheumatic fever are also found in antiphospholipid antibody syndromes.[83] However, the incidence of autoimmune diseases in rheumatic heart disorders is very rare or possibly mutually exclusive by negative feedback mechanisms, at least in the clinical experience of the author.

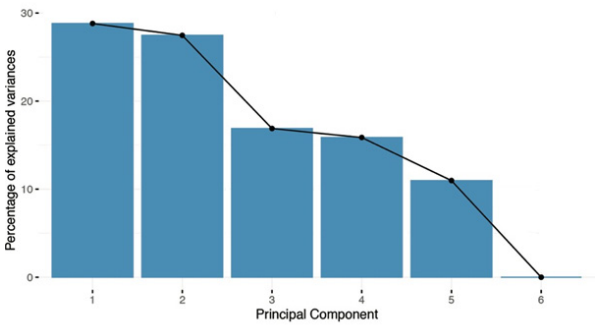

Fig. 2. Shows the variance explained by each principal component

Cancer prevention by S. pyogenes

Though certain viral infections can predispose patients for malignancies, infections can help to prevent malignancies.[84] This has been evaluated in the past as early as 1700's. William Coley in the 19th century has shown that *S. pyogenes*' vaccination can reduce the cancer progression, and improve the cure rates.[85] Hence, *S. pyogenes* vaccinations can be evaluated for this purpose. BCG vaccines are known to have anti-tumour activity in urinary bladder malignancies.[86]

Limitations and future perspectives

Further studies need to be performed to observe the immune changes in animals after direct *S. pyogenes* challenge and after the lysate administration. Specifically, the immune changes regulating the autoimmune disorders, cancer regulation, atherosclerotic processes, and host defense activities to viruses need to be studied in-depth in animal models.

Conclusion

Streptococcus pyogenes and its lysate has immunomodulation actions when tested with endothelial cells, which have pleiotropic functions. Further studies need to be performed to identify its potential benefits.

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ORIGINAL PAPER

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A prospective, interventional clinical study to evaluate the safety and efficacy of Liv.52 DS in the management of non-alcoholic fatty liver disease

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ABSTRACT

Introduction. Non-alcoholic fatty liver disease (NAFLD) is excessive fat build-up in the liver due to causes other than alcohol use.
Aim. To evaluate the clinical efficacy and safety of Liv.52 DS tablets in the management of NAFLD.
Material and methods. Prospective, interventional clinical study conducted on 60 patients of both sex, aged between 18-65 years, confirmed with NAFLD from clinical examination, laboratory test, ultrasound findings and those willing to give informed consent. All patients received Liv.52 DS at a dose of 2 tablets twice daily for 2 months. All patients were evaluated at baseline, end of 1st month, and end of 2nd month for liver function tests, hepatomegaly by ultrasound, NAFLD Fibrosis Score, lipid profile, hematology and biochemical investigations.
Results. Study data was analyzed with GraphPad Prism Software Version 6.07. Data of those patients who completed the study was considered for analysis. Significant improvement in hepatomegaly, liver enzymes was observed. NAFLD fibrosis score revealed no progression of liver fibrosis due to NAFLD during the study period. No abnormal lab values were recorded and there were no adverse events reported during the study.
Conclusion. Study concludes that Liv.52 DS is safe and beneficial in individuals suffering from NAFLD.
Keywords. ALT/AST, fatty liver, fibrosis, non-alcoholic fatty liver disease

Introduction

Non-alcoholic fatty liver disease (NAFLD) is excessive fat build-up in the liver due to causes other than alcohol use.[1] NAFLD, encompassing both simple steatosis and non-alcoholic steatohepatitis (NASH), is the most common cause of liver disease.[2-3] It may lead to complications such as cirrhosis, liver cancer, liver failure, or cardiovascular disease.[4]

NAFLD is the commonest cause of liver disease in Western countries; NAFLD is strongly associated with

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obesity, insulin resistance, hypertension and dyslipidemia and is now regarded as the liver manifestation of the metabolic syndrome. Rapid spread of the obesity 'pandemic' in adults and children, coupled with the realization that the outcomes of obesity-related liver disease are not entirely benign, has led to rapid growth in clinical and basic studies in NAFLD.[5]

Many patients with NAFLD remain undiagnosed and recognizing those at risk is the first step. Clinicians overly rely on abnormal liver enzymes to identify patients with NAFLD, so patients with significant liver disease can be overlooked, potentially missing opportunities for intervention. Although liver biopsy is the gold standard method for diagnosing and staging NAFLD, most patients can be effectively diagnosed non-invasively with tests that are routinely available in the clinic today.[6]

In terms of epidemiology, several studies have tried to quantify the true worldwide incidence of NAFL/NASH; however, due to extreme variations in study parameters and available testing, a clear and reliable occurrence rate is not currently available.[7] With that being said, estimates have been posited suggesting the incidence of NAFLD to be 20%-30% in Western countries and 5%-18% in Asia.[7] It is no surprise that the prevalence of NAFLD is increasing worldwide with each passing year, given the current trends in dietary irresponsibility and preponderance of a sedentary lifestyle.[7] Additionally, there has been a linear rise of NAFLD with that of diabetes and metabolic syndrome.[8]

In one study from the United States, it was shown that the incidence of NAFLD was 10% higher in overweight individuals compared to lean persons.[9] At present, the high prevalence and negative pathological consequences of NAFLD represent a significant economic burden for many countries. However, up to now, there is no effective procedure to treat the disease.[10-11]

The primary therapeutic approach is to recommend healthy lifestyle strategies that are focused on reducing body weight and increasing insulin sensitivity, including dietary and exercise Regimens.[12]

Traditional medicines are abundant sources of biologically active substances that can be applied to prevent human diseases.[13-14] Currently, an increasing number of studies have focused on herbal extracts or natural products, and many of these studies have discovered herbal products with potent effects against NAFLD.[15-16] Thus, herbal medicines are promising candidate drugs for the treatment of NAFLD. There are no specific reliable treatment options available for NAFLD. During clinical studies with Liv.52 DS, in various liver disorders, some of the patients with NAFLD were benefitted with Liv.52 DS.

Maity SG et al. evaluated the clinical efficacy and safety of Liv.52 DS tablets with UDCA in patients with Non-Alcoholic Steatohepatitis in a randomized, comparative clinical study, Liv.52 DS appear promising in the management of Non-Alcoholic Steatohepatitis.[17] In a clinical study conducted by Gosh S et al., inferred that Liv.52 DS appear promising in the management of Non-Alcoholic Steatohepatitis.[18]

In an *in vitro* by Vidyashankar to evaluate the quercetin effectively reversed NAFLD symptoms by decreased triacyl glycerol accumulation, insulin resistance, inflammatory cytokine secretion and increased cellular antioxidants in OA induced hepatic steatosis in HepG2 cells. Hence quercetin is promising to carry out more experimental and clinical studies to understand the molecular mechanism to overcome NAFLD symptoms.[19] In a study conducted by Vidyashankar to evaluate LHAE which could effectively reverse the molecular perturbations underlying NAFLD symptoms suggesting its importance to ameliorate OA induced hepatic steatosis in HepG2 cells. Hence treatment with LHAE could be a new perspective to carry out more experimental and clinical studies to understand the molecular mechanism to overcome NAFLD symptoms.[20]

Liv.52 DS Tablet is a polyherbal formulation consisting of extracts of *Capparis spinosa, Cichorium intybus,* Mandura bhasma, *Solanum nigrum, Terminalia arjuna, Cassia occidentalis, Achillea millefolium and Tamarix gallica.* Some preliminary studies demonstrated leads in NAFLD. Therefore, a clinical study was planned and carried out to further evaluate role of Liv.52 DS in the management of NAFLD.

Aim

To evaluate the safety and efficacy of Liv.52 DS in the management of Non-Alcoholic Fatty Liver Disease

Material and methods

This study was a prospective interventional clinical study conducted at Department of Internal Medicine, Faculty of Medicine, University of Sumatera Utara, Medan, Indonesia. The essential study documents were submitted to the Health Research Ethical Committee. All the study related documents were reviewed and approved with the vide approval number No.74/TGL/KEPK FK USU-RSUP HAM/2017. All the patients gave their informed consent for their active participation in the study.

A total of 60 male and female patients aged between 18-65 years with NAFLD from clinical examination, laboratory test, and ultrasound findings; and those who were willing to give informed consent were included in the study.

Alcoholic subjects were excluded in the study. Patients with severe metabolic disorders, carcinoma of liver or pancreas. Subjects with a known history of present condition of allergic response to similar pharmaceutical products, its components or ingredients in the test products, Subjects with pre-existing systemic disease necessitating long-term medications, genetic disorders, Subjects who has partic-

ipated in a similar clinical investigation in the past four weeks and those who refused to sign the informed consent form were excluded. Women of child bearing age and lactating women were also excluded from the trial.

At the initial visit, a detailed medical history and symptomatic evaluation was done. In addition, examination specific to the steatohepatitis with Hepatomegaly (enlarged liver) was done. The Subjects were instructed to take Liv.52 DS tablets 2 tablets twice daily for a period of 2 months. Subjects were evaluated at baseline, at the end of 1st month, and at the end of 2ndmonth for liver function tests including AST, ALT, ALP, GGT, serum bilirubin, albumin, hepatomegaly (by ultrasound) and NAFLD Score. All subjects underwent complete blood count, and biochemical investigations, blood sugar levels and lipid profile at baseline, Month 1 and Month 2.

Non-invasive NAFLD fibrosis score was calculated at each assessment visits to assess the severity of fibrosis due to NAFLD. NAFLD fibrosis score is calculated by a formula -1.675 + 0.037 × age (year) + 0.094 × BMI (kg/m^2) + 1.13 × IFG/diabetes (yes =1, no = 0) + 0.99 × AST/ALT ratio - 0.013 ×platelet count (×10^9/L) - 0.66 × albumin. NAFLD score was evaluated with a score of NAFLD Score < -1.455 = F0-F2, NAFLD Score -1.455 to 0.675 = indeterminate score and NAFLD Score > 0.675 = F3-F4.

All data were analyzed with GraphPad Prism for Windows version 6.07. A $p<0.05$ was considered as statistically significant. Respective statistical tests are mentioned with the summary table. A subgroup analysis was carried out to evaluate the role of Liv.52 DS in the Management of Non-Alcoholic Fatty Liver Disease in Diabetes Mellitus Subjects among the total included subjects.

All the patients were provided with a chart and requested to write down the date of taking the drug as well as record the occurrence of adverse effects, if any. At the time of follow-up visits at the end of 1st month, and at the end of 2ndmonth, the participants were requested to bring back the empty boxes of the study medication to ensure that they had consumed it.

Statistical analysis

Statistical analysis was carried out using GraphPad Prism, Version 6.07 for windows, GraphPad Software, San Diego, California, USA. Liver function tests, biochemical parameters, NAFLD score were analyzed using ANOVA followed by Tukey's multiple comparisons test. Hepatomegaly was analyzed by Repeated measures ANOVA followed by Dunnett's multiple comparisons test. The values are expressed as Mean ± SD.

Results

Sixty patients (34 females and 26 males) with a mean age of 48.2±12.3 years, with a mean weight of 77.03±9.24 kgs participated in this clinical study (Table 1). All the patients completed the study and their data was available for analysis. In the present study, there were total 17 patients who were diabetics and they have not been considered for statistical analyses.

Table 1. Demographic details

No. of Subjects	60
Age in years	48.2±12.3
Weight in kgs	77.03±9.24
Height in cms	159.3±7.7
Gender (Female: Male)	(34:26)

Table 2 explains the effect of Liv.52 DS on liver fibrosis as evaluated by NAFLD Fibrosis score. The interpretation is as compared to baseline. In the score F0-F2, there is a trend towards reduction in the fibrosis score at month 1, and further a trend towards reduction in the fibrosis score by month 2. Similarly people with indeterminate score also demonstrated that fibrosis score reduced with Liv.52 DS treatment. There is an trend towards reduction in the fibrosis but was not statistically significant. This signifies that Liv.52 DS demonstrates a trend towards reduction of the liver fibrosis associated with NAFLD (Table 2).

Table 2. Effect of Liv.52 DS on liver fibrosis (NAFLD fibrosis score)

Scale	N		Baseline	Month 1	Month 2
NAFLD Score <-1.455 = F0-F2	33	Mean	-2.886	-2.911	-2.915
		SD	1.044	1.061	0.8617
NAFLD Score -1.455 – 0.675 = indeterminate score	27	Mean	-0.650	-0.684	-0.849
		SD	0.484	0.543	0.667
NAFLD Score > 0.675 = F3-F4	0		0	0	0

Statistical test: ANOVA followed by Tukey's multiple comparisons test, Value in: Mean±SD

Formula for NAFLD Score: -1.675 + 0.037 × age (years) + 0.094 × BMI (kg/m^2) + 1.13 × IFG/diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio – 0.013 × platelet (×109/l) – 0.66 × albumin (g/dl)

NAFLD score was evaluated:

NAFLD Score< -1.455 = F0-F2

NAFLD Score -1.455 to 0.675 = indeterminate score

NAFLD Score > 0.675 = F3-F4

Table 3. Effect of Liv.52 DS on Hepatomegaly (number and %)

Liver size on Ultrasound	Baseline	Month 1	Month 2
	No. of Subjects (%)	No. of Subjects (%)	No. of Subjects (%)
Hepatomegaly > 16 cm	45 (75%)	43 (72%)	25 (42%)
No Hepatomegaly < 16 cm	15 (25%)	17 (28%)	35 (58%)

Effect of Liv.52 DS on number and percentage of subjects with hepatomegaly is given in Table 3 and Figure 1. At Baseline, 75% of subjects demonstrated hepatomegaly (Liver size >16 cm) and 25% subjects showed no hepatomegaly (liver size <16). After 2 months treatments with Liv.52 DS, only 42% people showed hepatomegaly and 58% subjects showed no hepatomegaly.

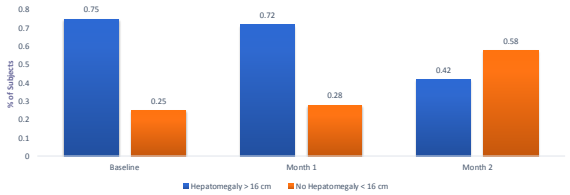

Fig. 1. Effect of Liv.52 DS on liver size by ultrasound measurement (expressed as % of individuals with hepatomegaly and no hepatomegaly)

Effect of Liv.52 DS on liver size is explained in Table 4 and Figure 2. At baseline liver size (cms) was 17.44 ± 1.9 reduced to 17.29 ± 1.77 at month 1 which further reduced 15.87 at the end of month 2 with statistical significance of p<0.0001.

Table 4. Effect of Liv.52 DS on Liver size (cms) expressed in mean ± SD

Liver size on Ultrasound	Baseline	Month 1	Month 2
Mean (cms)	17.44	17.29	15.87
SD	1.9	1.77	1.79
Std. Error of Mean	0.24	0.23	0.23
Significance		ns	p<0.0001[a]

Repeated measures ANOVA followed by Dunnett's multiple comparisons test
significance was fixed at <0.05
a: as compared to baseline
software: GraphPad Prism 6.07

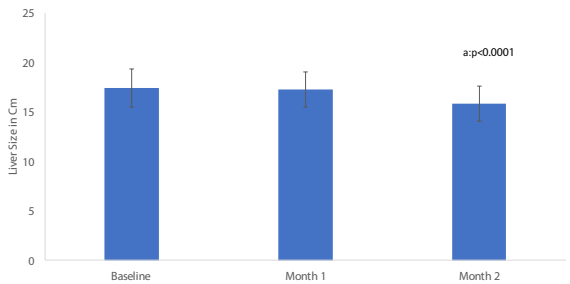

Fig. 2. Effect of Liv.52 DS on liver size by ultrasound measurement (expressed as cms (mean±SD) of individuals with hepatomegaly)

SGPT which was 60.33±13.04 at baseline reduced to 56.43±15.47 at month 1, which further reduced to 54.12±17.26 with a significance of p<0.0339 at the end of 2 months treatment of Liv.52 DS. SGPT which was 70.68±18.49 at baseline decreased to 64.13±22.07 at 1st month with a significance of p<0.0215 and further reduced to 61.9±22.7 with a significance of p<0.0022 at the end of 2nd month with Liv.52 DS (Table 5). There were no patients identified with hepatitis; although, ALT and AST levels were elevated at screening and was found to be reduced at the end of the study.

Table 5. Effect of Liv.52 DS on LFT (SGOT and SGPT)

Parameters	Baseline	Month 1	Month 2
SGOT (UI/L)	60.33±13.04	56.43±15.47	54.12±17.26 p<0.0339[a]
SGPT (UI/L)	70.68±18.49	64.13±22.07 p<0.0215[a]	61.9±22.7 p<0.0022[a]

Statistical test: ANOVA followed by Tukey's multiple comparisons test, significance was fixed at p<0.05, value in: mean±SD, a: as compared to baseline, SGOT – Serum glutamic-oxaloacetic transaminase, SGPT – Serum glutamic pyruvic transaminase

The hematological and biochemical investigations demonstrated that Liv.52 DS is a safe formulation. Although; high density lipoprotein, low density lipoprotein levels were within the normal range, total serum cholesterol and serum triglyceride levels were towards borderline high at screening and remained almost at same levels even at the end of the study (Table 6). There were no clinically significant adverse events either reported or observed during the entire study period. The overall compliance to the treatment was good and no treatment discontinuations were reported.

Table 6. Hematology and biochemical investigations

Parameters	Baseline	Month 1	Month 2	p value
Hemoglobin (g/dL)	13.07±2.15	12.96±2.25	13.09±2.15	ns
White Blood Count (/mm^3)	8445±1645	8455±2143	8229±1444	ns
Platelet count (/mm^3)	314950±77105	308002±65593	316150±51555	ns
Fasting glucose (mg/dL)	149.7±45.35	150.6±44.55	148.5±44.64	ns
Random Glucose (mg/dL)	182.8±61.42	184.1±43.64	192.4±29.26	ns
Gamma glutamyl transferase (UI/L)	40.52±14.05	40.68±11.69	38.42±10.42	ns
Alkaline Phosphatase (UI/L)	117.3±42.84	117.5±42.03	119±40.79	ns
Serum albumin (g/dL)	3.58±0.54	3.45±0.44	3.48±0.55	ns
Total bilirubin (g/dL)	0.68±0.22	0.60±0.27	0.77±0.25	ns
Direct bilirubin (g/dL)	0.35±0.13	0.31±0.15	0.36±0.15	ns
Total serum cholesterol (mg/dL)	233.3±29.33	235.3±28.46	228.9±33.91	ns
Serum triglycerides (mg/dL)	166.3±30.75	162.7±37.1	168±26.48	ns
High Density Lipoprotein (mg/dL)	41.23±5.82	41.22±4.99	42.32±4.78	ns
Low Density Lipoprotein (mg/dL)	160.5±30.27	162.3±30.8	160.3±30.1	ns

Statistical test: ANOVA followed by Tukey's multiple comparisons test, significance was fixed at p<0.05, value in: Mean ± SD. ns = Not significant

Discussion

Liv.52 DS Tablet is a hepatospecific formulation, designed for the management of liver disorders. It has a wide spectrum of therapeutic applications in liver disorders. It restores the metabolic efficiency of the liver

in various etiological forms of hepatocellular jaundice like infective and chronic active hepatitis, drug-induced hepatitis and alcohol-induced hepatic damage. It increases appetite. It corrects the hepatitis, and cirrhotic conditions, and in any hepatotoxic drug regimen. It is a supportive treatment as an adjuvant with hepatotoxic drugs. Therapy options include weight reduction in obese, good control in diabetics and exercise.[21]

Although there is no consensus for the treatment for NASH, effort needs to be made to prevent development of fibrosis, which results in cirrhosis and portal hypertension. As the pathogenesis of this condition is not clear, treatment has been largely empirical.[22]

Treatment should be focused on correction of the underlying metabolic syndrome. The role of specific pharmacologic treatment continues to evolve. Several large clinical trials using a variety of agents are currently under way and should provide additional treatment option for those with nonalcoholic steatohepatitis.[23]

In a study by Eguchi et al., who conducted pilot study of liraglutide effects in non-alcoholic steatohepatitis and non-alcoholic fatty liver disease with glucose intolerance in Japanese patients(LEAN-J); was aimed to evaluate the effect and action of liraglutide for biopsy-proven NASH. Subjects whose hemoglobin A1c levels failed to improve to less than 6.0% and/or whose alanine aminotransferase levels were not lower than baseline, received liraglutide at 0.9 mg/body per day for 24 weeks, after lifestyle modification intervention for 24 weeks. Study concluded that treatment with liraglutide had a good safety profile and significantly improved liver function and histological features in NASH patients with glucose intolerance.[24]

In a study by Belfort R et al., conducted placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. They randomly assigned 55 patients with impaired glucose tolerance or type 2 diabetes and liver biopsy-confirmed nonalcoholic steatohepatitis to 6 months of treatment with a hypocaloric diet (a reduction of 500 kcal per day in relation to the calculated daily intake required to maintain body weight) plus pioglitazone (45 mg daily) or a hypocaloric diet plus placebo. Before and after treatment, they assessed hepatic histologic features, hepatic fat content by means of magnetic resonance spectroscopy, and glucose turnover during an oral glucose tolerance test ([14C] glucose given with the oral glucose load and [3H] glucose given by intravenous infusion). The study concluded that the administration of pioglitazone led to metabolic and histologic improvement in subjects with nonalcoholic steatohepatitis. Larger controlled trials of longer duration are warranted to assess the long-term clinical benefit of pioglitazone.[25]

In a study by Cusi K et al., which is RCT with Long-Term Pioglitazone Treatment for Patients with Nonalcoholic Steatohepatitis and Prediabetes or Type 2 Diabetes Mellitus; all patients were prescribed a hypocaloric diet (500-kcal/d deficit from weight-maintaining caloric intake) and then randomly assigned to pioglitazone 45 mg or placebo for 18 months, followed by an 18-month open-label phase with pioglitazone treatment. Study concluded that long-term pioglitazone treatment is safe and effective in patients with prediabetes or T2DM and NASH.[26]

Eight active medicinal herbs viz., *Capparis spinosa, Cichorium intybus, Mandura bhasma, Solanum nigrum, Terminalia arjuna, Cassia occidentalis, Achillea millefolium* and *Tamarix gallica* were carefully selected during the product development. These herbs possess significant hepatoprotective activity and the results of the present study might be possibly due to the synergistic potential of these polyherbal actives.

Capparis spinosa

P-Methoxy benzoic acid from *Capparis spinosa* has potent hepatoprotective activity against chemically-induced hepatotoxicity, prevents elevation of malondialdehyde levels (plasma and hepatic) and enzyme levels (AST and ALT).[27-29] It improves the functional efficiency of the liver and spleen, with protective action on the histological architecture of the liver, and a salutary effect on liver glycogen and serum proteins.[30] Flavonoids of *Capparis spinosa* have significant antioxidant activity, as demonstrated by lipid peroxidation, bleaching of free radicals, and auto-oxidation of iron ions.[31]

Cichorium intybus

Cichorium intybus protects the liver against alcohol toxicity. It increases circulating leukocytes, splenic plaque-forming cells, hemagglutination titers, secondary IgG antibody response, phagocytic activity, natural killer cell activity, cell proliferation, and interferon gamma secretion.[32,33] Its hepatoprotective activity suppresses the oxidative degradation of DNA in tissue debris. It also has potent antioxidant action, as evident by its free radical scavenging effects, inhibition of hydrogen peroxide and iron chelation.[34]

Solanum nigrum

Solanum nigrum protects DNA against oxidative damage[35], and also acts as a potent scavenger of hydroxyl and diphenylpicrylhydrazyl radicals.[36] The cytoprotective effect of *Solanum nigrum* against gentamicin-induced toxicity showed a significant inhibition of cytotoxicity, and hydroxyl radical scavenging potential.[37]

Terminalia arjuna

Terminalia arjuna reduces cholesterol levels and is also useful in liver disorders.[38-39] It has potent antioxidant activity, which is due to its effects on lipid peroxidation.[40] Arjunaphthanoloside from *Terminalia arjuna* inhibits nitric oxide production, and terminoside A decreases inducible nitric oxide synthase levels in lipopolysaccharide-stimulated peritoneal macrophages[41]. It has strong antiviral activity, inhibiting viral attachment and penetration.[42] It also has supportive antibacterial activity.[43]

Cassia occidentalis

Cassia occidentalis has significant hepatoprotective effects in chemically-induced liver damage.[44] It modulates hepatic enzymes, which provides hepatoprotection.[45]

Achillea millefolium

Achillea millefolium is beneficial in chronic hepatitis[46] and has anti-hepatoma activity.[47]

Tamarix gallica

Tamarix gallica is a hepatic stimulant, digestive and hepatoprotective, and has a salutary effect on liver glycogen and serum proteins.[48]

Mandura bhasma

Mandura bhasma has hepatoprotective property and is beneficial in chemically-induced hepatotoxicity as it prevents changes in liver enzyme activities.[49] Mandura bhasma is a powerful hematinic and tonic.[50]

Prospective, randomized, double-blind, placebo-controlled clinical studies with large sample size (which we can consider as a drawback in the present study) will be helpful in drawing further conclusions related to the action of Liv.52 DS in non-alcoholic steatohepatitis.

Conclusion

The present study demonstrates that with Liv.52 DS treatment, there was a significant improvement in hepatomegaly, liver function parameters namely SGPT and SGOT. There was a trend towards improvement in NAFLD score which signifies improvement in liver fibrosis due to NAFLD. Hematology and biochemistry investigation results are within normal limits and there were no clinically significant adverse effects were reported during the clinical study. Subgroup analysis carried out in diabetic subjects further demonstrated beneficial effects in those populations suffering from NAFLD with respect to hepatomegaly and LFT levels. From the results of the study, it can be summarised that Liv.52 DS is safe and beneficial in individuals suffering from NAFLD. Further conclusions related to action of Liv.52 DS in non-alcoholic steatohepatitis can be drawn only with the conduct of randomized, double-blind, placebo-controlled clinical studies with larger sample size.

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ORIGINAL PAPER

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Effects of bridging and V-sitting exercises on pain intensity and disability of patients with non-specific chronic low-back pain

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ABSTRACT

Introduction. Exercises are important in the management of non-specific chronic low-back pain (NSCLBP).

Aim. The study compared the effects of bridging and V-sitting exercises on pain and disability of patients with NSCLBP.

Material and methods. 34 patients with NSCLBP recruited for the study were allocated into V-sitting (VSG) and Bridging Exercise group (BEG) equally. Participants in VSG and BEG groups performed V-sitting and bridging exercises respectively for 10 seconds, three times in a week for three weeks under a supervision of one of the authors. Each participants underwent ten sessions per a treatment regimen. Pain intensity and disability were assessed at the pre-intervention, second and third weeks using verbal rating scale and Rolland Morris Low Back Pain Disability Questionnaire prospectively.

Data were analyzed using descriptive and inferential statistics, alpha level was set at 0.05

Results. There was a significant reduction in the third week ($P < 0.001$) in both VSG and BEG group of pain intensity and disability comparing the pre intervention, second and third week values. There was a significant reduction in the 3rd week VSG's pain intensity ($F=27.34$ $P<0.001$) and disability ($F=14.96$, $P<0.001$) compared with BEG.

Conclusion. V-sitting and bridging exercises were effective in management of patients with NSCLP, but V-sitting seems more effective.

Keywords. bridging exercises, disability, low back pain, pain intensity, V-sitting exercise

Introduction

It has been stated that an unstable spine due to muscle weakness reduces endurance flexibility and the range of motion of the lower back, in addition to causing pain.[1] This on the other hand results into reduction in back muscles cross-sectional area on account of reduction and limitation to functional usage and physical activity.[2,3] Moreover, the physical causes of low back pain can

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

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be said to be muscle weakness and imbalance, produce as a result of changes in the postural mechanics, inadequate control of trunk muscle for proper stabilization and compensatory neuromuscular disorder.[4] Research has it that all this problems can be easily solved by trunk stabilization exercises and strengthening the lumber spine, which is a very valuable treatment in the management of Low Back Pain.[5,6]

Exercise therapy has been considered important from various researchers and reviews to be a key factor in the management of chronic low back pain.[7,8] Van Middelkoop, also reported that exercise therapy decreases pain intensity, alleviates disability, and improves physical functions for a long period, such as 12-months follow-up.[8] Some exercises for the management of low back pain includes: pelvic tilt, supine single and double knee to chest, supine lying alternate arm and leg, prone leg extension, prone single arm extension, prone alternate arm and leg extension, supine heel drag to extended arms, supine sit up, trunk hyperextension, abdominal hollowing, sit ups, curl ups, bridging and V-sitting exercises, William flexion exercises, bird dog, Basquet hang, side bridge, inclined sit up, crossed long lying sit up, bench trunk curl up to mention but few.[9-13] Most studies include that active exercise are valuable therapeutical approach to the management of low back pain, despite the lack of consensus on the optimal exercise technique, intensity or active intervention.[10,13] Moreover, most clinical protocols combine different exercises with various or multiple movement techniques and position.[5] Only few of these stabilization exercise position and techniques have been proven by studies to be effective in the management of chronic low back pain as whereby efficacy of one over the other is yet to be proven.[14]

The V- sitting posture stabilization exercise program is believed to eliminate the movements of other segments while slightly flexing the hip and slowly lifting the upper portion of the body, by contracting the trunk flexors and the back extensors simultaneously is an effective means of strengthening the abdominal muscles.[15] The diaphragm and the pelvic floor muscles that make up the roof and the floor respectively, contract rhythmically during the V-sitting position exercise, this contraction contributes to spinal stability with increased intra-abdominal pressure thus creating a rigid spine.[16] Hayashi, reported that exercises that involves the flexing of the trunk and the hip with the feet raised above ground level strengthens the trunk.[15] Researchers have demonstrated that exercise on an unstable or compromised support surface (sitting with ischial tuberosity alone with the leg suspended above the ground) further enhanced changes in the motor control system, increased the muscle activity, contraction speed, strength of spinal stabilization, and improved the harmony of neuromuscular reflex reactions.[17]

Bridging exercise is an exercise which control weight load by pressing the feet against a support surface, which plays a role in controlling body balance and power to maintain position.[18] It is performed to promote coordinated contraction of global muscle and local muscles in a position in which patients (with NCLBP) feel comfortable, as a result of the reduction pain.[19,20] It is done to increase the power of the hip extensors, and also for the purpose of achieving trunk stability.[21]

Stabilization exercises have been observed to involve combination of various movement and many activities in a single exercise treatment for the management of low back pain. Although, not so much have been done to rate the effect of one stabilization exercise over the other.[22,23] However, it is not clear if there can be an exercise with just a single movement technique that may be as effective as others and can prevent both therapist and patient from going through various exercise protocol and movement combinations.

Aim

This study was designed to compare the effectiveness of these two stabilization exercise protocol, bridging exercise (a multiple movement technique) and the V-sitting stabilization exercise protocol (a single movement technique) in terms of both pain and functional disability for the management of NSCLBP.

Material and methods

Participants

The quasi-experimental study employed participants with chronic low back pain patients referred for treatment at the Department of Physiotherapy, Obafemi Awolowo Teaching Hospitals Complex, Ile-Ife, Nigeria. Inclusion criteria and exclusion criteria included patients diagnosed with non-specific chronic low back pain, i.e pain of more than 3 months, however patients with low back pain but with history of metabolic bone diseases, history of hip pathology and systemic disease were excluded from the study. Purposive sample technique was used to recruit the participants using the sample size determination formula by Rosner to know how many patients were needed for the research.[24]

$N=4\sigma^2(zcrit+zpwr)^2/D^2$

where N is the total sample size σ is the assumed SD of each group (assumed, equal for the two groups) and this is assumed to be 4.

Zcrit is the standard normal deviate corresponding to the selected significance criterion (i.e 0.05(95%=1,960).

Zpwr is the standard normal deviate corresponding to the selected power (i.e.80=0.842)

D is the minimum expected difference among the two means D=4

N=4x4^2(1.96+0.842)^2/4^2

N=31.40 N is approximately equals to 31. For attrition,

10 percent of the sample size was added, which is 3 patients, this made 34 participants i.e 17 in each group

Research protocol

The following instrument were used in the study. (i) height meter; this is a wooden meter rule of three meters high calibrated in inches and centimetre. It was used to measure the height of the participants. (ii) Tape rule. A 150 cm long and 0.7 cm wide tape rule made from China was used to measure the circumference of the participants

(ii) A Bathroom Weighing Scale manufactured by the Hanson Company of Ireland in the year 2000 (0–120 kg) was used to measure the body weight of participants in kilogram to the nearest 1.0 kg.

(iii) A hand held stop-watch is an instrument that records the time both in minutes and in seconds and was used for the participant during the exercise protocol

(iv)Verbal Rating Scale (VRS): It consists of 6 scales raging from zero to 5, zero is no pain and five is the worst kind of pain. This tool was used for patients who can articulate pain in terms of words and expression of the level of the pain. The participants placed a check mark, best to the phrase that best describe the current intensity of pain .[25] A response of no pain was given a value of zero, 1= mild pain, 2= moderate pain 3= severe pain, 4= very severe pain, 5= worst possible pain. A response of no pain at all was assigned a value of zero while the minimum pain ever felt is assign a value of one and the highest and the unbearable pain is assign the value of 5. The validity of the VRS has been established in a study conducted Akinpelu et al where they tested the visual analogue scale, the VRS and the numerical rating scale in an experimental condition in which sound was used as the stimuli it was found that these tools are reliable for pain rating and measurement.[26]

(v) Rolland Morris Low Back Pain Disability Questionnaire (RMDQ): It is a 24 item questionnaire which involves the summation of the selected items with a total of 24. The questionnaires were specific on some of the daily activities of the patients which might have been affected by pain. This is ranging from I stay at home most of the time because of my back to I stay in bed most of the time because of my back. The clinical improvement of participants were graded based on analysis of serial questionnaire and percentage of improvement were calculated. The score of equal or greater than 14 represents a significant disability associated with significant outcome.[27] Greater level of disability associated with higher score. The participant was told to mark each appropriate question and the total number of marked statement is summed up. %of clinical improvement = (difference in score)/ initial score.

Ethical of approval

Ethical of approval (HREC NO: IPHOAU/12/530) was obtained from the Health Research and Ethics Committee of Institute of Public Health Obafemi Awolowo University, Ile-Ife before the commencement of the study, the nature and purpose of the study was explained to the participants and informed consent was obtained. Participants were allocated to each group consecutively. However, if a patient was discovered unable to do V- sitting based on the severity of the pain but able to do bridging, and vice versa, such patient was allowed to that group.

Measurements

Prior to the treatment, each of the height, weight, waist and the hip circumference of each participants were measured according to Marfell et al,. The initial pain intensity and functional disability were assessed using VRS, RMDQ respectively; at baseline, second week and third week of treatment session.[28]

Intervention

The V-Sitting Exercise Protocol: Participants started the exercise in a seated position with hands and foot on the couch. The abdominal muscles and core were contracted slowly with the two legs lifted up to an extended position at a 45-degree angle with the torso. The arms were slightly flexed to about 30% but not lifted from the couch. The protocol of Quinn was used with slight moldification[29]. Participants maintained the good core posture and a strong spine throughout the movement and to avoid rounding the shoulders forward, Fig I. Participants continued to breathe deeply during the movement. The position was held for 10 seconds, participants then returned to the starting position slowly while continuing to keep the abdominal muscles engaged and tight. The procedure was repeated 5 times for a start and progressed to 10 times before the end of the study

Bridging Exercises Protocol: Glute and side bridging exercise were employed in the study, using the protocols of Quinn.[29] For the glute bridging exercise, participant lied supine on the couch, with the knees bent and feet flat on the couch, the arms were on the abdomen pronated. The hips was lifted off the ground until the knees, hips and shoulder formed a straight line Fig II. The position was held for 10 seconds before easing back down. For side bridging exercise, participants lied on the side with the forearm on the couch under the shoulder, and the feet stacked together. The hip was lifted off the couch, which created a straight line from heel to shoulder keeping the head in line with the spine Fig III.

Data analysis

Data was analyzed using the IBM version 22 (statistical package for social science student). Independent –t- test

was used to compare the physical characteristics of the participants. Repeated measure of ANOVA was used to compare the mean values of the data among the treatment sessions within and between the two groups. The alpha level of 0.05 was set as a significant level.

Results

It was observed that there were no significant difference between the physical characteristics (p=0.320), of V-sitting and bridging Exercise groups as shown in table 1.

Table 1. Comparison of the physical characteristics of participants in V-sitting and bridging exercise group (N= 34)

Variables	V-sitting n=17 Mean±SD	Bridging N=17 Mean±SD	t	p value
Age(years)	53.00±11.31	57.92±12.76	-1.017	0.320
Height(m)	1.63±0.10	1.66±0.08	-0.763	0.453
Weight(kg)	74.23±9.94	70.32±10.09	0.973	0.341
BMI (kg/m^2)	28.06±3.23	25.59±2.58	2.113	0.046
Wc (cm)	87.00±17.99	90.15±11.45	-0.527	0.603
HpC (cm)	103.42±8.59	103.00±13.26	0.920	0.927
WHR	0.84±0.17	1.15±0.88	-0.662	0.515

The results of the comparison of mean values the outcome measures for V-sitting exercise group were shown in table 2.

Table 2. Comparison among pretreatment, 2nd and 3rd week, pain intensity and disability of subjects in V- sitting group (N=34)*

Variables	Mean± SD	F	P
Pain intensity			
Pretreatment	3.42 ±0.66^a		
2nd week	2.25±0.62^b	44.420	0.001
3rd week	1.50±0.52^c		
Disability			
Pretreatment	54.51±14.15^a		
2nd week	35.07±11.44^b	27.409	0.001
3rd week	17.71±10.67^c		

*Key: Post Hoc analysis: mean difference with alphabets (a,b,c,d,e,) with different alphabets mode are significantly difference but those with the same alphabets mode were not significantly different.

There was a significant reduction (F=44.420; p<0.001) in the pain intensity when the pretreatment (3.42 ±0.66) values was compared with 2nd (2.25±0.6216) and 3rd week (1.50±0.52) values. There was also a significant reduction (F=27.409; p<0.001) in the level of disability, when the disability of pretreatment, (54.51±14.15) 2nd (35.07±11.44) and 3rd (17.71±10.67) week were compared. Post hoc analysis revealed that for the pain intensity and disability, the reduction was significant from pretreatment to 2nd and 3rd week in that order. Furthermore, the results in the bridging exercise group was presented in table 3, from the table, there was a significant different reduction (F=24.182; p<0.001) in pain intensity among the pretreatment, (3.38±0.6504) 2nd (2.38±0.6504) and 3rd week (1.62±0.6504) values.

Table 3. Comparison among pretreatment, 2nd and 3rd week pain intensity and disability of bridging exercise group subjects (N= 34)*

Variables	Mean± SD	F-ratio	P-value
Pain intensity			
Pretreatment	3.38±0.6504^a		
2nd week	2.38±0.6504^b	24.182	0.001
3rd week	1.62±0.6504^c		
Disability			
Pretreatment	53.21±16.33^d		
2nd week	38.46±13.62^e	10.461	0.001
3rd week	29.49±9.076^f		

*Key: Post Hoc analysis using LSD = mean difference with alphabets (a,b,c,d,e,) with different alphabets mode are significantly difference but those with the same alphabets mode were not significantly different

Similarly, considering the disability, a significant reduction (F=10.46; p<0.001) was observed when the pretreatment (53.21±16.33) 2nd (38.46±13.62) and 3rd (29.49±9.076) week variables were compared. Comparing the two groups as shown in table 4, there was no significant different in the pretreatment pain intensity and disability between p>0.05. With regard to the pain intensity, but there was a significant reduction (27.34, P=0.000) when the values of the 3rd week of the V sitting (1.50 ±0.52) and Bridging (1.62±0.65) exercises were compared. With respect to the disability, there was also a significant reduction (14.96, P=0.000) at the 3rd week when the V-sitting exercise group (17.71±10.6) was compared with bridging exercise group (29.49 ±9.08) as shown in table 4.

Table 4. Comparison among the pretreatment, 2nd and 3rd week of v-sitting and bridging exercise group pain intensity and disability (N=34)*

	VSG n=17			BEG n=17			F	P
Variable	PreRx	2nd week	3rd week	PreRx	2nd week	3rd week		
PI	3.42 ±0.66^a	2.25 ±0.62^b	1.50 ±0.52^c	3.38 ±0.65^a	2.38 ±0.65^d	1.62 ±0.65^c	27.34	0.000
DIS	54.51 ±14.15^a	35.07 ±11.44^b	17.71 ±10.68^c	53.21 ±16.33^a	38.46 ±13.62^d	29.49 ±9.08^e	14.96	0.000

*Key: VSG = V-sitting Exercise Group, BEG = Bridging Exercise group. PI = Pain Intensity, DIS = Disability, PreRx= Pretreatment, Post Hoc analysis of LSD= mean difference with alphabets (a,b,c,d,e,) with different alphabets mode are significantly difference but those with the same alphabets mode were not significantly different

The effect size of V-sitting exercises on pain intensity (Π^2_p = -0.103) and disability ($\Pi^2_{p=}$ -0.596) were significantly more than that bridging exercise when compared as shown in table 5.

Table 5. Magnitudes of effect size using partial Eta Square between the VSG and BEG (N=34)*

Variables	M3V	M3B	DM3	SD1	SD2	SD1+SD2	PETA (Π^2_p)
PI	1.5	1.62	-0.12	0.52	0.65	1.17	-0.103
DIS	17.71	29.49	-11.78	10.68	9.08	19.76	-0.596

*Key: M3V= Means for 3rd week V-sitting group, M3B= Mean value for Bridging exercise. DM3 = Difference between the means SD 1= Standard Deviation for V-sitting variables, SD2 = Standard deviation for Bridging variables

Discussion

The specific objectives of this study were to, determine the effect of V- sitting and bridging exercises on pain intensity and disability of patients with chronic low back pain, also to compare the effects.

The study observed a significant reduction on pain intensity and disability of subjects between the pretreatment and 3rd week in subjects with V-sitting exercise. This is inferred that V-sitting exercise is effective in the management of non-specific chronic low back pain. There is little evidence on the effectiveness of V-sitting exercise posture as a core stabilization exercise. However, Guimeres et al., pointed out in their research that investigated twelve different forms and modifications of abdominal muscles exercises which included curl-up, sit-up, V-sit exercises among others, that V-sit has the least activity involved and produces the highest level of muscle action potential in both the upper and lower part of rectus abdominis muscle with minimal level of activation in the rectus femoris as compared with other abdominal muscles exercises.[30] This was considered to be as a result of the fact that, rectus abdominis origin or insertions was not fixed as compared with other abdominal exercises in the ascending phase of the trunk and the lower limb. Mcgill has however pointed out that core stability is achieved by the ability of the abdominal muscle to create sufficient stiffness via simultaneous co-contraction and coordinated contraction of the abdominal muscles.[31] He also stated that compromised task of daily living is not compromised by insufficient strength but probably insufficient endurance and control. V-sitting posture exercise reduces disability by promoting endurance level of both the abdominal muscle and spine musculatures. Studies reported that an endurable muscle reduces the risk of back troubles in the future.[32,33] Lumber stabilization exercise including V-sitting exercises increases the strength and size of the erector spinea muscle and reduces the risk of having low back pain.[34]

It was observed that there was a significant reduction between the pretreatment, 2nd and the 3rd week of treatment, in pain and disability of subjects who received bridging stabilization exercise. This is an indication that bridging exercises were also effective in ameliorating the pain intensity and disability of patients with low back pain. Increment in muscle strength and balance in lumbar spine and relief of pain could be achieved by stabilization exercise, functional exercise, resistance exercise, and rehabilitation exercise.[35-39]

Bridge exercises have been reported to be the commonest used exercise protocol among the trunk stability exercises though in different positions with the intension to strengthen the co-activation of trunk muscles.[40,41] In addition, the superficial and deep trunk muscles were activated, gluteal and lower leg muscles were strengthened in appropriate ratio during the bridge exercises.[21] This was achieved by the usage of glute bridging exercises in the course of the study. Literature has reported that in patients with chronic low back pain, there is weakness of spinal extensors and abdominis, specifically transversus abdominis.[42] Trunk stability exercises were done to protect the spine from re occurrence of muscle damage, instability of the spine which may lead to pain and spinal degenerative changes.[41] These were achieved from our study using glute bridging exercises, hence there was reduction in pain intensity and disability index.

One of the great contributors muscles to the spinal stability is quadratus lumborum, it is as well a strong lateral stabilizer of the spine.[43] In the course of the side/lateral bridge exercise, there was strengthening of the lateral musculature specifically quadratus lumborum and the oblique group, which is another reason while there were significant improvements in the outcome measures of bridging exercises group. Exercise of sufficient duration and intensity results in the release of peripheral and central beta-endorphin which have been associated with changes in pain sensitivity.[44]

Regarding the comparison between the V-siting exercise and bridging exercise group, it was observed that there was no significant different between the anthropometric characteristics with participants in the V-sitting and bridging exercise groups. This indicated that the subjects in the two groups were comparable. Any difference obtained after intervention between the two groups was due to intervention not attributed to anthropometric variation.

This study observed a significant reduction when the 2nd week of treatment in both pain of the subjects that underwent the V-sitting exercise was compared with those subjects in the bridging exercise group. This result is in accordance with the works of other authors that specific stabilization exercise can reduce low back pain intensity and disability, or in combination with

other adjunct therapy.[45,46] Stabilization exercise also prevent recurrent episodes of low back pain.[47,48] The results of this research showed that the use of stabilization exercises is effective in the reduction of pain-related disability in NSCLBP patients. The results of this study conforms to the study of Akodu et al., who reported that stabilization exercise was effective in the management of pain and functional disability in patients with NSCLBP.[48] Shakeri et al, in their study, which focused on the effect of lumbar stabilization exercises on pain and disability in women with menstrual low back pain, the results showed that lumbar stabilization exercises improve pain and disability.[49] As well Nava-Bringas et al., in their study on the adherence to a stability exercise program in patients with CLBP, reported that there was reduction in pain, with functional improvement, and that the improvement presented more quickly than the control following adherence to a lumbar stabilization exercise program.[50]

A recent focus in the physiotherapy management of patients with back pain has been the specific training of muscles surrounding the spine (deep abdominal and lumbar multifidius), considered to provide dynamic stability and fine control to the lumbar spine.[51] A specific exercise treatment approach appears to be more effective than others commonly prescribed conservative treatment programs in patient with chronically symptomatic spondylosis or spondyloloisthesis.[52] Stabilization or core stability exercises have been suggested to reduce symptoms of low back pain and disability in patient with low back pain and form an effective treatment.[53]

Our study observed that V-sitting exercise reduced pain intensity and disability more than bridging exercises as shown in the magnitudes of the effect size using partial eta square. This may indicate that V-sitting exercise is more effective that bridging exercises. Possibly it can be inferred that the strengthening effects of V-sitting exercises is more than that of bridging exercises. By virtue of the protocol of the two exercises, V-sitting exercises strengthened the abdominal and spinal muscles as well as diagram. These are group of muscles that stabilize the low back vertebrae. The bridging exercises considering all the exercises strengthen the trunk muscles in general and pelvic muscles. Vera-Garcia et al, demonstrated that exercise on an unstable or compromised support surface like V-sitting exercises where patient is sitting with ischial tuberosity alone with the leg suspended above the ground further enhanced changes in the motor control system, increased the muscle activity, contraction speed, strength of spinal stabilization, and improved the harmony of neuromuscular reflex reactions.[17] V- sitting posture stabilization exercise eliminate the movements of other segments by slightly flexing the hip and slowly lifting the upper portion of the body, and isometrically contracting the trunk flexors and the back extensors simultaneously, an effective means of strengthening the abdominal muscles.[15] V-sitting position exercise, contributes to spinal stability with increased intra-abdominal pressure thus creating a rigid spine.[54]

Looking at it critically, it will be observed that exercises carried out in our study were majorly isometric in nature. Studies have reported that there are hypo-analgesic effects of isometric exercises especially on the contracting body part, the contralateral and a distant body part to the contracting one.[55] The implication is that central inhibitory pain mechanism is activated when muscles are contracting in a static position.[56] The process is carried out by increase in concentration of beta-endorphins, attention mechanism, activation of diffuse inhibitory controls or interaction of systems which regulate the pain.[56] In addition, pain perception was reduced as a result of isometric exercises which activates the secretion of endogenous opioid system in the brain.[43]

Conclusion

It can be concluded from this study that both the V-sitting exercise posture and glute and side line bridging exercises were effective in ameliorating pain intensity and disability of patients with non-specific low back pain. V-sitting exercise seems to have reduced pain intensity and disability better than bridging exercise.

Recommendations

Based on the outcome of this study, it can be recommended that V-sitting exercise can be incorporated for patient with non-specific chronic low back pain. However, if patient reports aggravating symptoms or unable to do it, bridging exercise can be employed.

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ORIGINAL PAPER

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Prevalence and predictive factors for exclusive breastfeeding in the first 6 months among mothers attending Primary Health Care Centers in Cairo, Egypt

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ABSTRACT

Introduction. Although most organizations recommend breastfeeding for at least one year due to its well-known beneficial effects, the prevalence of exclusive breastfeeding in many developing countries was quite low.

Aim. To identify prevalence and predictive factors affecting exclusive breastfeeding in Primary Healthcare (PHC) Centers in Cairo, Egypt.

Material and methods. A cross-sectional study, among mothers attended first six months immunization sessions in three PHC Centers were selected via a convenient sample, A total sample of 180 mothers, 60 from each PHC Center, were subjected to an interview questionnaire. The questionnaire used was previously validated and pre-tested. Data collection took the period from April to August 2019. Comparison between exclusive and nonexclusive breastfeeding according to possible risk factors was done.

Results. The frequency of breastfeeding among the participants was 90.6%; however the total exclusive breastfeeding frequency was only 39.4%. Our logistic regression model showed that exclusive breastfeeding decreased with progressive increase in infant's age (OR 0.74), and mothers with good knowledge about proper practice of breastfeeding adhered more to exclusive breastfeeding (OR 2.51). Also, it showed that, during working hours, mothers who fed their infants other than breast milk adhered less to exclusive breastfeeding (OR 0.19).

Conclusion. The prevalence of exclusive breastfeeding is quite low. The predictive factors for exclusive breastfeeding are, younger infant's age, good knowledge of the mothers about proper practice of breastfeeding, and mothers insisting on breastfeeding during working hours.

Keywords. breastfeeding, cross sectional, infants formula, primary healthcare, risk factors

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Introduction

Breastfeeding is the ideal method suited for the physiological and psychological needs of infants not only because breast milk has great nutritional values, but also it is clean and contains antibodies that protect the baby against many common childhood diseases, beside that it is always at the right temperature, inexpensive and nearly every mother has more than enough of this high quality food for her baby.[1] Breastfeeding is a challenge for health professionals, regardless of their specialization, as they have to face a demand that requires skill and sensibility, for which they are not prepared.[2] In Egypt, the Egyptian Demographic and Health Survey (EDHS) 2014 showed that exclusive breastfeeding is common but not universal in very early infancy among infants under two months of age, 71% receiving only breast milk. However, the proportion of exclusively breastfed drops off rapidly among older infants. By age 4-5 months, only 13% of children were exclusively breastfed.[3]

Family Physicians understand the advantages of family-centered care and are well positioned to provide breastfeeding support in that context as they provide comprehensive care to the whole family.[4] Health professionals need to be better trained to work on promoting breastfeeding, whether by health and medical schools or by healthcare administrators, in order to consolidate multi-professional teams committed to maternal-infant health.[2] Post discharge primary care support for breastfeeding mothers and infants can increase breastfeeding rates and duration.[4] Family physicians should have the knowledge to promote, protect, and support breastfeeding as the family physicians have great role in supporting breastfeeding.[4] Clinicians can support post discharge breastfeeding by assessing milk production and milk transfer; evaluating an infant's latch to the breast; identifying maternal and infant anatomic variations that can lead to pain and poor infant weight gain; knowing the indications for frenotomy; and treating common breastfeeding-related infections, dermatologic conditions, engorgement, and vasospasm.[4] Infants who are breastfed have a decreased risk of atopic dermatitis and gastroenteritis, and have a higher IQ later in life. Additional benefits in infants have been noted in observational studies.[4] Maternal benefits of breastfeeding include decreased risk of breast cancer, ovarian cancer, postpartum depression, hypertension, cardiovascular disease, and type 2 diabetes mellitus.[4] The best way to assess milk supply is by monitoring infant weight and stool output during wellness visits. Proper positioning improves latch and reduces nipple pain. Frenotomy is controversial but may reduce pain in the short term.[4] Breast milk does not sufficiently resource the infants' daily necessities of definite nutrients like iron and vitamin D, and therefore complementary feeding is required.[5] The beneficial health effects of breastfeeding for new-born explain the national and international supports to initiate and continue breastfeeding for all new-born.[6] World Health Organization 2017 (WHO) and UNICEF (2017) strongly recommending to start breastfeeding during first hour of birth for establishing breastfeeding exclusively in the initial 6 months of life and continuing breastfeeding up to 2 years of age and beyond along with complementary feeding.[6] Full breastfeeding is breastfeeding either exclusively or predominantly. Exclusive breastfeeding means only breast milk is allowed to be fed to the baby in addition to vitamins and medications if indicated. On the other hand, partial breastfeeding includes other feeding methods in addition to breastfeeding (i.e. bottle, cup, lact-aid) regardless of content.[6,7] Many of the problems associated with discontinuation of breastfeeding can be escaped by counseling and health education.[7] The AAF and the U.S. Preventive Services Task Force recommend primary care interventions to support breastfeeding and improve breastfeeding rates and duration.[4] It is important for the health professionals to consider the mother's cultural background an influence on the decision to breastfeed. However, the professionals must be willing to share their knowledge with the family and form a social network to provide support and encouragement for nursing mothers to overcome obstacles[8]. The members of the nursing mothers' social network, including the health professionals, are capable of interfering the decision to breastfeed, by inspiring and supporting the initiative, by transferring knowledge and cultural values, or family traditions collective with growing disinterest and discouragement, due to the burden on the nursing mother in relation to how to feed the child.[9]

Aim

This study aimed at identification of prevalence and predictive factors affecting exclusive breastfeeding in Primary Health Care (PHC) Centers in Cairo, Egypt.

Material and methods

A cross-sectional study was conducted on three PHC Centers among 97 PHC units and centers (1/3 in low socioeconomic areas, 1/3 in intermediate socioeconomic areas. and 1/3 in high socioeconomic areas) in Cairo, Egypt; Saraya Al-Koppa, El-Mhkama and Al-Matarya. Data collection was done from April to August 2019. The study population included mothers paired with their infants, were attending the first 6 months immunization sessions in three PHC centers. Infants with specific health problems who needed special feeding programs were excluded. Infants with any obvious congenital anomalies, features of genetic diseases or if they had a medical history of any metabolic errors or physical problems were also excluded.

A convenient sample was used for selecting the three Primary Health Care Centers (one representative for every socioeconomic area) and systematic random sample

for selecting participant mothers for data collection. The estimated sample size was 174 infants with their mothers (approximated to 180), thus 60 were the needed from each center. As exclusive breastfeeding prevalence was 13% in the first 4-5 months, according to the most recent Egypt Demographic and Health Survey.[3] The official records of Cairo governorate showed that the catchment areas of the three selected PHC have yearly 25,000 newborns.[10] Sample size was calculated with precision 5% and 95% confidence interval specified limits. Sample size was calculated using EPIDAT version 4.2 program.

Data collection was done via questionnaire, which was adopted from questionnaire for monitoring breastfeeding mothers in baby friendly hospitals, it was translated into Arabic, for word and back translation were carried out ,reviewed by committee from family medicine and paediatric departments and pre-tested before using it.[11] It comprises 51 main questions with some subsidiary questions investigating personal and medical characteristics of the mothers and their infants, in addition to mother's knowledge, health education and breastfeeding practice. This was conducted in the form of interview questionnaire. Standard of living was calculate and classified into high, medium and low.[12]

Ethical considerations

Administrative approval of the Primary Healthcare Centers, where data collection took place was obtained. Ethical approval was obtained from research Ethics Committee of Faculty of Medicine, Ain Shams University in 21.04.2019.Verbal informed consent from study participants was obtained before answering the questionnaire and the questionnaire was anonymous.

Statistical analysis

The collected data were processed and coded before being analyzed using the IBM SPSS program (Statistical Package for Social Sciences) for Windows Version 23.0. Initial comparisons between exclusive and non-exclusive breastfeeding participant mothers, according to possible risk factors, using the Student's t-test/Mann-Whitney test for continuous variables and Pearson's chi-square X^2 test for categorical variables. Level of significance has been set at P-value > 0.05: Non-significant; P-value ≤ 0.05: Significant; P-value < 0.01: Highly significant.

The adjusted predictive factors for Exclusive Breastfeeding were obtained by logistic regression analysis. The dependent variable was performance or no performance of exclusive breastfeeding in all the participants. All variables described previously were considered as possible candidates for the final model. The initial multivariable model construction consisted of the preliminary selection of variables using a manual purposeful selection method and a relatively high significance level (alpha approximately 0.10). Subsequently, the resulting model was reduced using a likelihood ratio test with a significance level of 0.05. Before accepting a final model, the interactions as well as confounding factors were evaluated. The calibration of the final model was assessed using the Hosmer and Lemeshow goodness-of-fit test, and its discrimination was assessed by the area under the receiver operator characteristic (ROC) curve.

Results

Our study showed that, the mean age of participant mothers was 29.4 years (SD=5), ranged between 18 and 42 years, and there was no significant association between exclusivity of breastfeeding and sociodemographic data of the mothers (Table 1).

There was significant association between chronic disease of the mother and exclusivity of breastfeeding, but no significant association between it and other medical data of the mothers (Table 2).

The mean age of participant infants' is 3.7 (SD=1.5) months, with highly significant association between increasing infants' age and nonexclusive breastfeeding, indicating that the age of the infant can influence the decision of the mother to exclusive breastfeeding. The mean infants' weight is 6.0 (SD=1.3) kilograms with no significant association with exclusivity of breastfeeding in our study. There was no significant association between exclusivity of breastfeeding and infant's gender, order among siblings, infant maturity, admission to incubator, and incubation period (Table 3).

The study showed that, the frequency of breastfeeding among the participants was (90.6%), but the total (0-6 months) exclusive breastfeeding frequency was (39.4%), (Figure 1), and its dropping could be referred to certain predictive factors which are clearly evidenced in our study such as improper breastfeeding practice (Table 4).

As not all of mothers are aware about early initiation of breastfeeding and most of them started breastfeeding late and only 32% of the exclusively breastfeeding mothers initiate breastfeeding within the first hour after birth, beside their poor level of perception about proper baby positioning and latching. Although 100% of the exclusively breastfeeding mothers replied that they are aware that successful breastfeeding is painless but most of them are not aware about prevention and early management of it if it happened as only 18.3% of the exclusively breast feeding mothers seek medical help for painful breastfeeding. In spite of 94.4% of exclusively breastfeeding mothers know about feeding on demand, but only 57.7% of exclusively breastfeeding mothers identifies early infants cues for feeding, and very little percent of the mothers feed for 15 minutes per each feed as all of these are vital practical steps for successful breastfeeding techniques that is supposed not to be missed, added to that low awareness rate about dealing with common breastfeeding problems especially among

Table 1. Comparison between exclusive and non-exclusive breastfeeding regarding sociodemographic data of mother domain to be breastfeeding

	Variables	Total No. 180 No. 71	Exclusive breastfeeding (mother's milk only) No. 109	Non-exclusive breastfeeding	P-value	Sig. *
Mother's age	Age in years (Mean ± SD)	29.4±5	28.7±4.35	29.97±5.46	0.086	NS
Mother's education	Do not read or write	17 (9.4%)	7 (9.9%)	10 (9.2%)	0.36	NS
	Primary	4 (2.2%)	0 (0.0%)	4 (3.7%)		
	Preparatory	5 (2.8%)	3 (4.2%)	2 (1.8%)		
	Secondary	49 (27.2%)	20 (28.2%)	29 (26.6%)		
	University	86 (47.8%)	35 (49.3%)	51 (46.8%)		
	Higher Education	19 (10.6%)	6 (8.5%)	13 (11.9%)		
Mother's occupation	Housewife	52 (28.9%)	16 (22.5%)	36 (33.0%)	0.18	NS
	Employee	128 (71.1%)	55 (77.5%)	73 (67.0%)		
Standard of living	High	3 (1.7%)	1 (1.4%)	2 (1.8%)	0.903	NS
	Medium	152 (84.4%)	61 (85.9%)	91 (83.5%)		
	Low	25 (13.9%)	9 (12.7%)	16 (14.7%)		
Living statues	Living alone	134 (74.4%)	52 (73.2%)	82 (75.2%)	0.56	NS
	with the family of the husband	45 (25.0%)	19 (26.8%)	26 (23.9%)		
	with another family	1 (0.6%)	0 (0.0%)	1 (0.9%)		
Living with husband	No	3 (1.7%)	1 (1.4%)	2 (1.8%)	0.66	NS
	Yes	177 (98.3%)	70 (98.6%)	107 (98.2%)		
Smoking	Smoker	2 (1.1%)	1 (1.4%)	1 (0.9%)	0.635	NS
Number of children	1	62 (34.4%)	24 (33.8%)	38 (34.9%)	0.216	NS
	2	54 (30.0%)	26 (36.6%)	28 (25.7%)		
	3	39 (21.7%)	15 (21.1%)	24 (22.0%)		
	4	16 (8.9%)	5 (7.0%)	11 (10.1%)		
	More than four	9 (5.0%)	1 (1.4%)	8 (7.3%)		

P-value > 0.05: Non- significant; P-value ≤ 0.05: Significant; P-value < 0.01: Highly significant

* Chi-square test; Independent student's t-test

Table 2. Comparison between exclusive and non-exclusive breastfeeding regarding mothers medical data

	Variables	Total no. = 180 No. = 71	Exclusive breastfeeding (mother's milk only) No. = 109	Non-exclusive breastfeeding	P-value	Sig. *
Chronic diseases of the mother	Yes	10 (5.6%)	1 (1.4%)	9 (8.3%)	0.091	NS
	No	170 (94.4%)	70 (98.6%)	100 (101.7%)		
Number of pregnancies	1	56 (31.1%)	22 (31.0%)	34 (31.2%)	0.397	NS
	2	54 (30.0%)	25 (35.2%)	29 (26.6%)		
	3	34 (18.9%)	12 (16.9%)	22 (20.2%)		
	4	20 (11.1%)	9 (12.7%)	11 (10.1%)		
	More than four	16 (8.9%)	3 (4.2%)	13 (11.9%)		
Type of delivery	Vaginal delivery	20 (11.1%)	7 (9.9%)	13 (11.9%)	0.063	NS
	Painless vaginal delivery	18 (10.0%)	13 (18.3%)	5 (4.6%)		
	Caesarean without general anesthetic	5 (2.8%)	2 (2.8%)	3 (2.8%)		
	Caesarean with general anesthetic	91 (50.6%)	33 (46.5%)	58 (53.2%)		
	Assisted vaginal delivery(ventose or forceps)	46 (25.6%)	16 (22.5%)	30 (27.5%)		
Complications during pregnancy	Yes	80 (44.4%)	30 (42.3%)	50 (45.9%)	0.633	NS
	No	100 (55.6%)	41 (57.7%)	59 (54.1%)		
Complications during delivery	Yes	10 (5.6%)	5 (7.0%)	5 (4.6%)	0.35	NS
	No	170 (94.4%)	66 (93.0%)	104 (95.4%)		
Complications after delivery	Yes	13 (7.2%)	2 (2.8%)	11 (10.1%)	0.08	NS
	No	167 (92.8%)	69 (97.3%)	98 (89.9%)		
Previous exclusive breast feeding experience	1	28 (15.6%)	15 (21.1%)	13 (11.9%)	0.096	NS
	2	19 (10.6%)	10 (14.1%)	9 (8.3%)		
	3	5 (2.8%)	3 (4.2%)	2 (1.8%)		
	4	1 (0.6%)	1 (1.4%)	0 (0.0%)		
	More than four	1 (0.6%)	0 (0.0%)	1 (0.9%)		
	There is no	126 (70.0%)	42 (59.2%)	84 (77.1%)		

P-value > 0.05: Non-significant; P-value < 0.05: Significant; P-value < 0.01: Highly significant

*: Chi-square test; Independent student's t-test

Table 3. Comparison between exclusive and non-exclusive breastfeeding regarding factors related to infant domain to be breastfed

Variables		Total no. = 180 No. = 71	Exclusive breastfeeding (mother's milk only) No. = 109	Non-exclusive breastfeeding	P-value	Sig. *
Infant age	Age in months (Mean ± SD)	3.7±1.5	3.27±1.51	4.02±1.49	0.001	S
Infant's gender	Female	97 (53.9%)	44 (62.0%)	53 (48.6%)	0.079	NS
	Male	83 (46.1%)	27 (38.0%)	56 (51.4%)		
Infant weight	Infant weight in kg	(6.0±1.3)	5.94±1.17	6.06±1.39	0.546	NS
Infant's order among other siblings	0	3 (1.7%)	0 (0.0%)	3 (2.8%)	0.22	NS
	1	65 (36.1%)	23 (32.4%)	42 (38.5%)		
	2	58 (32.2%)	29 (40.8%)	29 (26.6%)		
	3	31 (17.2%)	11 (15.5%)	20 (18.3%)		
	4	14 (7.8%)	6 (8.5%)	8 (7.3%)		
	5	6 (3.3%)	2 (2.8%)	4 (3.7%)		
	6	2 (1.1%)	0 (0.0%)	2 (1.8%)		
	7	1 (0.6%)	0 (0.0%)	1 (0.9%)		
Full term or preterm	full term	163 (90.6%)	67 (94.4%)	96 (88.1%)	0.198	NS
	Preterm	17 (9.4%)	4 (5.6%)	13 (11.9%)		
Incubated infant	Yes	21 (11.7%)	6 (8.5%)	15 (13.8%)	0.346	NS
	No	159 (88.3%)	65 (91.5%)	94 (86.2%)		
Incubation duration	Median (IQR)	1 (1–3)	1 (1 – 3)	1 (1 – 3)	0.769	NS
	Range	1 – 62	1 – 7	1 – 62		

* Chi-square test; Mann-Whitney test

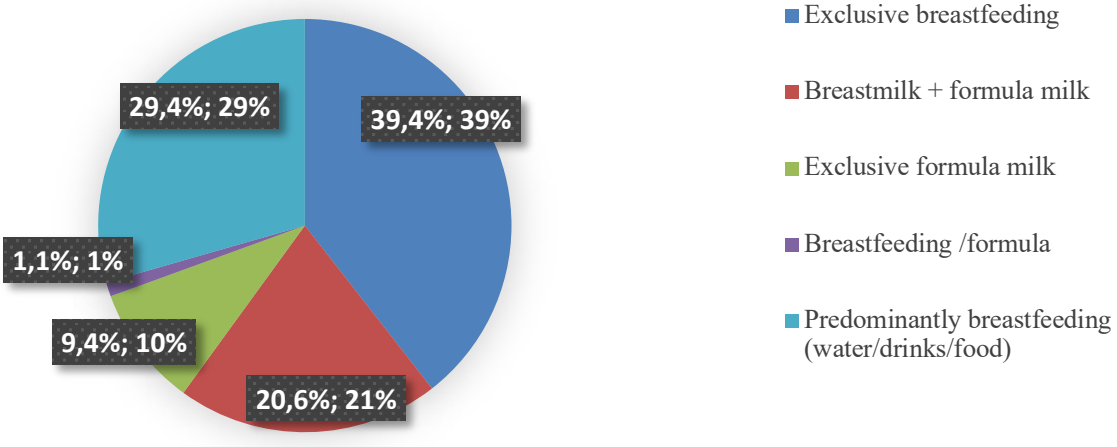

Fig. 1. Frequency of breastfeeding among total participants

mothers for the first. There was highly significant association between exclusivity of breastfeeding and infant positioning and latching during breastfeeding, with significant association as regard frequency of breastfeeding per day, but there is no significant association as regard other feeding practices (Table 4).

There was highly significant association between exclusivity of breastfeeding and employee taking their infants to work, feeding them direct breastfeeding, employee feeding formula milk or starting drinks or food to their infants during working hours, added to that no one of the employee mothers practicing milk expression (Table 5).

Our logistic regression model showed that exclusive breastfeeding decreases with progressive increase in infant's age with OR 0.74, mothers with good knowledge about proper practice of breastfeeding (e.g. infant's chin should be immersed in mother's breast and most nipple halo in the infant lower lip) adhered more to exclusive breastfeeding with OR 2.51. Our multivariate analysis showed that during working hours, mothers who fed their infants other than breast milk adhered less to exclusive breastfeeding with OR 0.19, and it is very obvious in this study, although there is high frequency of breastfeeding among our sample (Table 6).

Table 4. Comparison between exclusive and non-exclusive breastfeeding regarding breastfeeding practice

Variable		Total no. = 163 No. = 71	Exclusive breastfeeding (mother's milk only) No. = 92	Non-exclusive breastfeeding	P-value	Sig.*
Start breastfeeding Immediately after birth	Yes	2 (1.2%)	1 (1.4%)	1 (1.1%)	0.683	NS
Start breastfeeding within one hour of birth	Yes	44 (27.0%)	23 (32.4%)	21 (22.8%)	0.72	NS
Start breastfeeding more than one hour of birth	Yes	77 (47.2%)	36 (50.7%)	41 (44.6%)	0.436	NS
Start breastfeeding a day after birth?	Yes	36 (22.1%)	13 (18.3%)	23 (25.0%)	0.307	NS
Identify early infant cues for feeding	Yes	88 (54.0%)	41 (57.7%)	47 (51.1%)	0.398	NS
Identify intermediate infant cues for feeding	Yes	104 (63.8%)	46 (64.8%)	58 (63.0%)	0.818	NS
Identify late infant cues for feeding	Yes	147 (90.2%)	66 (93.0%)	81 (88.0%)	0.296	NS
Infant attached to mother body and belly	Yes	144 (88.3%)	69 (97.2%)	75 (81.5%)	0.002	HS
Infant chin is immersed in mothers breast and most nipple halo in the infant lower lip	Yes	132 (81.0%)	63 (88.7%)	69 (75.0%)	0.029	S
Mother nipple in the mouth of the infant	Yes	153 (93.9%)	71 (100.0%)	82 (89.1%)	0.005	HS
Not painful and swallowing	Yes	153 (93.9%)	71 (100.0%)	82 (89.1%)	0.005	HS
Correct infant position	Yes	19 (11.7%)	8 (11.3%)	11 (12.0%)	0.547	NS
Frequency of breastfeed per day	3 times	9 (5.5%)	0 (0.0%)	9 (9.8%)	0.001	S
	4 times	7 (4.3%)	2 (2.8%)	5 (5.4%)		
	5 times	2 (1.2%)	0 (0.0%)	2 (2.2%)		
	6 times	3 (1.8%)	0 (0.0%)	3 (3.3%)		
	8 times	2 (1.2%)	2 (2.8%)	0 (0.0%)		
	depending on the needs of the child	140 (85.9%)	67 (94.4%)	73 (79.3%)		
Start adding other foods with breastfeeding	Mean ± SD	2.68 ± 1.57	4.00 ± 0.00	2.65 ± 1.57	0.400	NS
	Range	1 – 5	4 – 4	1 – 6		
Reduce feeding from the painful breast	Yes	22 (13.5%)	12 (16.9%)	10 (10.9%)	0.356	NS
Little of mother milk to relive nipple pain	Yes	7 (4.3%)	5 (7.0%)	2 (2.2%)	0.241	NS
Seeking medical help for painful nipple	Yes	26 (16.0%)	13 (18.3%)	13 (14.1%)	0.47	NS
Feeds from one or both breast	From one breast	10 (6.1%)	2 (2.8%)	8 (8.7%)	0.189	NS
	From both breasts	153 (93.9%)	69 (97.2%)	84 (91.3%)		
Reasons of breast feeding from one breast	Health problems for the child	2 (1.2%)	1 (50.0%)	1 (12.5%)	0.255	NS
	Health problems for mother	4 (2.5%)	1 (50.0%)	3 (37.5%)		
	Other	4 (2.5%)	0 (0.0%)	4 (50.0%)		
Duration of breast feeding session	5 minutes	20 (12.3%)	7 (9.9%)	13 (14.1%)	0.767	NS
	10	41 (25.2%)	17 (23.9%)	24 (26.1%)		
	15	14 (8.6%)	6 (8.5%)	8 (8.7%)		
	20	2 (1.2%)	1 (1.4%)	1 (1.1%)		
	more than 20 minutes	1 (0.6%)	1 (1.4%)	0 (0.0%)		
	as your child needs	85 (52.1%)	39 (54.9%)	46 (50.0%)		

P-value > 0.05: Non-significant; P-value ≤ 0.05: Significant; P-value < 0.01: Highly significant

* Chi-square test

Table 5. Comparison between exclusive and non-exclusive breastfeeding regarding breastfeeding practice among employee mothers

Variable No. = 128		Total no. No. = 54	Exclusive breastfeeding (mother's milk only) No. = 74	Non-exclusive breastfeeding	P-value	Sig.
Employee taking their child to work	Yes	52 (40.6%)	51 (94.4%)	1 (1.4%)	0.000	HS
	No	76 (59.4%)	3 (5.6%)	73 (98.6%)		
Employee feeding baby directly from the breast	Yes	52 (40.6%)	51 (94.4%)	1 (1.4%)	0.000	HS
	No	76 (59.4%)	3 (5.6%)	73 (98.6%)		
Employee feeding baby formula milk	Yes	62 (48.4%)	12 (22.2%)	50 (67.6%)	0.000	HS
	No	66 (51.5%)	42 (77.8%)	24 (32.4%)		
Employee feeding baby other (mention...)	Yes	14 (10.9%)	2 (3.7%)	12 (16.2%)	0.025	S
	No	114 (89.1%)	52 (96.3%)	62 (83.8%)		

*Not: All employee mothers are not expressing, nor storing their breast milk

Table 6. Predictive factors of exclusive breastfeeding in the multivariable analysis

Variable	Odds ratio	95% Confidence interval	P value
Infant's age in months	0.74	0.57 – 0.96	0.022
Mother knows that infant's chin should be immersed in mother's breast and most nipple halo in the infant lower lip	2.51	0.79 – 7.95	0.118
During working hours, mothers feed her babies other than breast or industrial milk.	0.19	0.04 – 0.94	.0.041

Hosmer and Lemeshow goodness-of-fit test: 0.961. Discrimination (area under the receiver operating characteristics curve): 0.57.

Discussion

This study included total number of 180 Egyptian mothers paired with their infants, were recruited from three PHC centers, their ages ranged from 18 to 42 years with a mean age of 29.4 (SD 5) years. Mother age has no association with exclusivity of breastfeeding in this study, but, another studies revealed that young mothers were non-exclusively breastfeeding their children more than older mothers.[13,14] In contrast, Labib and El-Shafei found increase in exclusive breastfeeding rate among women in the ≤25 age, referring it to their eagerness to be fully engaged in motherhood acts.[15]

About 47.8% of the mothers had university education, 71.1% of the mothers were employee with no significant association with exclusivity of breastfeeding, but another researchers found that all these social factors enhance the choice of nonexclusive breastfeeding.[16]

Nearly 73.9% of mothers were living in a separate house, and it showed no association with exclusivity of breastfeeding in this study. In other studies mothers-in-law and some grandmothers were supporting the mothers to feed their babies breast milk exclusively and dispirited the mother of giving pre lacteal feeds but others advised starting liquid or solid foods before six months as evidenced.[17,18] About 98.3% of participants have their husband with them with no association with exclusivity of breastfeeding in this study.

A previous study showed that single mothers tend to choose artificial feeding, another study reviled that most of the fathers in general are very supportive of feeding breast milk and disheartening the mother from giving formula milk and few fathers did not interfere with the mother's choice of feeding even the help given by men is low in traditional societies and usually men not fully involved in child feeding practices.[13,18] About 1.1% were current smoker, one third of the mothers had only one child, the mean age of participant infants was 3.27 (SD 1.5) months for exclusively fed infants and 4.02 (SD 1.491) month for nonexclusive breastfed infants with highly significant association between higher infant's age and non-exclusivity of breastfeeding.

Most of the infants were full term (90.6%) and mean infants' weight was 5.9 (SD 1.1) kilograms for exclusive breastfed and 6.06 (SD 1.39) kilograms, for non-exclusively breastfed infants, with no association with breastfeeding exclusivity .But another study proved that incubated infants were more liable for artificial feeding than to be exclusively breastfed.[8] The BFHI Ten Steps are targeted to the term infant population only, so, extra and modified policies to encourage and care breastfeeding in the neonatal intensive care are desired.[19]

Noticeably there was high percent (50%) of cesarean sections among our sample, 70% of the mothers had no previous exclusive breastfeeding experience. Most of the mothers started breastfeeding late, no one of employee mothers expressing her breast.

There are well established international recommendations for exclusive breastfeeding for the baby's first six months of age, followed by the addition of complementary foods to be continued breastfeeding through the baby's first year, and continuation of breastfeeding for as long as desired by both mother and infant.[3,6,20,21] In contrary, in the present study the frequency of breastfeeding among participants was 90.6%, and the frequency of exclusive breastfeeding dropped to 39.4%, which indicates clear defect in exclusive breastfeeding among infants in their first six months of age, this could be referred to many predictive factors which were clearly evidenced in this study such as defective breastfeeding practices as proper infant positioning and latching during breastfeeding.

The study logistic regression model showed that, exclusive breastfeeding decreased with progressive increase in infant's age, while mothers with good knowledge about proper practice of breastfeeding (e.g. infant's chin should be immersed in mother's breast and most nipple halo in the infant lower lip) adhered more to exclusive breastfeeding. The study multivariate analysis showed that during working hours, mothers who fed their infants other food than breast milk adhered less to exclusive breastfeeding. This could reflect poor knowledge about the importance of breast milk in comparison of other types of infants feeding in the first 6 months of their lives.

Study limitations

Before reaching conclusions based on the present results, it is necessary to consider a number of potential objections to the methodology. Epidemiologic studies conducted at healthcare centers are considered methodologically suspect by many investigators. Controlled clinical trials provide more evidence, but analyzing data from observational studies, case-control or cohort studies, is sometimes desirable, especially in studying the predictive factors of a practice as in our case.

Conclusion

The prevalence of exclusive breastfeeding is quite low, a problem seen in most of developing countries. The predictive factors for exclusive breastfeeding are, younger infant's age, good knowledge of the mothers about proper practice of breastfeeding, and mothers insisting on breastfeeding during working hours.

Recommendations

Great efforts are needed for raising exclusive breastfeeding rate via proper health education and sufficient practical training of the mothers about proper breastfeeding practice. More studies and researches are needed to investigate and clarify other causes of low exclusive breastfeeding rate. All Egyptian organizations and institutions involved in child health and wellbeing should collaborate to support breastfeeding through the following actions:

1. Research Investment to support the progress of targeted evidence-based strategies for social and behavioral change.
2. Professional and public education to resolve the actual obstacles to breastfeeding exclusively and successfully.
3. Strengthening of systems and policies which help working mothers to breastfeed their infants exclusively up to 6 months of age, via supplying mothers with enough support
4. Enough training and targeted supervision to reconstruction the health workers capacity for proper maternal and family support and counseling.
5. Building successful model of baby friendly environment and breastfeeding support groups in different health facilities in Egypt and should work towards national implementation of international guidelines and recommendations supporting proper breastfeeding.

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ORIGINAL PAPERR

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Assessment of the state of knowledge of bloodborne infections, occupational exposure and post-exposure prophylaxis and study of exposure to potentially infectious materials among students of selected medical faculties in Poland

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ABSTRACT

Introduction. Occupational exposure to potentially infectious material (PIM) is a serious problem for healthcare workers, including medical students.

Aim. We assessed the state of knowledge about occupational exposure and frequency of exposure among students of selected medical faculties in Poland.

Material and methods. Retrospective analysis with proprietary questionnaires.

Results. Only 34.5% from 753 respondents correctly indicated bloodborne pathogens and 9.3% PIM. There were 84 reports of exposure, mostly during intravenous injections. 10.4% students claimed probable occupational exposure which was not reported. Most common reason for not reporting was fear of negative supervisor reaction.

Conclusion. Student's knowledge of this matter is poor. Significant percentage of students has never participated in occupational exposure training. Occupational exposure was experienced by surprisingly large number of students. Students are afraid to report the incidents. Additional education would be useful in reducing exposure risk.

Keywords. knowledge, materials, occupational exposure, post-exposure prophylaxis, potentially infectious, students

Introduction

Since the first occupational infection by the human immunodeficiency virus (HIV) in 1984, occupational accidents involving biological material have been a prominent issue in public health.[1] Occupational exposure is defined as a contact of mucosa, conjunctiva or damaged skin with potentially infectious material (PIM), which occurred at work.[2] Due to the frequent

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contact with patient's body fluids and secretions, healthcare workers are particularly exposed to PIM. This issue concerns also medical students, who attend many procedures within the framework of academic course.[3] Not only blood is regarded as a PIM, the risk of infection occurs also in case of contact with other biological materials, such as: cerebrospinal fluid; pleural/peritoneal/pericardial fluid, amniotic fluid or any exudation containing blood. Despite the possibility of transmission over 20 pathogens with PIM, a particular role is attributed to hepatitis B virus (HBV), hepatitis C virus (HCV) and HIV, due to serious health consequences and possible further spread.[4] It is estimated that the risk of infection after a contact with PIM is about 0.3% for HIV, varies from 0.5 to 3% for HCV and may reach 40% for HBV.[5] In the literature, there are limited polish studies concerning occupational exposure among medical students, having regard to the students' state of knowledge, exposure epidemiology or prevention methods.[6]

Aim

We have undertaken a study with purpose to assess the state of knowledge regarding occupational exposure and post-exposure prophylaxis (PEP) and to estimate the scale of a PIM exposure among students of selected medical faculties in Poland.

Material and methods

The subject of the study was to assess the state of knowledge regarding occupational exposure and post-exposure prophylaxis (PEP) and to estimate the scale of a PIM exposure among students of selected medical faculties in Poland. For this purpose, an anonymous survey consisting of 33 questions was constructed. Respondents were asked about: field of study, year of study, university, sex, age (5 questions), issues concerning occupational exposure and PEP (7 questions to assess respondents' state of knowledge), their own experience regarding occupational exposure and PEP (21 questions).

Addressees were students of medical faculties most at risk of occupational exposure, i.e. medicine, dentistry, nursing, midwifery and emergency medicine of all medical universities in Poland. The study was conducted in two ways - stationary in paper form given to all students before classes, mainly at the University of Rzeszow, and electronically - using social media and Google Forms. The invitation to participate was also sent via e-mail to the dean's offices of medical universities - in this way all students of the above-mentioned faculties in Poland had equal chances to participate. It was carried out throughout the country from February to March 2020.

The study was a non-interventional, anonymous, voluntary survey research. Every respondent agreed to participate in the study by starting to complete the questionnaire. Moreover, participation in it did not involve any financial gain or material benefits. All students' rights including the protection of sensitive data, were preserved and respected by the research team according to GCP and Declaration of Helsinki requirements. The study was approved by Institutional Review Board (Bioethics Committee of the Regional Medical Chamber in Rzeszow).

Table 1. Demographics of the study population (age, sex, university, year of study)

Study population n=753	N	%
Age mean ± SD, range (year)	22.2 ± 3.88, 19-58	
Sex:		
Male	124	16.5
Female	629	83.5
Field of study:		
Medicine	274	36.4
Nursing	196	26.0
Midwifery	166	22.0
Emergency Medicine	94	12.5
Dentistry	23	3.1
University:		
University of Rzeszow	398	52.9
Medical University of Lublin	68	9.0
Medical University of Lodz	47	6.2
Public Higher Medical Professional School in Opole	43	5.7
Medical University of Silesia	33	4.4
Medical University of Bialystok	33	4.4
University of Warmia and Mazury in Olsztyn	29	3.9
Wroclaw Medical University	16	2.1
Nicolaus Copernicus University, Collegium Medicum in Bydgoszcz	13	1.7
State School of Higher Vocational and Economic Education in Jaroslaw	13	1.7
Medical University of Warsaw	12	1.6
Jagiellonian University Medical College in Krakow	12	1.6
Poznan University of Medical Sciences	10	1.3
Medical University of Gdansk	6	0.8
University of Technology and Humanities in Radom	6	0.8
Others	14	1.9
Year of study:		
1st year of the Bachelor's programme/long-cycle Master's programme	162	21.5
2nd year of the Bachelor's programme/long-cycle of the Master's programme	172	22.8
3rd year of the Bachelor's programme/long-cycle of the Master's programme	173	23.0
4th year of long-cycle Master's programme/1st year of the Master's programme	120	15.9
5th year of long-cycle Master's programme/2nd year of the Master's programme	115	15.3
6th year of long-cycle Master's programme	11	1.5

Results

753 students took part in the study (437 in electronic form, 316 answers in paper form). Answers were obtained from a representative group for the population of medical students of several dozen of the largest universities in Poland. We included students of selected medical

faculties: medicine (n=274), dentistry (n=23), nursing (n=196), emergency medicine (n=94) and midwifery (n=166). The responses were obtained from medical students from all years of study – both undergraduate and graduate. The respondents were from 19 to 54 years old, 96.6% of them were in the age range 19-24. The average age was 22 years. The ratio of women to men was approximately 5:1 (Table 1).

Knowledge about post-exposure prophylaxis

According to the obtained data, 33% of students declare that they did not undergo any PEP training during their studies (Figure 1). Respondents were asked how they assessed their knowledge of what to do after exposure to PIM on a scale of 1 (no knowledge) to 5 (full knowledge). Most students rated their knowledge at 4 points (n = 278) and 3 points (n = 266). The average rating was 3.29 points.

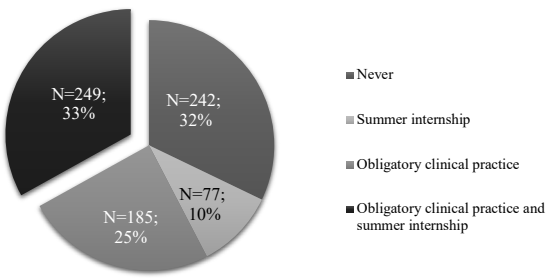

Fig. 1. Have you ever had training in post-exposure prophylaxis?

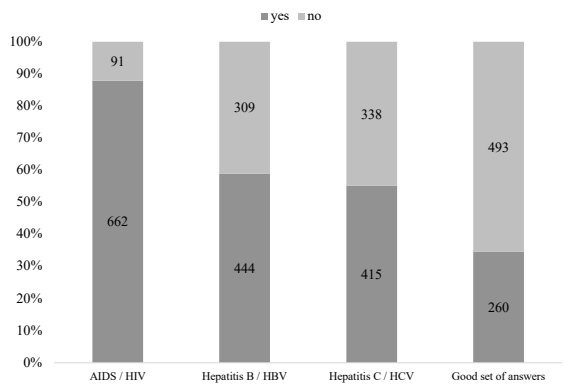

Fig. 2. What diseases associated with exposure to infectious material can you be exposed to in healthcare facilities? (AIDS - Acquired Immune Deficiency Syndrome, HIV - Human Immunodeficiency Virus, HBV – Hepatitis B Virus, HCV – Hepatitis C Virus)

The first open question asked to list diseases particularly vulnerable for medical students due to exposure to blood or body fluids. 34.5% of respondents indicated three viruses: HIV, HBV and HCV, 11.7% responded with "hepatitis" without specifying the type. Other responses that have often been mentioned include tuberculosis, human papillomavirus (HPV), Clostridium difficile or Staphylococcus aureus infection (Figure 2).

In the next three multiple-choice closed questions including both correct and incorrect answers, 95.8% of students indicated percutaneous needlestick injury contaminated with blood as a situation requiring PEP, 89.4% marked conjunctival contact with potentially infectious material, 46.9% - superficial injury with needle considered as uncontaminated with blood, 13.5% - exposure of intact skin to PIM. 43.7% of respondents marked a set of answers: conjunctival contact with potentially infectious material and intradermal cut with a needle contaminated with blood (Figure 3).

The next multiple-choice closed question concerned activities that should be performed immediately after the exposure. 77.2% of students indicated rinsing a damaged skin thoroughly with water, 47.0% disinfection the skin with non-alcoholic agent, 37.2% disinfection with alcoholic agent, 11.8 % marked stopping the bleeding and 9.7% applying pressure on the wound. The set of answers including rinse damaged skin with plenty of water and disinfect the skin with an alcohol-free agent selected 30.1% of respondents (Figure 4).

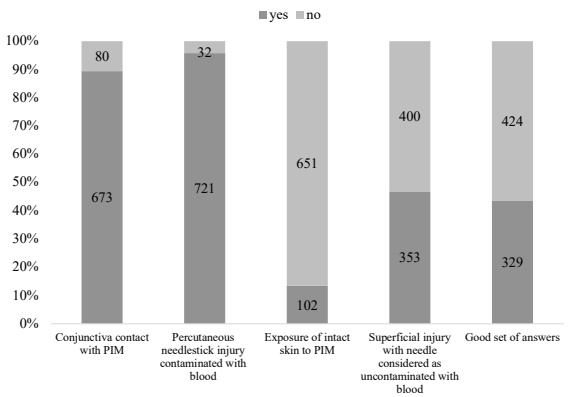

Fig. 3. What events require post-exposure prophylaxis? (PIM – potentially infectious materials)

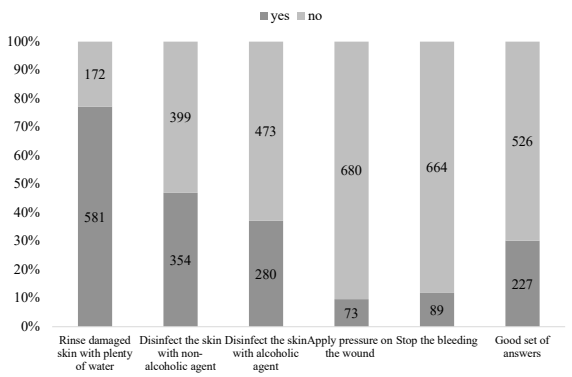

Fig. 4. What should you do after percutaneous injury with potentially contaminated needle?

When it comes to materials considered as PIM, 99.1% indicated blood, 82.2% marked semen, 74.4% faeces, 72.4% cerebrospinal fluid, 60.8% amniotic fluid, 16.1% sweat and 15.1% tears. (Figure 5).

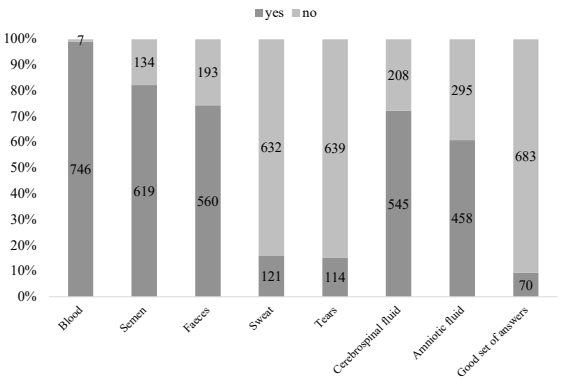

Fig. 5. What could be potentially infectious material?

The last question regarding the time when the post-exposure procedure should be implemented – 73.2% indicated the answer "no later than 24 hours", 17.7% "no later than 48 hours", 8.1% "no later than 72 hours" and 1.1% marked the answer "does not matter". (Figure 6).

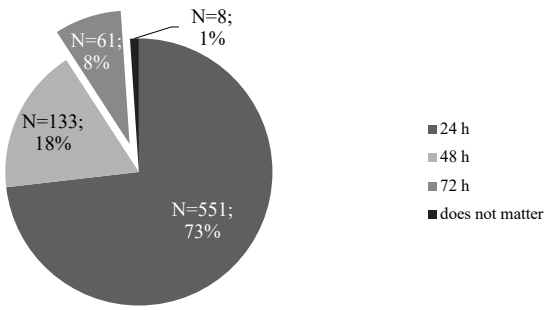

Fig. 6. How many hours should post-exposure prophylaxis begin?

For better illustration and comparing the subjective and objective students' knowledge about post-exposure procedures, a five-point scale (0-5 points) was used, according to which points were awarded for giving the correct answer (Table 2). The table presents also the objective and subjective knowledge depending of the year of study and field of study.

Occupational exposure

The second part of the survey concerned the frequency of occupational exposure among students and the course of proceedings after it occurred. Only students who declared that they had experienced the exposure participated in it. It turned out that 11.3% (n=85) of the respondents at least once experienced the occupational exposure, while 7.3% marked the answer "Probably yes". Exposures most often occurred during holiday internships (41.2%). The highest percentage of exposed respondents was found among dentistry students - 26.1% of all people from this faculty experienced exposure (Table 3). The data show that nearly 25% of exposed people have never participated in the PEP training.

Table 2. Comparison of subjective and objective knowledge assessment and objective knowledge depending on the year and field of study

Points	Subjective knowledge assessment		Objective knowledge assessment	
	N	%	N	%
1	34	5	19	2.5
2	113	15	54	7.2
3	226	35	158	21.0
4	278	37	403	53.5
5	62	8	119	15.8
Average score	3.29		3.73	

Year of study	Average subjective score	Average objective score	Faculty	Average subjective score	Average objective score
I	2.86	3.35	Medicine	3.31	3.97
II	3.37	3.71	Nursing	3.38	3.56
III	3.34	3.63	Midwifery	3.30	3.78
IV	3.52	4.06	Emergency Medicine	3.11	3.29
V	3.45	4.00	Dentistry	3.13	3.78
VI	3.64	4.73			

When it comes to the activities during which the exposure occurred, the most often there were various types of injections (intravenous, intramuscular, central), as well as blood collection – 64% in total. The second most frequently mentioned activity was surgery assistance – 11.8% of cases. The infectious material that students came into contact with most often was blood (91.8%). The incidents were most often reported to the academic teacher (35.3%), internship supervisor (18.8%) and chief of department or ward nurse (17.6%). The post-exposure procedure was initiated only in 67% of cases (n=57). The most common answers to the question about the reasons for lack of commence of post-exposure procedures were: 'no need' and 'the patient was considered healthy'.

All respondents were also asked if they had experienced the exposure but did not report it. As many as 76 people answered affirmatively (10.1%). To the question 'Were you/ would you be afraid to report an occupational exposure?' 85 respondents (11.2%) answered 'yes'. Among the reasons often mentioned were fear of the reaction of superiors, the need to incur costs, as well as the lack of time to undergo a long post-exposure procedure. 7.3% of respondents avoid carrying out invasive procedures for fear of exposure.

Table 3. Occupational exposure

Have you ever experienced occupational exposure?	N	% of all students
No	613	81.4
Yes	85	11.3
Probably yes	55	7.3
Do you think that you have experienced occupational exposure but did not report it?		
No	677	89.9
Yes, once	57	7.6
Yes, several times	19	2.5
When the occupational exposure occurred?		% of exposed students (n=85)
During summer internship	35	41.2
During obligatory clinical practice	32	37.6
During extra activities e.g. on call	18	21.2
Field of study:		% of students in each field of study
Medicine	43	15.7
Nursing	10	5.1
Midwifery	20	12.0
Emergency Medicine	6	6.4
Dentistry	6	26.1
Year of study:		% of students in each year of study
1st year of the Bachelor's programme/long-cycle Master's programme	10	6.2
2nd year of the Bachelor's programme/ ong-cycle of the Master's programme	12	7.0
3rd year of the Bachelor's programme/long-cycle of the Master's programme	17	9.8
4th year of long-cycle Master's programme/1st year of the Master's programme	14	11.7
5th year of long-cycle Master's programme/2nd year of the Master's programme	30	26.1
6th year of long-cycle Master's programme	2	18.2

Primary prevention

The last part of the questionnaire focuses on exposure prophylaxis. Respondents were asked whether the academic teachers or superiors inform about the patient's potential infection - an affirmative answer was given by 73% (n=550). In open question about whether they decide to take extra precautions when dealing with HBV/ HCV/ HIV infected patient, most students (56.6%) do not apply additional security.

Moreover, our study checked how many students have been ever tested for HBV/ HCV/ HIV except for cases where PEP was initiated. The collected data shows that relatively large proportion of students had tested on their own, while a smaller percentage of tests was funded by universities. Very large percentage of students (over 50% in the case of HCV and HIV and over 40% in the case of HBV) who had run the tests was from the 1st and 2nd year of master's degree studies. It turned out that 43.4% of respondents (n=33), who believe they had exposure but did not report it, were tested for those three infections. The last question was to check what proportion of medical students decided to test the level of anti-HBs antibodies. A division into fields of studies was made, which showed that the percentage of students who checked the level of vaccine antibodies was similar for individual fields – only in the case of emergency medicine this percentage was much lower (Table 4).

Table 4. Primary prevention

How often do you have a contact with PIM?	N	% of all students
Everyday	68	9.0
Couple times a week	260	34.6
Couple times a month	186	24.7
Couple times a year	162	21.5
Less	37	4.9
Never	40	5.3
What additional measures do you use in contact with an HIV/HBV/HCV-infected patient?		
Gloves	115	15.3
Double-gloving or triple-gloving	67	8.9
Greater caution	62	8.2
Other (masks, glasses, protective clothing)	84	11.2
None	426	56.6
Have you been tested for HIV/HBV/HCV (not as part of PEP)?		
No	443	58.8
Yes, HBV – funded by the university	71	9.4
Yes, HBV – on my own	175	23.2
Yes, HCV – funded by the university	34	4.5
Yes, HCV – on my own	113	15.0
Yes, HIV – funded by the university	24	3.2
Yes, HIV – on my own	115	15.3
Students tested for HIV/HBV/HCV by fields		% of all students in a given field
Medicine	113	41.2
Nursing	77	39.1
Midwifery	68	41.0
Emergency Medicine	45	47.9
Dentistry	9	39.1
Have you ever checked level of post-vaccination anti-HBs antibodies?		% of all students
Yes	195	26.0
No	558	74.0
Students who checked level of post-vaccination anti-HBs antibodies by fields		% of all students in a given field
Medicine	58	21.2
Nursing	34	17.3
Midwifery	39	23.5
Emergency Medicine	7	7.4
Dentistry	5	21.7

Discussion

Medical students are a group particularly at risk of accidental exposure to infectious material because they do not have adequate experience, but they are willing to

learn new, invasive procedures – they are also required to prepare for their future profession. Students of medicine, nursing, midwifery, dentistry and emergency medicine are particularly vulnerable, because they relatively often have contact with PIM while taking part in invasive medical procedures. To date, no scientific studies have been published regarding the scale of occupational exposure among young adepts of medical faculties in Poland. There are also few publications regarding this issue in other countries. Most commonly these are studies on a very small group of students or students are included as a smaller proportion of all health care workers of a given centre. Therefore, the risk of occupational exposure among medical students is often underestimated and its scale is unknown due to the lack of official data on the number of reported incidents.

Our study revealed that at the root of the problem of occupational exposure and PEP may be poor students' knowledge. As many as 30% of respondents stated that they have never had training in post-exposure prophylaxis, despite carrying out the same study program throughout Poland. It revealed that a large proportion of students do not know how to behave in contact with infectious material and what to include to PIM. In the part of the survey containing questions about knowledge about occupational exposure and post-exposure prophylaxis, students confirmed their subjective poor assessment of own knowledge. In an open question referring to diseases related to occupational exposure, only slightly more than 30% of respondents correctly listed hepatitis B, hepatitis C and HIV infection, and another 10.8% mentioned HIV/ AIDS and 'hepatitis'. Students mentioned also incorrect answers such as tuberculosis, human papillomavirus (HPV), *Clostridium difficile* or *Staphylococcus aureus* infection. Respondents also had a problem determining the situations that require the implementation of PEP. Although about 90% correctly marked conjunctival contact with potentially infectious material and percutaneous needle injury with contaminated blood as incidents requiring PEP, many students also marked a superficial injury with a needle deemed uncontaminated and, surprisingly, exposure of intact skin to potentially infectious material (respectively 46.9% and 13.5%). To sum up, only 43.7% of respondents selected the correct set of answers (conjunctival contact with potentially infectious material and intradermal cut with a needle contaminated with blood). In terms of potentially infectious materials, students' knowledge was very poor. Only 9.3% of respondents correctly marked all the answers! - blood, semen, cerebrospinal fluid and amniotic fluid. Interestingly, 7 respondents concluded that blood is not a PIM. It turned out that less than 75% respondents know that cerebrospinal fluid and amniotic fluid (including 77.6% of midwifery students who have frequent contact with amniotic fluid) may also be responsible for occupational exposure. Faeces proved to be particularly problematic, because almost 75% of the students considered them to be PIM. It should be emphasized that, according to the Occupational Safety and Health Administration (OSHA), faeces are not potentially infectious material in themselves – they become PIM only when contaminated with blood.[7] In terms of PEP implementation time, only 8.1% of the students correctly indicated the answer 'no later than within 72 hours. The PEP procedure is not known to all students - only every third respondent knows how to proceed after exposure to PIM. It is worth noting that more or less equal number of students would disinfect the wound with an alcoholic and non-alcoholic agent. According to WHO guidelines, immediately after exposure to the infectious material, the place should be rinsed thoroughly with water and disinfected with a non-alcohol or soap-containing disinfectant, and in the case of conjunctiva – rinsed several times with running water or saline.[8] It is very important not to use strong solutions (alcohol, iodine) and not to squeeze the wound area, as this may result in additional irritation and increased penetration of microorganisms into the tissues. Also, the blood flowing out should not be stopped, as the free outflow of blood may limit the entry of pathogens.[9] In summary, the knowledge status of students can be described as moderate (average score 3.73 points on a scale of 0 to 5). However, analysing individual questions the knowledge of the respondents is very poor – just like in other foreign publications on knowledge in the field of occupational exposure and PEP.[10,11] Our study shows that awareness and knowledge generally increase with the year of study, which was confirmed by a higher percentage of correct answers and the highest score among students of 6th year (3.35 points on the first year compared to 3.73 points on the last year). This is related to the gradual acquisition of knowledge during clinical classes and the role of observation and modelling on teachers. When it comes to field of the study, medicine students achieve the best score (3.97 points) and the worst result was obtained by emergency medicine students (3.29).

Students subjectively assessed their knowledge at the beginning of the questionnaire at 3.29 on a scale of 0 to 5, which can be described as moderate, and thus according to the actual state of their knowledge. It is very important issue because high self-esteem combined with low actual knowledge can be a risk. In such situations, students could opt out of further PEP training and would potentially pose a threat as medical personnel.

The results of our study indicate that more than 1 in 10 students experienced professional exposure. The result cannot be compared with other studies, because, so far, no studies have been published exclusively concerning students of various medical faculties and exposure to infectious material among them, both in Poland and in other countries. However, considering much rarer students' contact with PIM in comparison to regular

healthcare workers, we can conclude that this is a large percentage of them. This is worrying, because the problem of occupational exposure among students is not addressed worldwide, while concerning so many young people. The percentage of respondents who experienced such an event increases with the year of study as the number of incidents accumulates throughout the entire duration of study. Regarding fields of study, our study revealed that the largest percentage of occupational exposure concerns dental students - up to a quarter of them experienced an incident. The problem of exposure to potentially infectious material in dental students is widely discussed in the professional literature. Due to the specific and difficult technical conditions during invasive procedures and the actual performing of them by less experienced students of the last years of study only supervised by academic teachers, professional exposure occurs very often. According to published studies, percentage of dental students who have experienced exposure to biological material during their study period ranges from 19.1% to even 80.0%.[12-16] Surprisingly, in our study the smallest percentage of events concerned nursing and emergency medicine students. These data differ from those usually published, according to which nursing and emergency medicine students are most vulnerable to occupational exposure due to frequent contact with potentially infectious materials while performing invasive procedures.[12,17] We are not able to explain this fact unlike this is the result of the growing knowledge about occupational exposure in recent years and the resulting greater attention and caution when performing invasive procedures. Following this way of thinking, a high percentage of occupational exposure among medicine students may result from sporadic participation in invasive procedures and little experience in this field. This is confirmed by the fact that almost half of the incidents took place during holiday practices.

It should be emphasized that 10% of respondents admitted that they had experienced the occupational exposure but did not report it – a quarter of it repeatedly. This shows the problem of low reporting starting during studies, but fully developed among healthcare professionals. Available publications show that up to 80% of occupational exposures are not reported.[17-19] Fortunately, the results in our study are much lower. According to the limited data, reporting in Poland oscillates around 45-70%, including only about 20% of employees who report professional exposure each time. The most common reasons for concealing such incidents include lack of time, too much formalities, recognition of such events as a normal element of work, and no obligation to report exposure.[20-22]

Similarly to available publications, most of the exposures concerned needlestick injuries.[13,18,23,24] It is worth reminding here about replacing the needle cover after use (usually completely unnecessary action) so often causes needle injuries and possible bloodborne infection.[17,25,26] According to OSHA, recapping needles is generally incorrect.[27] In our study almost all incidents related to blood exposure, occurred in surgical and ob-gyn wards, what is undoubtedly connected with the number of invasive procedures performed. The same conclusions were obtained in other publications on student exposures.[28] As for PEP, the procedure was implemented only in 60% of cases. In the remaining cases, the patients were subjectively considered healthy and uninfected. It is worrying that one respondent after reporting the exposure was laughed at and ignored and three other students indicated that they had not informed anyone about the incident. Such situations may lead to further unreported exposures in the future and to possible infection eventually. Among the respondents only one person reported the presence of a disease related to occupational exposure, without indicating which one.

Statements of respondents about the reason for not reporting incidents are just as contentious. Most (almost 50 students) admitted that they would be afraid to report occupational exposure due to the negative assessment from the academic teacher and other students. Nervousness, mocking, shouting, evaluation as incompetent, inattentive, too fearful were also indicated. Similar results can be found in available publications.[17] Such situations are completely inappropriate and should be eliminated from the healthcare environment. According to the principle 'a good rescuer is a living rescuer', healthcare workers should mind own health as well as their colleagues including young medical adepts. Atmosphere of safety and trust should be created, and students should be assured that professional exposure can happen to any employee, even those most experienced and skilled. Other reasons for not reporting were a lot of formalities, confusion and too much time spent on the entire post-exposure procedure. Attention was also paid to several responses regarding the costs of PEP and possible treatment. Pursuant to the law in force in Poland, in the case of occupational exposure financing of post-exposure proceedings is the responsibility of the employer or the commissioning entity.[29] The results show that some of the respondents are not familiar with the applicable regulations for healthcare professionals (including medical students). It is dangerous because in the event of an exposure a student may not report it with unreasonable fear of incurring costs – and expose self to falling ill. It is therefore important to educate students about their rights and obligations to increase their sense of safety and minimize the phenomenon of low reporting of such incidents. Almost every tenth respondent indicated avoiding invasive procedures for fear of potential infection. Higher percentage of affirmative answers occurred in the group of people who have already experienced occupational exposure, which shows how traumatic this event is and how much it affects the further learning process and professional work.

More than half of the students do not use any additional protective measures when the patient has confirmed HIV/HCV/HBV infection. Other respondents pointed to putting on gloves (even double or triple), masks, special protective clothing and greater caution. Obviously, the use of masks, glasses, protective clothing is indicated for the prevention of contact with PIM, however the use of double and triple gloves can be controversial. This results in significant stiffening of the material and less comfort of work, which can lead to less precise movements and increase the risk of an exposure incident. According to WHO recommendations, double gloves should be used only when participating in longer surgical procedures (>30 minutes), especially orthopaedic ones, and in case of expected contact with large amounts of blood or other body fluids.[30] Numerous publications indicate that double gloves usually provide greater safety than a single layer.[31] Additional exercises and getting used to working in a double layer will not affect the effectiveness of manual work and will allow to maintain greater safety.

Almost 75% of respondents confirmed that in the case of performing an invasive procedure in a patient with confirmed HIV/ HBV/ HCV infection, they were informed about it by the academic teacher before conducting the procedure. It is very important because it allows student to use additional protective measures and leads to increased vigilance and greater precision in performing the procedure.[32,33]

About 75% of respondents have never checked their own anti-HBs vaccine antibodies. The minimum protective level of antibodies is generally considered as 10 IU/L. In Poland there are no recommendations for routine determination of antibody levels after vaccination, but it is measured in employees of some healthcare facilities. There are also no legal regulations for people of the medical profession who have not obtained a protective level of antibodies. The literature suggests the usefulness of performing such tests in healthcare workers, as well as among medical students, because the very fact of being vaccinated against HBV in childhood may not show actual immunization.[34] Slightly more than half of the surveyed students reported that they had carried out their own tests for HBV/HCV or HIV infection, while at the university's expense – 17% (not under PEP). The largest percentage of tests performed for these viruses was present among emergency medicine students (47%). It turns out that a very large percentage of tested students are from second-degree studies - more than 50% in the case of HCV and HIV and more than 40% in the case of HBV. Midwifery, nursing and emergency medicine students often take up work after completing their undergraduate studies, so it is possible that they did tests when undertaking work in healthcare facilities.

Conclusions

Medical students are particularly vulnerable to occupational exposure to potentially infectious material due to frequent performance or assistance in invasive procedures and not fully developed precision and fluency in their performance. It is therefore important to have knowledge of occupational exposure and post-exposure prophylaxis. The results collected in this study point to insufficient emphasis placed on these issues during studies, which results in unsatisfactory knowledge of the studied population. It seems that deficiencies in theoretical knowledge, as well as a small awareness of the right to implement post-exposure prophylaxis, cause fear of reporting occupational exposures among students. In addition, fear of negative evaluation by the academic teacher, ridicule, or too much commotion associated with submitting the exposure incident may be the reasons for inadequate reporting. The analysis of the data revealed that in the studied population 11% of students have experienced occupational exposure. Despite the fact that the time of exposure was not correlated with the year of study, it is worth emphasizing that training in post-exposure prophylaxis should be mandatory at the beginning of studies because most students take part in invasive procedures on first year clinical practice or holiday internships after 1st year of study. The training could be repeated on the third or fourth year, when great part of bachelor graduated take their first job.

Therefore, it is important to build an atmosphere of safety and trust and to properly educate students in this matter – submitting exposures and implementing prophylaxis can protect against the occurrence of a serious chronic disease.

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REVIEW PAPER

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What's new in airway secretions clearance for adults? A systematic review

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ABSTRACT

Introduction. Airway clearance techniques are an essential part of routine respiratory physiotherapy, enabling bronchial secretion clearance—the mucus overproduction and retaining results in lung function deterioration and disrupts effective pulmonary rehabilitation. Several mucus clearance methods are included in the physiotherapy daily routine of patients with chronic lung conditions; nevertheless, new techniques and approaches are continuously developed.

Aim. Thus, this systematic review summarizes novel airway clearance techniques applied in patients with chronic pulmonary conditions.

Material and methods. The PubMed, Cochrane Library, and PEDro databases were searched from 2010 to 2021, and studies were selected based on eligibility criteria.

Analysis of the literature. 101 patients from five studies describing four different techniques were included. Novel techniques were non-invasive ventilation, intrapulmonary percussive ventilation, trachea vibration, and PEP-sound wave combination. Significant improvements were noted for ventilation homogeneity (NIV), saturation (NIV), respiratory rate (IPV), and diffusion capacity (VL), whereas cardiovascular function and exercise endurance did not change significantly.

Conclusion. The presented methods are considered to have similar effectiveness as well-known airway clearance techniques. However, the systematic use of presented methods in routine pulmonary rehabilitation must be preceded by in-depth investigation to provide no-bias results.

Keywords. bronchial mucus, rehabilitation, respiration

The list of abbreviations:

AD – autogenic drainage, **CF** – cystic fibrosis, **COPD** – chronic obstructive pulmonary disease, **COVID-19** – coronavirus disease 2019, **CPT** – chest physiotherapy, **FET** – forced expiratory technique, **FEV1** – forced expiratory volume in one second, **FVC** – forced vital capacity, **HR** – heart rate, **IPV** – intrapulmonary percussive ventilation, **LCI** – lung clearance index, **MEF25** – maximal expiratory flow at 25 % of the forced vital capacity, **MEF75** – maximal expiratory flow at 75 % of the forced vital capacity, **NIV** – non-invasive ventilation, **PAP** – positive airway pressure, **PEDro** – Physiotherapy Evi-

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dence Database, **PEP** – positive expiratory pressure, **RR** – respiratory rate, **SpO_2** – oxygen saturation, **TV** – trachea vibration, **VAS** – visual analogue scale, **VC** – vital capacity, **VL** – VibraLung® acoustical percussor

Introduction

Mucus expectoration is critically important in pulmonary rehabilitation, especially for patients with chronic obstructive pulmonary conditions such as Chronic Obstructive Pulmonary Disease (COPD), asthma, cystic fibrosis (CF), bronchiectasis, and more.[1-4] These diseases are characterized by chronic airway inflammation and mucus overproduction, leading to severe obstruction. Moreover, distal airway occlusion, ciliary function disorder, and often ineffective cough are key problems with proper clearing secretions, leading to lung function deterioration.[5]

Airway clearance techniques (ACTs) are an essential part of respiratory physiotherapy. These techniques allow for effective mucus evacuation and subsequently enable efficient respiratory muscle training. There are many different techniques applied depending on patients' needs, cooperation or readiness. Some of them, such as postural drainage and chest percussion, are simple and do not require much patients' involvement, but at the same time are regarding as time-consuming, often uncomfortable, and considered less effective when compared with other techniques.[6-8] Moreover, developed secretion clearance devices replaced the head-down postural drainage positions with sitting positions in many countries.[7] Routine treatment includes: (1) volitional breathing based techniques, such as forced expiratory technique (FET), active cycle of breathing technique (ACBT), and autogenic drainage (AD); (2) positive expiratory pressure (PEP) based techniques, such as PEP, Hi-PEP, and oscillating PEP; (3) oscillation based technique, such as high-frequency chest wall oscillation.[9-16] Several reviews and overviews synthesized studies on safety, effectiveness, and quality of life of patients with chronic pulmonary diseases following routinely applied ACTs protocols.[1-3] Nevertheless, respiratory rehabilitation is still developing fast because the number of patients with severe respiratory conditions is growing continuously.[17] Furthermore, nowadays, pulmonary physiotherapy is facing a high burden of COVID-19 patients and survivors, including patients with chronic pulmonary condition exacerbations.[4,18-21] Therefore, novel ACTs, including, but not limited to, methods designed especially for patients unable to use hand-held devices, are of great importance.

Aim

This review aims to look through novel ACTs to summarize their usefulness in everyday pulmonary physiotherapy practice.

Material and methods

Search strategy

A systematic search of PubMed, the Cochrane Library, and Physiotherapy Evidence Database (PEDro) databases was undertaken for years from 2010 to 2021 to look for records involving the phrase "airway clearance techniques" and additional phrases: "novel"; "new"; "state of the art"; "chest physiotherapy"; "chronic pulmonary condition".

Inclusion and exclusion criteria

After duplicates removal, the retrieved publications were screened critically and independently by authors. Publications were included if they mentioned innovative airway clearance techniques in adults, discussed secretion clearance effectiveness, were classified as the randomized controlled study, cohort study, or observational study, and have been written in English or Polish. Publications were excluded if they did not have enough quantitative data in the results section and mentioned only commonly known airway clearance techniques, such as the active cycle of breathing technique, autogenic drainage, positive expiratory pressure, oscillating positive expiratory pressure, and high-frequency chest wall oscillation.

If the information presented in the title, abstract, or keywords suggested the publication might contain data relevant for this review, the full version of the article was downloaded for further investigation. The study exclusion decision was made based on all authors' opinions, and publications not meeting the inclusion criteria were excluded from the analysis.

Study quality appraisal

Extracted data included study design, population (sample size, age, disease), the study's aim, applied protocols (method, therapy duration, individual settings), and results, especially mucus secretion analysis. The primary focus was to check the actual impact of the applied method on mucus secretion. Therefore, from the final analysis, we excluded the studies that did not mention sputum/mucociliary clearance quantitative information (e.g., sputum wet/dry weight, ventilation improvement etc.). The methodological quality assessment was performed using the PEDro scale designed for randomized studies. The tool contains eleven questions scored one point each regarding the applicability of the trial (criterion 1), internal validity (criteria 2-9), and presence of statistical data (criteria 10-11).[22, 23]

Analysis of the literature

Quality appraisal results

The results of the quality assessment are presented in Table 1.

Table 1. The PEDro scale quality assessment results

Study	Rodriguez et al. [24]	Stanford et al. [25]	Paneroni et al. [26]	Kamimura et al.[27]	Wheatley et al. [28] (part I)	Wheatley et al.[28] (part II)
Eligibility Criteria	Yes	Yes	Yes	Yes	Yes	Yes
Randomly Allocated	Yes	Yes	Yes	Yes	Yes	Yes
Concealed Allocation	Yes	Yes	Yes	Yes	Yes	Yes
Similar Groups at Baseline	Yes	Yes	Yes	No	No	No
Blinding of Subjects	Yes	No	No	No	No	No
Blinding of Therapists	No	No	No	No	No	No
Blinding of Assessors	Yes	Yes	No	No	No	No
Data from > 85% of Subjects	Yes	No	Yes	No	Yes	Yes
Intention to Treat	No	Yes	Yes	Yes	Yes	Yes
Statistical comparision	Yes	Yes	Yes	No	Yes	No
Measures of Variability	Yes	Yes	Yes	Yes	Yes	No
Final score	9/11	8/11	8/11	5/11	7/11	5/11

The studies included in the analysis ranged from 5 to 9 on the PEDro scale with a median score of 7. The manuscript authored by Wheatley et al. reported two different studies' designs; therefore, it was divided for improved study quality evaluation.[28] All reported publications scored particularly poorly in blinding of subjects, therapists, and assessors. However, usually, physiotherapeutic interventions requiring patients' commitment need to be carefully explained, and often the proper training should be provided before the intervention, which limits blinding possibilities.

Characteristic of included studies

The summary of database search results is presented in Figure 1. A systematic search of databases identified 4521 records. After duplicates removal, 3841 records were screened based on the title, abstract and key words, and 3798 articles were excluded. 34 full articles were evaluated, and subsequently, 5 articles were included for the review. The reasons of 29 articles exclusion were: (1) lack of information about the sputum secretion (24 publications); (2) lack of any quantitative information about sputum/mucociliary clearance (4 publications); (3) study design (1 publication).

All included publications were randomized studies, 4 randomized crossover studies, and 1 randomized controlled trial.

Reviewed publications recruited 101 patients: 67 diagnosed with CF, 22 with bronchiectasis, 6 with bronchial asthma, 5 with COPD, and 1 with chronic bronchitis. The age of participants ranged from 17 to 93 years. The number of male participants was 52 and female participants 49. The rehabilitation for most individuals was performed either by patients alone at home (n=44) or organized as ambulatory treatment (n=46). Only one publication reported inpatients intervention (n=11).[28]

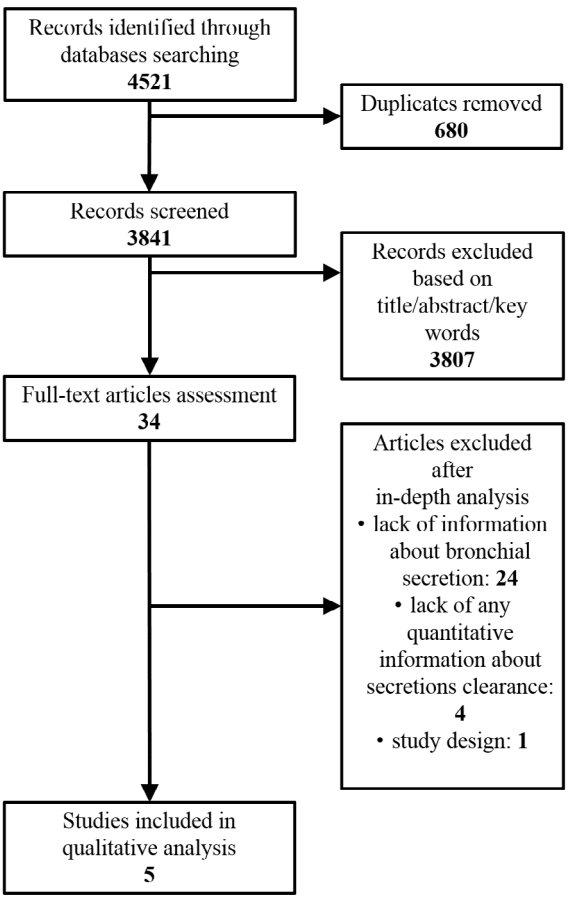

Fig. 1. Flow chart with summary of database search

The summary, including study design, aim, material and methods, results, and key findings, is presented in Table 2.

Techniques description

Five included studies reports four novel airway clearance techniques: non-invasive ventilation (NIV), in-

Table 2. Summary of reviewed manuscripts

Author	Rodriguez et al.[24]	Stanford et al. [25]	Paneroni et al. [26]	Kamimura et al.[27]	Wheatley et al. [28]
Study design	RCT	RCS	RCS	RCS	RCS
Tested technique	NIV-bilevel PAP	NIV	IPV	Cervical trachea vibration (TV method)	Sound waves + PEP (VibraLung® Acoustic Percussor-VL)
Aim	To investigate NIV efficacy as ACT in comparison to standard treatment	To investigate NIV efficacy as ACT in comparison to standard treatment	To investigate IPV efficacy as ACT in comparison to standard treatment	To investigate TV method efficacy as ACT in comparison to standard treatment	To investigate efficacy of VL as ACT in comparison to standard treatment
Population	Experimental group: 16 patients with CF 8M, 8F 28±11 y Control group: 16 patients with CF 8M, 8F 33±9 y	14 patients with CF 7M, 7F 35.5±17.1 y	22 patients with bronchiectasis 12M, 10F 64.4±8.9 y	12 patients: bronchial asthma=6, COPD=5, chronic bronchitis=1) 5M, 7F 54–93 y	Study I: 10 outpatients with CF 7M, 3F 25–34 y Study II: 11 inpatients with CF 5M, 6F 17–29 y
Treatment design	Setting: home treatment Duration: 12 weeks Frequency: 2 sessions (60 min)/day Experimental group: – Inhalation of bronchodilators and hypertonic saline 7% for 10 minutes – Autogenic drainage for 15 minutes – NIV – bilevel PAP (expiratory pressure 10 cm H_2O, inspiratory pressure 20 cmH_2O); 2 minutes breathing – Huffing (FET technique) Full cycle was repeated during 60 minutes Control group: – Inhalation of bronchodilators and hypertonic saline 7% for 10 minutes – Autogenic drainage for 15 min – PEP – 10 breaths through PEP face mask (10–20 cm H20) – Huffing (FET technique) Full cycle was repeated during 60 minutes	Setting: out-patient Duration: 2 days (1 day experimental treatment – 1 day control treatment) Frequency: 2 sessions (30 min)/day Experimental treatment: – 10 NIV breaths –settings determined individually – 4 'huffs' or coughs Control treatment: – usual ACT – 4 'huffs' or coughs	Setting: out-patient Duration: 2 days (1 day experimental treatment – 1 day control treatment) Frequency: 1 session (30 min)/day Experimental treatment: – IPV session in sitting position: 3 active cycles (2 phases low pressure–high frequency; 1 phase high pressure–low frequency) – Cough after each cycle Control treatment: – Combination of forced expiration postural drainage (prone, right-lateral decubitus, left–lateral decubitus), percussion and vibration (10 minutes each position) – Cough after each position	Setting: home treatment Duration: 12 weeks (4 weeks–experimental treatment – 4 weeks washout period – 4 weeks standard treatment) Frequency: 2 sessions (5 min)/day Experimental treatment: electronic larynx applied to cervical trachea to generate transcutaneous vibration at 80 Hz Control treatment: oscillating PEP (Acapella ®) standard treatment	Study I Setting: out-patient Duration: 2 days (1 day experimental treatment – 1 day control treatment) Frequency: 1 session (20 min)/day Experimental treatment: VibraLung breathing with sound waves (PEP + sound waves) Control treatment: VibraLung breathing without sound waves (PEP) Study II Setting: in-patient Duration: 12 days Frequency: 4 sessions (30 min)/day Experimental treatment: HFCWO 2 sessions/day + Vibralung breathing 2 sessions/day Control treatment: HFCWO 4 sessions/day
Outcome measures	1. Sputum: LCI (before and after completing the study) 2. Pulmonary function: FEV1, FVC [%] (before and after completing the study) 3. Exercise endurance: 6MWT [m]	1. Sputum: 24-h sputum wet weight [g]; 2. Pulmonary function: FEV1, FVC [l], MEF25, MEF75 [l/s], SpO_2[%], WoB and EoC 3. Treatment satisfaction: VAS [points]	1. Sputum: sputum wet and dry weight [g] 2. Cardiopulmonary function: SpO_2 [%], HR [beats/min], RR [breaths/min] 3. Dyspnea: VAS [%] 4. Sensation of phlegm encumbrance: VAS [%] 5. Discomfort: VAS [%]	1. Sputum: expectoration difficulty recorded daily – VAS 2. Pulmonary function: FEV1 [%], VC [%] 3. QoL: SGRQ, SF–36	Study I&II 1. Sputum: wet weight [g], pellet weight [g], dry weight [g] 2. Pulmonary function: FVC [l]; FEV1 [l]; FEV1/FVC [%]; SpO_2 [%]; DM/V_c 3. Cardiovascular function: HR [beats/min], stroke volume [ml]

Results	Experimental group 1. LCI pre 10.2±2.37; post 9.2±2.55 2. FEV1% pre 43±12; post 41±12 FVC% pre 64±12; post 61±16 3. 6 MWT pre 553±69; post 559±95 Control group: 1. LCI pre 9.69+2.5; post 9.76+2.5 2. FEV1% pre 55±15; post 54±13 FVC% pre 78±13; post 78±12 3. 6 MWT pre 539±55; post 553±77 Experimental vs control: 1. LCI* (p=0.01) 2. FEV1 (p=0.52), FVC (p=0.25) 3. 6MWT (p=0.76)	Experimental vs control treatment: 1. Sputum: Exp. 48.1±30.8; Ctr. 49±29.4; p=0.84 2. Pulmonary function: no significant difference in FEV1, FVC, MEF25, MEF75, EOC and WoB; SpO2*: Exp. 95.7±2.3; Ctr. 94±2.5; (p=0.004) 3. Treatment satisfaction: no significant difference	Experimental vs control treatment: 1. Δ Sputum: wet weight = 3.0 g (p=0.58); dry weight = –0.31 g (p=0.26) 2. Δ Cardiopulmonary function: SpO_2 = 0.6 (p=0.35); HR = –0.4 (p=0.82); RR* = –1.6 (p=0.047) 3. Dyspnea: Exp. pre 35%±29%; post 23%±20% (p=0.004)* Ctr. pre 33%±27%; post 27%±26% (p=0.09) 4. Sensation of phlegm encumbrance: Exp. pre 47±35%; post 27±32% (p=0.001) Ctr. pre 48±1%; post 37±35% (p=0.03) 5. Discomfort: Exp. 23±17%; Ctr. 40±27% (p=0.03)*	Experimental treatment: 1. Sputum: expectoration difficulties decreased during usage 4/12 patients 2. Pulmonary function: FEV1% pre 66.8%; post 66.3% (p=0.7334); VC% pre 87.5%; post 89.6% (p=0.1294) 3. QoL: SGRQ pre 48.4; post 54.1 (p=0.4238); SF–36: PCS pre 31.3; post 39.9 (p=0.1099); MCS pre 51.9; post 49.5 (p=1.000); RCS: pre 41.3; post 34.6 (p=0.5693) Control treatment: 1. Sputum: expectoration difficulties decreased during usage 5/12 patients 2. Pulmonary function: FEV1% pre 69.2%; post 67.1% (p=0.6089); VC% pre 90.7%; post 88.9% (p=0.0957) 3. QoL: SGRQ pre 54.2; post 49.9 (p=0.4238); SF–36: PCS pre 36.0; post 36.9 (p=0.8501); MCS pre 50.0; post 51.5 (p=0.2095); RCS: pre 50.1; post 51.4 (p=0.5186) Device preference and effectiveness: 6 patients rated TV method and 5 patients rated Acapella® as more effective; VAS: Exp. 60 (20–80); Ctr. 50 (20–100) p=0.9257	Study I: 1. Sputum: wet weight: Exp. 10.5; Ctr. 10.0 (p=0.25); dry weight: Exp. 0.58; Ctr. 0.67 (p=0.57) pellet weight: Esp. 5.9; Ctr. 4.4 (p=0.25) Experimental treatment: 2. Pulmonary function: FVC: pre 4.1; post 4.0 (p=0.25) FEV1: pre 2.6; post 2.5 (p=0.13) FEV1/FVC: pre 61; post 61 (p=0.71) SpO_2: pre 97; post 98 (p=0.41) DM/V_C: pre 0.72; post 0.76 (p=0.04)* 3. Cardiovascular function: HR: pre 89; post 88 (p=0.24) Stroke volume: pre 43; post 39 (p=0.38) Control treatment: 2. Pulmonary function: FVC: pre 4.1; post 4.0 (p=0.38) FEV1: pre 2.6; post 2.7 (p=0.43) FEV1/FVC: pre 64; post 64 (p=0.59) SpO_2: pre 98; post 98 (p=0.59) DM/V_C: pre 0.68; post 0.72 (p=0.28) 3. Cardiovascular function: HR: pre 95; post 89 (p=0.02)* Stroke volume: pre 38; post 32 (p=0.16) Study II: results reported as supplementary figures, no quantitative data provided
Key findings	NIV significantly improved ventilation homogeneity and has similar effectiveness as PEP. NIV is safe in long–term application	NIV significantly improved oxygen saturation. NIV has similar effectiveness in sputum clearance as standard treatment but the study is unpowered (small number of participants)	IPV presented similar to CPT effectiveness in airway clearance, oxygen saturation, and heart rate. IPV significantly improved breathing and was better tolerated by individuals	The TV method presented similar to oscillating PEP effectiveness in promoting sputum expectoration and quality of life improvement, but the study is unpowered (small population, lack of objective airway clearance results e.g. sputum weight)	The single intervention of the VL presented similar to PEP effectiveness in sputum expectoration and ventilation parameters. The VL seems to promote diffusion whereas PEP improves cardiac function

Abbreviations used in table only: RCT – randomized controlled trial; RCS – randomized crossover study; M – male; F – female; y – years; FET – forced expiratory technique; EoC – Ease of sputum Clearance questionnaire; WoB – Work of Breathing questionnaire; SGRQ – St George Respiratory Questionnaire; SF–36 – The Short Form (36) Health Survey; PCS – physical component summary; MCS – mental component summary; RCS – role–social component summary; DM/V_C – functional unit of diffusion; HFCWO – high–frequency chest wall oscillation

trapulmonary percussive ventilation (IPV), cervical trachea vibration (TV method), and combination of sound waves with positive expiratory pressure (VL- VibraLung® acoustical percussor).[24-28]

NIV and IPV are well-known pulmonary rehabilitation methods applied in exacerbations of chronic respiratory conditions and acute pulmonary events.[26,29,30] NIV covers all non-invasive ventilation types, providing positive airway pressure that alleviates pulmonary exacerbation, reduces breathing work, and enhances tidal volumes, which is suggested effective in secretion mobilization.[29,31,32] IPV was initially applied to treat smoke-induced lung damage, but its ability to deliver a small burst of high-flow gas, imitating tidal volumes, was suggested to effectively clear airway secretions.[33-35] Besides, IPV promotes respiratory function and reduces hospitalization.[36]

Two remaining techniques employ airway oscillation mechanisms based on resonance effect. This effect promotes chest wall movements, and therefore secretions mobilization and airways clearance.[37] Cervical trachea transcutaneous stimulation (tracheal vibration- TV method) is normally used to generate voice after laryngectomy, but it was also suggested to augment airway oscillation, which reduce mucus viscosity, and therefore promote mucociliary clearance.[27,38,39] VibraLung® acoustical percussor is a device combining standard positive expiratory pressure (4-5 H_2O) with additional sound waves applied at various ranges of frequencies.[40] Its efficacy as an airway clearance technique is based on acoustic theory, suggesting a relationship between airway segment size and frequency applied to promote airway oscillation.

Manuscripts outcomes summary

The effectiveness of secretion clearance was assessed in all included publications. Three studies discussing NIV, IPV, and VL reported sputum collection: wet weight, wet and dry weight, and wet, dry, and pellet weight, respectively. One study, examining NIV-bilevel PAP, evaluated lung clearance index (LCI), indicating ventilation homogeneity.[24-26,28] The paper investigating the TV method reported expectoration difficulty recorded daily on the Visual Analogue Scale (VAS) by patients.[27] No significant difference was detected in sputum weight (wet, dry, or pellet) for NIV, IPV, and VL methods than standard treatment regimens (CPT, PEP, and oscillating PEP). NIV-bilevel PAP (positive airway pressure) significantly reduced LCI values (p=0.01), whereas expectoration difficulties after a single treatment with the TV method decreased in 4 among 12 patients.[24,27]

Pulmonary functions

Respiratory functions were measured in all included studies. Four research reported spirometry volume (FVC, VC, FEV1) and flow (MEF25, MEF75) parameters, three studies oxygen saturation (SpO_2), one study respiratory rate (RR), and one study diffusion capacity.[24-28] No significant differences were reported for spirometry results, comparing both pre-post treatment results and experimental-control treatment results. However, two sessions of NIV significantly improved oxygen saturation (p=0.004), a single intervention of IPV reduced respiratory rate (p=0.047), and a single VL session improved diffusion capacity (p=0.04).[25,26,28]

Cardiovascular functions

Among all reviewed manuscripts, two reported the impact of selected ACT on heart rate, and one of them additionally the impact on stroke volume. Obtained results were statistically insignificant.[26,28]

Exercise endurance and dyspnea

Only one publication discussed the impact of airway clearance technique on exercise endurance, measured with a 6-minute walk test (6MWT); however, no difference between the experimental and control group was recorded. The dyspnea, measured with VAS, was also reported by just one publication, and it was significantly reduced after a single session of IPV (p=0.004).[24,26]

Users impressions and quality of life

Two manuscripts included user impressions: one discussed discomfort during treatment[26] [ref], and the other treatment satisfaction, both reported as VAS score results. Only one publication discussed the impact of applied treatment on quality of life. While treatment satisfaction and quality of life were similar for experimental and standard approaches, the discomfort during the IPV session was significantly decreased (p=0.03).[25-27]

Study limitations

This systematic review has some limitations. Firstly, the number of included studies is insufficient to draw solid conclusions. However, the authors decided to include only publications with insufficient quantitative data, especially the absence of sputum/lung clearance outcomes, and exclude studies on commonly known airway clearance techniques (ACBT, AD, PEP, oscillating PEP, HFCWO). Moreover, the final decision was based on the authors' subjective opinion, hence some of the publications could have been accidentally excluded. Secondly, the methodological quality of included studies was reduced by poor blinding of subjects, therapists, and assessors. Thirdly, occurring inconsistencies in study duration, frequency of daily intervention, and measured outcomes among all studies limited the possibilities of analysis. Nevertheless, this systematic review is an insightful investigation of the state of the art ACTs, emphasizing additionally the necessity to further study designed techniques.

Conclusion

Most of the novel methods discussed in this systematic review improved secretion clearance, and therefore lung function, but neither routine treatment nor novel technique appears to be superior. Among five, three studies investigated a single physiotherapy session, which substantially limits the diagnostic approach, but at the same time shows the immediate intervention effect. Not a single study presented a significant increase in sputum expectoration; however, it has been demonstrated that long-term application of NIV-bilevel PAP improves ventilation homogeneity, considering NIV as an efficient airway clearance technique. Moreover, just a single NIV session improves oxygen saturation and provides good preparation for subsequent pulmonary rehabilitation. Similarly, a single session of IPV significantly decreases respiratory rate and dyspnea, promoting successful respiratory training. Furthermore, patients considered IPV more comfortable when compared to standard CPT. Two oscillation-based methods presented similar efficacy to widely used ACTs, with only one significant improvement of diffusion capacity increased by VL.

Several limitations need to be addressed before these methods will be considered everyday treatment. Firstly, the number of participants should be increased. Secondly, the study duration should be increased to a long-term course of the chosen method since a single session is insufficient to draw a solid conclusion. Thirdly, many outcome measures need to be carefully revised to provide no bias information.

Although the number of limitations occurs, the presented methods' effectiveness is considered similar to well-established airway clearance techniques, suggesting the possibility to include them as a routine treatment after in-depth investigation.

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REVIEW PAPER

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Natural properties of lycopene and its application in medicine

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ABSTRACT

Introduction. The subject of the article is a chemical compound belonging to the carotenoid family, i.e. lycopene.

Aim. The aim of this study was to describe interest among scientists regarding lycopene due to its unique properties, general availability and potential for wide application in medicine.

Material and methods. This article is a review in which the properties of lycopene, its chemical structure and sources (especially in the form of tomato fruits and its products), as well as its important role and importance in medicine, is presented.

Analysis of the literature. Lycopene is an interesting compound with interesting properties. It may prove to be an important and readily available means of preventing and fighting cancer (especially prostate cancer, uterine cancer and breast cancer). Additionally, lycopene can counteract cardiovascular diseases that are common nowadays.

Conclusion. The data indicates increased number of papers regarding applications of lycopene in medicine.

Keywords. carotenoid, lycopene, medicine

Introduction

Lycopene is a chemical compound from the carotene group, belonging to the carotenoid family. Due to its belonging to this family and its characteristic intense red color, it is often used as a dye in food products under the name E160d. It is not synthesized by the human body, therefore it must be taken with food.

Due to the fact that lycopene is a natural dye found in plants, it is found in fruits and vegetables that are available and eagerly eaten every day, especially those with a red color. We can find it in vegetables and fruits such as pepper, watermelon, pink grapefruit, peach, papaya tree, guava, strawberry, papaya, or rosehip.[1,2] However, tomatoes are a particularly rich source of lycopene. Its content changes during the ripening of tomato fruits and depends on the growing conditions and the microclimate (mainly temperature and sunlight). In addition, sun-ripened tomatoes are the richest in lycopene, not greenhouse tomatoes or tomatoes.[1-3] According to Clinton, the content of lycopene, depending on the variety and ripeness of tomatoes, ranges from 0.9 to 4.2 mg/100g.[4] On the other hand, McClain and Baush report that in yellow tomatoes lycopene is about 0.5 mg/100g, and in intensely red tomatoes it can be even ten times more - 5 mg/100g.[3-5]

In addition to the (total) absolute lycopene content of the raw or processed raw material, its bioavailability

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

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is also important. Lycopene is found mainly under the skin of tomatoes, and the process of grinding and cooking them causes the release of lycopene. Moreover, under the influence of temperature, lycopene in tomatoes or its products is transformed into the form of trans-lycopene, where it is more efficiently absorbed from the gastrointestinal tract, which is related to its better solubility in fats in this form.[6]

Aim

The aim of this study was to presents the natural properties of lycopene and its application in medicine

Material and methods

Literature search was done to identify appropriate methodology and design of the study. The search of literature was helpful to mark the map of interest.

Analysis of the literature

The processing of tomatoes generates large amounts of by-products (mainly seeds and skins), which until recently were only additives for livestock feed. The by-products of tomato processing, however, turned out to be an important source of lycopene. Lycopene obtained from the remains of tomatoes is used as a supplement in the production of functional food.[7]

Synthetic lycopene is also available. It is a mixture of geometric isomers of lycopene (double bonds of trans configuration) and is obtained by condensation of synthetic intermediates, commonly used in the production of other carotenoids used in food. Due to the inability to obtain crystalline lycopene in an aqueous solution and its high susceptibility to the negative effects of light and oxygen, it is not suitable for industrial purposes. Only properly transformed material is placed on the market and intended for consumption. Additionally, attempts are still being made to produce lycopene with the participation of Mycobacterium aurum bacteria, which are classified as non-pathogenic microorganisms *Blakeslea trispora*.[8]

Lycopene is an unsaturated hydrocarbon containing forty carbon atoms in the molecule of the formula C40H56. It owes its intense red color to the chromophore system, which strongly absorbs radiation in the visible light range with a wavelength of λ = 444, 470 and 502 nm.[9]

This compound, as an unsaturated polyene hydrocarbon, is composed of eight isoprene residues (having five carbon atoms). In total, the isprene residues form a carbon chain of forty carbon atoms containing two unconjugated and eleven conjugated double bonds. This makes seventy-two geometric isomers of lycopene possible. Both lycopene ion rings are open, so this compound does not have the properties of β-carotene (provitamin A).

Lycopene is the main carotenoid, which (unlike β-carotene), when absorbed in the intestine, is not converted to retinol, nor is it (unlike other carotenoids) a substrate for cyclin-dependent dioxygenase.[10]

The double bonds in lycopene can be isomerized from the all-trans position to the mono- or poly-cis isomers by exposure to light, heating, or chemical reactions. The presence of many conjugated double bonds makes lycopene distinguishable among carotenoids with the strongest antioxidant properties and participates in the creation of an antioxidant barrier in living organisms. It neutralizes free radicals more effectively than β-carotene, α-tocopherol or lutein. Additionally, it has been shown that lycopene not only neutralizes free radicals, but also has the ability to regenerate other antioxidants.[11]

Lycopene tends to accumulate in cell membranes, which increases their fluidity and permeability, which indirectly modifies the antioxidant response pathways in the cell. Moreover, lycopene, being an effective scavenger of free radicals, protects lipids of cell membranes against their oxidation and degradation (i.e. lipid peroxidation).

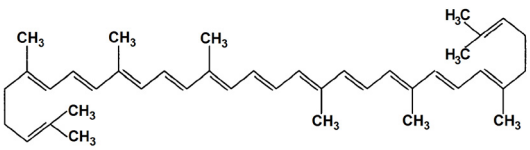

Fig. 1. Lycopene structure

The structure of lycopene carries properties that are used in many areas, including in cosmetics (cosmetics for skin and hair care), food production (improving the color of dishes), or in medicine (where the properties and applications will be described in more detail). Scientists emphasize the antioxidant properties of lycopene in particular. Antioxidation consists in neutralizing the action of harmful compounds formed during all metabolic processes in the body. These compounds are called free radicals.[12]

Free radicals are groups of atoms or molecules containing one or more unpaired electrons on the last shell, which makes them unstable and very reactive.[13] In addition, they are dangerous for the body because they cause an avalanche of new radicals which, striving for a stable electronic system, react with various cells and damage their structures, thus disrupting their functions. As a consequence, it leads to changes in the genetic material and the occurrence of pathological conditions.

Lycopene belongs to the group of carotenoids, which are characterized by strong antioxidant properties, however, the antioxidant activity of lycopene is three times higher compared to other carotenoids. Due to the presence of conjugated double bonds, lycopene reacts extremely efficiently with atomic oxygen and re-

active oxygen species. The active forms of oxygen include both free radicals, e.g. superoxide anion ($O_2^{\bullet-}$), hydroperoxide radical ($HO_2^{\bullet}$), hydroxyl radical ($OH^{\bullet}$) and singlet oxygen ($^1O_2$), ozone (O_3), and hydrogen peroxide (H_2O_2). Many conjugated double bonds in the lycopene molecule make it participate in the creation of the antioxidant barrier of the human body and show a protective effect against the genetic material.[16]

Lycopene, as an exceptionally effective free radical scavenger, also plays a particularly important role in the prevention and prevention of civilization diseases, including reducing the risk of cardiovascular diseases. The etiology of most cardiovascular diseases is primarily associated with the process of atherosclerotic changes. Its essence is the deposition of atherosclerotic plaque on the walls of arteries, which leads to narrowing of blood vessels and many diseases. Plaque formation is a complex and lengthy process in which low-density lipoproteins (LDL) play an important role. They penetrate into the arterial endothelium and undergo gradual oxidation Frequent consumption of lycopene, and thus a higher concentration of this compound in the blood, prevents the oxidation of the LDL cholesterol fraction and lowers its overall level, thus reducing the risk of developing cardiovascular diseases.

The antioxidant properties of lycopene also contribute to reducing the risk of senile cataracts, autoimmune and neurodegenerative diseases, infertility, rheumatoid arthritis and carcinogenesis, which is associated with the anti-cancer and anti-inflammatory properties of lycopene.[18-20]

Reducing the frequency of carcinogenesis by using the addition of lycopene (scavenger of free radicals, reactive oxygen species) results from the reduction of the size of the formation efficiency of cancer-causing substances and the inhibition of unnatural cell division in the body of humans and animals.[21]

In developed countries, cancer is now becoming the leading cause of premature death. Therefore, methods of preventive disease prevention are still being sought. Based on epidemiological studies, food producers are increasingly using raw materials of plant origin containing substances that prevent the formation of cancer. One of the substances of plant origin with this effect is lycopene.[22]

Prostate cancer is the second most common cancer diagnosed in men worldwide and is the sixth most common cause of cancer death. Risk factors that influence the development of prostate cancer include age, race, genetics, obesity, and diet. Perhaps an additional factor leading to the development of this disease is a mutation in the genes that control cell differentiation and growth. Moreover, the chronic inflammatory process in bacterial prostatitis can lead to the formation of oxygen free radicals, which results in DNA damage and mutation formation, as is the case with exogenous mutagenic factors. Studies conducted on men indicate a relationship between the content of lycopene in the serum and the incidence of prostate cancer. Those of the respondents whose consumption of lycopene was at the level of about 33 mg/day showed half the risk of developing prostate cancer compared to the group that consumed much less of it, within 13 mg/day. The results of clinical trials also suggest that supplementation with lycopene 15-30 mg/day reduces the incidence of benign prostatic hyperplasia and prostate cancer.[23] Lycopene may inhibit the growth of lung and kidney cancer cells.[24]

A similar relationship can be observed in studies on the incidence of breast cancer, ovarian cancer and endometrial cancer in women. Women with the highest serum lycopene levels (0.59-1.58 g/dL) had an 85% reduced risk of developing endometrial cancer compared with patients with serum lycopene levels 0.36-0.51 g/dL. In addition, lycopene used during radiotherapy of women with breast cancer has a protective effect and reduces the side effects of irradiation within the irradiated skin.

Lycopene, being an effective scavenger of free radicals, also inhibits the aging process of the skin, increases resistance to solar radiation, which is manifested by less reddening of the skin during sunbathing. Lycopene added to the diet protects human skin against UVA and UVB rays (i.e. rays that generate free radicals). Therefore, it reduces the frequency of skin melanoma.[25]

The health-promoting properties of lycopene can be used both in the prevention and treatment of cardiovascular diseases.[26] The blood LDL-lowering properties of lycopene have been shown to inhibit the atherosclerotic process in the arteries.

Supplementation with 60 mg of lycopene daily for 3 months leads to a reduction in LDL levels by 14% In addition, lycopene helps maintain normal blood pressure values, and its effect is greater in the group of subjects with systolic blood pressure values above 140 mm Hg Additionally, consuming lycopene above 12 mg/day lowers systolic blood pressure by an average of 4.95 mm Hg which partially prevents the progression of arterial hypertension.[27,28]

Conclusion

Lycopene is an interesting compound with interesting properties. It may prove to be an important and readily available means of preventing and fighting cancer (especially prostate cancer, uterine cancer and breast cancer). Additionally, lycopene can counteract cardiovascular diseases that are common nowadays. Increased interest in lycopene and its properties in use may contribute to the improvement of the quality of life of people in society in the field of civilization diseases, and further exploration of knowledge and research on it may be beneficial for the field of medicine.

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REVIEW PAPER

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Naturally occurring photosensitizers and photodynamic therapy: laser or sun?

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ABSTRACT

Introduction. Our understanding of photodynamic therapy (PDT) is crucial for the applications of this treatment. In this physical phenomenon occurs light absorption by the applied photosensitizer, which results in its excitation to higher electron levels. After activation, a series of complex physicochemical processes take place in the tissues,

Aim. The aim of this study was to describe the basic naturally occurring photosensitizers used in PDT techniques. This study clarified applications of photosensitizers.

Material and methods. This study was used basic information about PDT reaction and the selective destruction of the tumor by photooxidation: a photosensitizer, a light source and oxygen. The papers reviewed here are based on Medline/Pub.

Analysis of the literature. Photodynamic therapy is an innovative form of treatment. This method is not a commonly used therapeutic and therapeutic tool, but a supplement to many already tested and analyzed techniques. Thanks to continuous research, this method has a wider and wider range of applications in medicine.

Conclusion. The data indicates increased number of papers regarding applications of PDT in medicine.

Keywords. diagnosis, oxygen, photodynamic therapy

Introduction

Photodynamic therapy is a therapeutic tool used in the treatment of diseases (bacterial, viral and fungal) and neoplasms in the field of dermatology, urology, gynecology and dentistry.[1] It can also be used as a supplement to aesthetic medicine treatments. The foreground components are photosensitizers and light - as the central element of this method. The idea of using light (a commonly available and life-giving element) as a healing tool was born in antiquity.[2] The Egyptians, worshiping the sun god (Ra), believed that all prosperity, prosperity and health come from the sun. phototherapy (aka heliotherapy) to restore health. Most often, this form of treatment was used in chronic skin diseases.[3-5] Phototherapy at that time was characterized by great interest and widespread use. With the abandonment of ancient beliefs and the fall of the Western Roman Empire, this therapy ceased to be practiced. It was returned to it in the 1830s. A significant increase in research and published articles took place only in the 20th century. Pho-

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

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todynamic therapy is a synthesis of photochemical and photophysical processes occurring in the tissue after light exposure and after the introduction of a photosensitizer.[6-7] In this therapy, several most important stages can be distinguished.8 The scheme is presented below (Figure 1).

Photodynamic therapy is a targeted method of destroying cancer cells. Contrary to chemotherapy, it "spares" healthy cells adjacent to diseased cells.[8-10] The selection of an appropriate photosensitizer and light of a specific and appropriate wavelength is extremely important in the general procedure of therapy. There is no single, strictly defined procedure for carrying out the procedure. The entire procedure depends primarily on the type of lesion and the available photosensitizer resources.

Aim

The paper presents the classification of photosensitizers, examples and characteristics of natural photosensitizers and a description of photodynamic therapy.

Material and methods

The literature review was prepared on the basis of the databases contained in the International Medical Library PubMED, the specialized search engine Google Scholar, and on the basis of the ScienceDirect scientific literature platform. Searches were conducted for "photodynamic therapy", "natural photosensitizers", "hypericin", "hypericin photodynamic", "curcumin photodynamic therapy" and "hypocrellin photodynamic therapy". In the PubMED library alone, over 30,000 publications appeared under the slogan "photodynamic therapy". The articles for the review were used from all three sources and selected on the basis of abstract analysis and the availability of the entire material/publication. The publications concern current research of scientists and research groups in the field of photodynamic therapy. Systematic reviews describing the breakthrough historical discoveries of this form of treatment and the characteristics of natural photosensitizers found in nature were also used.

Analysis of the literature

Based on the literature review, it can be concluded that photodynamic therapy is highly effective in lesions and in the initial stages of skin cancer.[11] It is a non-invasive form of treatment, the so-called a non-surgical tool that uses light and photosensitizers.[12] Photosensitizers accumulate in disease cells, in places of the tumor, and are then irradiated with laser light or visible light - followed by absorption.[13,14] singlet (1S0) to the excited singlet state (1S1). Much of the energy is lost through direct photon emission in the excited state. The so-called the phenomenon of fluorescence. A small part of the energy is used in the process of transition to the triplet excited state (3T1). The active form of the photosensitizer in the triplet state, reacting with oxygen, causes the formation of active oxygen species, i.e. singlet oxygen. These molecules cause apoptosis - programmed cell death.[14-20] Interpretations of the entire procedure can be reduced (simplified) to the statement that photodynamic therapy is the conversion of light energy into toxic, reactive oxygen species.[21]

The appropriate selection of the photosensitizer is extremely important, because it affects the effectiveness and efficiency of the treatment therapy.6 Photosensitizers are characterized by such properties as: minimal harmfulness/toxicity to the patient's body, speed of removal (reconstitution in the tissue) and the number of potential side effects.[22] Currently there are known three generations of photosensitizers (Table 1). These photosensitizers are widely available on the pharmaceutical market and intended for specific clinical applications.

Over the years, research groups around the world have started the process of incorporating natural photo-

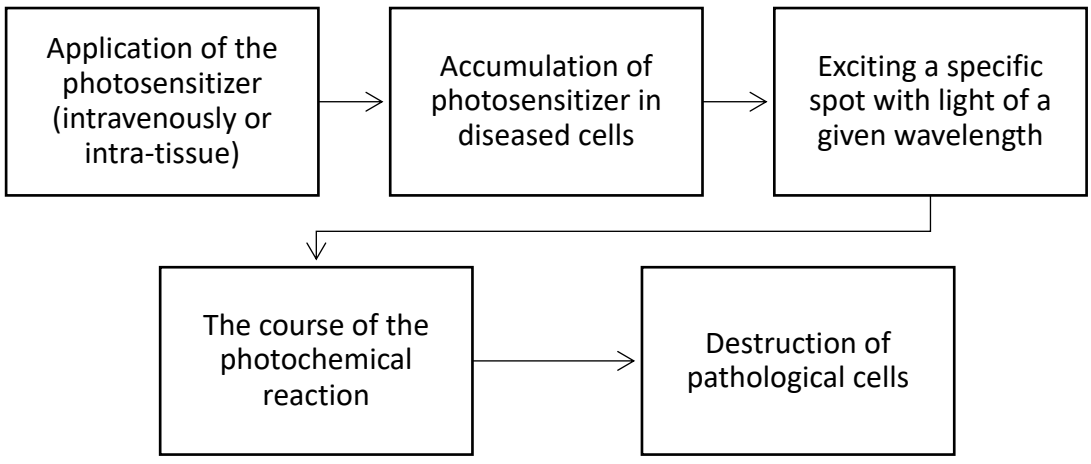

Fig. 1. The most important stages of photodynamic therapy

Table. 1 Generations of photosensitizers

First generation photosensitizers	Second generation photosensitizers	Third generation photosensitizers
Hematoporphyrin derivatives (HpD, Photofrin)	5-aminolevulinic acid and its esters, a benzoporphyrin derivative, lutetium texaphyrin, temoporfin, tin ethiopururine and taloporfin sodium	Combination of photosensitizers with monoclonal antibodies
Application: urology (bladder cancer); pulmonology; cancers of the head, neck and esophagus	Application: incl. in surface lesions within the oral cavity	Purpose: non-neoplastic vascular changes

Table. 2 Characteristics of natural photosensitizers

Name feature	Hypericin	Hypocrelin	Curcumin
Source	St. John's wort	Extracted from mushrooms Hypocrella bambusae	*Curcuma longa L*
Absorbed wavelength	590	600-900	405-435
Application	Tumors of the bladder, ovary, breast, colon, esophagus, psoriasis	Lung cancer, skin diseases	Neoplasms of the breast, cervix, stomach, diseases of the surface vessels and respiratory tract

sensitizers (common in nature) into the photodynamic therapy procedure (Table 2). Such an example is hypericin. Hypericin is a hydrophobic molecule that requires a special carrier. [23,24] It is obtained from a plant belonging to the St. John's wort family, called St. John's wort. This plant belongs to the group of skin medicinal plants.[25] As a photosensitizer, hypericin has excellent properties, relatively low toxicity and antiviral activity.[26,27] It absorbs wavelengths (590nm). It has been confirmed to be important in the treatment of conditions such as cutaneous T-cell lymphoma, psoriasis, bladder and ovarian cancer.[28] Based on literature data, it is known that hypericin inhibits the growth of ATL cells, i.e. adult T-cell leukemia, leading to apoptosis of viral transcription. Moreover, according to the latest research, hypericin can control tumor growth and affect the amount of interleukins in colon cancer cells.[28] It exhibits a cytotoxic effect in esophageal, colorectal, bladder and breast cancer cell lines.[29] It has also been confirmed that this photosensitizer does not exhibit acute toxicity. in the absence of light. [30] Thanks to numerous studies, hypericin is gaining more and more importance in photodynamic therapy, improving the procedure and increasing the effectiveness of this method.

Another type of natural photosensitizer is hypocrelin. It is widely used in the treatment of skin diseases. It is characterized by high efficiency, increased metabolism and low toxicity in the absence of light.[31] It exhibits effective antiviral antimicrobial activity, while antifungal activity is analyzed in research centers and discussed.[32] Absorbs light in the wavelength range from 600-900nm, which makes its use limited. Hypocrelin is also being analyzed in terms of its effectiveness in the treatment of keloid fibroblasts by mitochondrial apoptosis and analyzed for antitumor efficacy, e.g. in the lungs.[33,34] In the last 4 years, research centers have emerged to develop and use other hypokrelin derivatives in photodynamic therapy, aimed at obtaining a photosensitizer with new, unusual properties.

Curcumin, obtained from the Ostryża long root, is also a natural photosensitizer. It exhibits antiviral and antitumor activity.[35] It absorbs light in the wavelength range 405-435nm. In recent years, the use of curcumin in the treatment of oral infections has been analyzed, thus confirming its antifungal and antibacterial activity.[35] Curcumin also has some limitations. It exhibits low solubility, which requires its use in the form of nanometer-sized emulsions.[36] Studies have shown high activity in the treatment of breast, cervical and stomach cancers (including its non-cancerous forms) in surface vessel diseases and in lower respiratory tract infections.[36-40] The table 2 below presents the most important information about the three most popular natural photosensitizers.

The selection of an appropriate photosensitizer alone does not determine the success and effectiveness of treatment. It is also important to choose the right laser. The basic criteria are the maximum and the wavelength range of the photosensitizer used. The depth of radiation penetration depends not only on the wavelength, but also on the type of tissue being treated. The lack of a standard research protocol in therapy opens up opportunities and flexible development paths for research teams working to obtain the best results and the highest treatment effectiveness.[41]

To sum up: the origin of the idea of photodynamic therapy is due to the observations and discoveries of the healing properties of the Sun - the central star of the Solar System. Currently, it is known that ultraviolet (UV) radiation, the source of which is the Sun, in addition to its positive effects, can also cause a number of dangerous skin changes, leading to the development of cancer cells. It is important to mine, the so-called The "golden

mean" and skilful use of the properties of solar radiation. Although the effectiveness of treating any skin lesions with laser photodynamic therapy is very high, you should still be aware of the negative effects and avoid excessive exposure to solar radiation.

Conclusions

Photodynamic therapy is an innovative form of treatment. Although it was pioneered by the Egyptians in ancient times, a significant development of research took place in the twentieth century. This method is not a commonly used therapeutic and therapeutic tool, but a supplement to many already tested and analyzed techniques. Thanks to continuous research, this method has a wider and wider range of applications in medicine. The wide list of advantages proves that photodynamic therapy can become an increasingly used tool in the field of aesthetic medicine and in the treatment of selected neoplasms.

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CASUISTIC PAPER

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Lian Gong for treatment of fibromyalgia – a case study

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ABSTRACT

Introduction. As a Complementary Integrative Practices (CIPs), Lian Gong has been increasingly used in the practice of Physiotherapy.

Aim. This study aims to verify the effects of Lian Gong in a patient with fibromyalgia.

Description of the case. In this case study the patient (one patient is evaluated) was diagnosed with fibromyalgia 29 years ago and sought care following discontent with previous treatments. Pain intensity was assessed with a visual analog scale (VAS), quality of life with SF-36, and the pressure pain threshold algometer (PPT). There were 16 visits with two weekly 60-minute sessions. When the patient was reevaluated, a Global Perception of Change (GPC) scale was added to assess general health. A folder was submitted to the patient for follow-up of exercises at home and asked to return after 4 weeks (follow-up period).

Conclusion. The results indicate improvement in pain, functional capacity and general health. As a first therapy treatment, Lian Gong proved promising results in one case of fibromyalgia. The possible benefits when combined with other forms of care should be explored by clinical trials to expand knowledge of health benefit potential.

Keywords. fibromyalgia, modalities of physiotherapy, Qigong, quality of life, rehabilitation, traditional chinese medicine

Introduction

Fibromyalgia is a chronic, rheumatic disease of undefined cause, likely to occur due to abnormalities in neuroendocrine regulation and as a response to stress, and has a higher prevalence in women between 40 and 60 years of age.[1] Despite the rheumatology approach, there are common neuropathic symptoms (paresthesia, numbness, and thermal sensitivity). Its incidence is 2-4% in the general population, with diffuse muscle pain as its main characteristic.[2] Genetic pre-disposition, emotional and cognitive factors, body-mind relationship, and ability to cope with stressful situations are all characteristics that contribute to the onset of fibromyalgia, but its etiology remains uncertain.[3]

Besides the above, there are other symptoms such as fatigue, sleep disorders, depression, changes such as hyperalgia, allodynia, morning stiffness, and headache (which can be exacerbated by hormones), physical and mental stress, temperature changes, changes in diet and sleep window.[1] The diagnosis is based on a set of symp-

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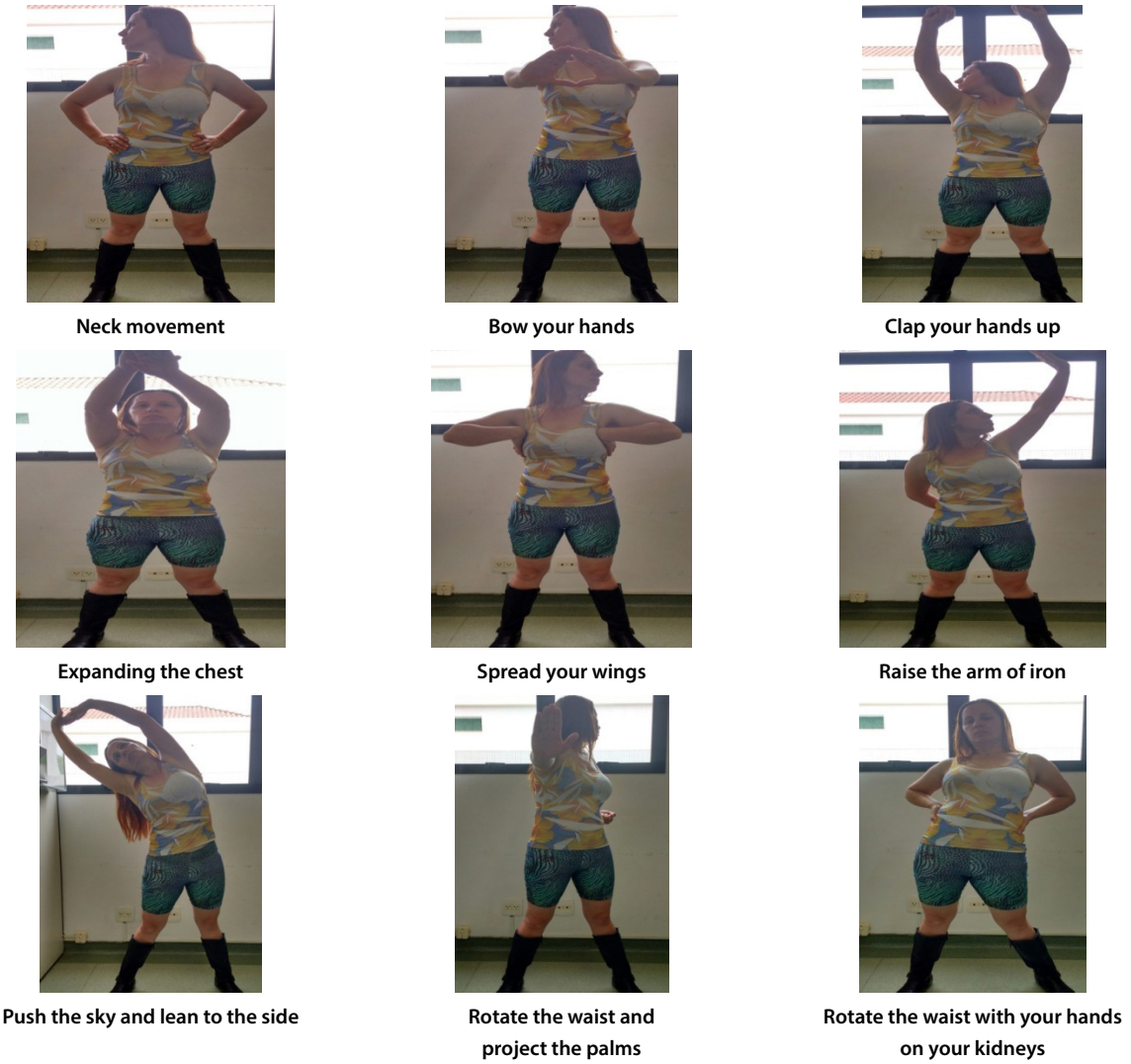

Fig. 1. Booklet with representation of the exercises to be performed by the participant

toms that need to be present for at least three months, with pain in both hemicorps, tender points in eleven (or more) specific areas, generalized fatigue, depression and anxiety.[3]

As there is no cure for fibromyalgia, the treatment is based on symptom control and improves quality of life with drug management and non-pharmacological therapies. Physical exercise is part of the forms of treatment indicated in the multidisciplinary approach, as well as low power laser, balneotherapy and cognitive--behavioral therapy. Besides the usual practices of aerobic activity, strengthening and stretching, alternative and complementary therapies (Acupuncture, Massage, Meditation, Tai Chi Chuan, Shiatsu, among others) are applied in the management of fibromyalgia. A recent literature review on the use of such therapies in fibromyalgia has pointed to promising results, however, with the exception that these effects were based on small studies, but given the low risk, they could be considered an adjunct form of treatment. Although Lian Gong was briefly mentioned, there were no major considerations on the subject.[1] In recent years, there has been considerable growth in the use of resources beyond conventional Western medicine in rheumatic patients, yet scientific evidence of these practices remains under investigation. Lian Gong is considered one of these mind--body interventions that are part of the list of alternative treatments possibilities, consisting of gentle movements, breathing exercises, and meditation, producing gains in muscular power and endurance, flexibility, balance, and affecting psychological factors such as distress and anxiety.[1,5–8]

Lian Gong is of Chinese origin, has a long history (more than five thousand Years), and is practiced by more than 60 million people in China. Lian Gong can be defined as the ability to release, strengthen, and direct Qi "life energy" through specific exercises, harmonizing breathing, posture, body movements, and mind. Qi flows through meridians and its balance determines good overall health according to Traditional Chinese

Fig. 1A. Booklet with representation of the exercises to be performed by the participant

Medicine (TCM).[8–10] With this line of reasoning, the technique would be in a position to accelerate the self-healing process[6]. They are simple, smooth movements, with a choreographed, rhythmic routine, with respiratory control and can be adapted depending on the needs of the practitioner.[6,7] Although its effectiveness continues to be investigated, it is aimed at improving physical and mental well-being and quality of life.[8]

In Brazil, one of the strategies incorporated for health promotion by the National Policy of Integrative and Complementary Practices (NPICP), better known as "CIPs", published in the form of Ministerial Ordinances No. 971 on May 3, 2006 and No. 1,600 on July 17, 2006.[9] Some Brazilian cities have implemented, within their health policies, the use of Lian Gong for the population.[10,11]

While the effects of Lian gong in the West are still being explored, this resource is indicated in cases of fibromyalgia.[8]

Aim

The objective of this study was to present the effects of the technique, as a single therapy, in a patient with fibromyalgia that had been ongoing for decades.

Description of the case

The present study is characterized as a case study, being previously approved by the Research Ethics Committee of Centro Universitário Lusíada – UNILUS under number 2,243,010. The patient chosen was on the waiting list of the UNILUS Physiotherapy Clinic, with a diagnosis of fibromyalgia.

The patient E.M.L, 46 years, with 1.63 cm height and 67 kg, had been diagnosed at 17 years of age, and had performed physiotherapeutic treatment (infrared, TENS and hydrotherapy) and medications (muscle relaxant and antidepressant) before, but remained dissatisfied with the results.

Before the evaluations began, the procedures were explained to the patient and the informed consent form

Fig. 1B. Booklet with representation of the exercises to be performed by the participant

was signed. We applied the visual analog pain scale (VAS - ranging from 0 (no pain) to 10 (maximum pain possible), SF-36 quality of life questionnaire (0 - 100, which evaluates functional capacity, physical aspects, pain, general health status, vitality, social, emotional and mental health) and the pressure pain threshold (PPT) (Tester® - Kgf) on the 18 points related to the tendon points of individuals with fibromyalgia (adding the maximum pressure supported at all points, to generate a single final value).[5,9,16]

We also analyzed which Lian Gong movements the patient would be able to perform correctly or need only minimal adjustments, and then the treatment plan was constituted. After the second meeting, the sessions lasted 60 minutes, performing exercises that included the axial and appendicular skeleton, respecting the painful limit. It is therefore recommended that the session be made of six exercises in three series, totaling 18 exercises. For the evaluation of the exercises applicable to the patient in question, each movement was harmoniously performed three times, with respiratory control and following the work sequence of the "anterior and posterior part" of Lian gong's manual, totaling about 20 exercises per session.[11] The treatment was performed for eight weeks, twice a week, reaching 16 sessions.

In the reevaluation, in addition to the items mentioned above, the Global Perception of Change (GPC) scale was added (ranging from -5 to + 5, positive values indicate greater satisfaction), helping to analyze how efficient the treatment was.[13] After the reevaluation and the end of the clinic visits, the patient received a booklet with photos (figure 1) showing how to perform the exercises, which she was already familiar with, so that she could continue at home and return for a new reevaluation after four weeks (follow-up). She was also guided to access the following links in case of doubt in the execution: https://www.youtube.com/watch?v=F-gQkYSAmJ6o https://www.youtube.com/watch?v=rb-XOUiVFLDo.

The other instrument used for general pain assessment, VAS, showed a significant pain reduction effect, occurring 50.9% and 58.4% of reduction at completion and follow-up after treatment, respectively. The SF-36 questionnaire recorded increases (Functional Capacity and General Health Status), stabilization (Physical, Social, Emotional and Mental Health Aspects) and decrease (Vitality) of its values (Table 1).

Table 1. Quality of life assessment through SF-36. Higher scores indicate improved quality of life

	Begin	End	Follow-up
Functional capacity	80	80	90
Physical aspects	100	100	100
Improvement of pain	51	51	51
General health status	30	47	57
Vitality	95	90	90
Social aspects	100	100	100
Emotional aspects	100	100	100
Mental health	84	92	84

The evaluation of the pain pressure threshold recorded a progressive increase in the capacity to withstand a pressure of 21.6% at the end of clinic visits and a total of 23.1% when returning for follow-up evaluation. The improvement in pain accompanied by VAS and PPT, probably participation in the positive response, was noted and maintained at the end and the return to the last evaluation of the study, by grading its improvement by +3 on the GPC scale (Table 2).

Table 2. Parameters linked to pain and general improvement*

	Begin	End	Follow-up
VAS	5.3	2.6	2.2
PPT	1315	1600	1620
GPC	*	+3	+3

*VAS – visual analogue scale, PPT – pressure pain threshold, GPC – global pain change

Discussion

Use of Lian Gong has proven useful as an add on therapy in the treatment of fibromyalgia in the case studied. These findings corroborate those found in other studies, which describe positive results with Lian Gong among the possibilities of alternative and complementary therapies when treating fibromyalgia.[14] The fact that it does not prescribe any specific device or tool for its performance, makes its practice valid in any environment and time. Through TCM, Lian Gong would be able to elevate the body's physical energy by promoting the fluidity of Qi and blood circulation, as showed by the increased electrical conductivity of meridians. It has already proven effective in preventing bone mineral loss, reducing oxidative stress, increasing antioxidant enzymes, homeostasis of the autonomic nervous system, activation of immune system cells, such as in reducing pain, improving balance, flexibility, agility, strength, fatigue, quality of life and sleep.[4,5]

This potentially justifies the improvements in overall pain, functional capacity, and general health status of the SF-36. Generalized chronic pain is the main complaint of fibromyalgia patients and is related to decreased quality of life. This association has previously been demonstrated in the comparison between the Fibromyalgia Impact Questionnaire (FIQ) and the VAS.[15] Despite the short intervention period (8 weeks) and follow-up (4 weeks), these were already sufficient for pain to be minimized at half of the initial assessment, corroborated by improvement in the other instruments (PPT and GPC). Since with the severity of the pain, there is a reduction in functional capacity. The painful improvement is assumed to have contributed to the increase in Functional Capacity in the SF-36.

The SF-36 was lower than the initial value, only in the item Vitality, there was improvement in pain measured both by the VAS and the pressure gauge and satisfaction with the proposed treatment via the Global Perception of Change scale. This way, it is believed that this variable did not present much of a change because the data from the other domains initially already presented high values. Since diffuse pain is usually the main complaint of these patients and although it has not been altered via the SF-36, the benefit achieved by other assessment instruments (VAS, PPT and GPC) stands out. This improvement in pain complaint, added to the increase in Functional Capacity and the General Health Status, generates a promising idea of the effects of Lian Gong on fibromyalgia.

Commonly used in association with VAS, the pressure algometer in the comparison of individuals with fibromyalgia to rheumatoid arthritis, dyspareunia and controls, shows lower pressure tolerance values, i.e., they feel more pain with smaller stimuli.[2] The characterization of fibromyalgia is generalized pain in 4 out of 5 regions (4 quadrants and axial).[20] The increase in pain pressure threshold with the practice of Lian Gong generates optimism and the need for studies with a larger number of participants and time, to determine how much improvement is expected to the longevity of a mind-body exercise routine.

GPC is easily and quickly applied, described as clinically relevant in cases of fibromyalgia when related to the patient's general clinical picture and previously used with EVA and body map (18 common tender point sites). The higher the values obtained through the GPC, the better the pain (via VAS and painful points) and all other measures of fibromyalgia severity.[13] What was found was in fact improvement of the painful condition (VAS and PPT) and the GPC.

Since fibromyalgia is not only musculoskeletal issue, there is psychoemotional involvement, Lian Gong fits the demand to be a mind-body therapy.[4] Mental Health of the SF-36 showed this effect during the face-to--face period of the intervention, but it returned to initial values during the four weeks of follow-up. Therefore, the result may have been the engagement of the professional during the sessions and not restricted to the technique. It is noteworthy that there are indications for the exercise to be performed regularly and even daily, in the present study the indicated practice was regular, but with 2 sessions per week.[8]

Since fibromyalgia is not a curable disease, therapies that help control symptoms and improve quality of life are the focus for these patients.[1] The benefits gained from the practice of Lian gong, its adaptability, and low cost, have caused this alternative to spread and conquer followers in Brazil.[10] CIPs such as Lian Gong, act in the promotion and integrality of health, surpassing the rooted curative model as the assertive one. It is common for Lian Gong to be practiced in groups, providing socialization, strengthening of the professional-patient relationship, and amplification of the individual's notion of his or her general state of health through contact with others in similar conditions. The use of Lian Gong in cases of fibromyalgia strengthens the understanding of the need for active participation in the care process adding to medical approaches. Although the publication of the National Policy of Integrative and Complementary Practices in SUS was made in 2006 and Lian Gong is not a new therapy, both the training of professionals focused on these practices and the scientific basis require investment.

The methodology used for monitoring the case reported here, while effective in reflecting symptomatic changes, could have been more complete if it had used specific instruments that evaluated sleep, fatigue, anguish, anxiety, and depression, which are to the painful complaint and the SF-36 items are to be addressed.[1,4,5] In addition to this limitation, it should be taken into consideration that this is a case study and cannot extrapolate the findings to all patients with fibromyalgia. Clinical trials will be more reliable in this response and it is worth noting the need for a control group to evaluate the real effects of this therapy, both its evaluation as an isolated form of treatment, as proposed here, although it is commonly described as adjunct. Progress has been made in proposing a placebo form of Lian gong.[4]

Conclusion

Lian Gong stands out for its adaptability, easy application, low demand for the necessary structure, low cost, and the main one, promising results through fibromyalgia. With the organization of health systems to adhere to self-applicable or simple inclusive care, Lian Gong seems to successfully meet the purpose of CIPs.

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CASUISTIC PAPER

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Persistent hyperthyroidism in a patient after total thyroidectomy: the thyroid anatomy has implications for treatment

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ABSTRACT

Introduction. Grave's disease (GD) can be treated using three modalities: anti-thyroid medications, radioactive iodine therapy (RAI), or surgery. If surgery is selected, total thyroidectomy is the procedure of choice. Patients with hyperthyroidism frequently have an enlarged thyroid gland, occasionally with a pyramidal lobe.

Aim. We point the usefulness of thyroid scintigraphy, which provides valuable information regarding the thyroid anatomy.

Description of the case. The manuscript presents a case report of 43-year-old woman with unstable Grave's disease, who underwent thyroidectomy and developed persistent hyperthyroidism postoperatively. She was referred by an endocrinologist to a nuclear medicine outpatient clinic for RAI therapy. I-iodide scintigraphy revealed two foci with excessive tracer accumulation. One of the foci in the middle of the neck corresponded to the pyramidal lobe.

Conclusion. The thyroid anatomy anomalies can lead to unnecessary implications for treatment. Identifying the pyramidal lobe preoperatively and removing it from patients requiring total thyroidectomy may decrease the recurrence rate of hyperthyroidism. Thyroid scintigraphy is a useful diagnostic tool to visualize the pyramidal lobe.

Keywords. Grave's disease, pyramidal lobe, scintigraphy, thyroidectomy

Introduction

Grave's disease is a kind of hyperthyroidism in which thyroid hyperplasia and toxicosis occur in response to high levels of antibodies to the TSH receptors. GD can be treated using three modalities: anti-thyroid medications, RAI, or surgery. The choice of treatment for GD requires presenting all short- and long-term side effects of the treatment options to the patient.

RAI therapy or total thyroidectomy are options for those resistant to medication or suffer from relapse of symptoms. Approximately one in four patients with GD may undergo total thyroidectomy. Patients with hyperthyroidism frequently have an enlarged thyroid gland, occasionally with a pyramidal lobe.[1] On the other hand detection of pyramidal lobe in patients with hyperthyroidism very often indicates an autoimmune reason of thyrotoxicosis. The pyramidal lobe (PL) is a remnant of the thyroglossal duct that extends superiorly from the isthmus and can reach the level of the hyoid bone. It is more common in men than in women, most commonly

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develops left of the midline, and has multiple anatomical variations. Based on anatomical studies, the frequency of pyramidal lobe development is between 15% and 75%.[2,3] In a study conducted by Wahl et al., pyramidal lobe was observed to have left lobe origin at 53%, right lobe origin at 39% and isthmus at 8%.[4] We report a patient who underwent total thyroidectomy and developed persistent hyperthyroidism postoperatively.

Aim

We point the usefulness of thyroid scintigraphy, which provides valuable information regarding the thyroid anatomy and can be helpful not only for endocrinologists but also for surgeons.

Description of the case

A 43-year-old woman presented to the nuclear medicine outpatient clinic with a few-months history of weakness, increased sweating and emotional lability. She was referred by an endocrinologist to a nuclear medicine outpatient clinic for possible RAI- therapy and it was our first meeting with the patient. The woman underwent total thyroidectomy 10 months prior due to unstable GD for three years (unsuccessfully treated with thyrostatic drugs). Preoperative ultrasound of the thyroid gland revealed a goiter with a volume about 55.5 ml.

Thyroid scintigraphy was not performed before surgery. She was smoking cigarettes. The choice of surgical treatment was associated with a large volume of the thyroid gland , high levels of antibodies (TRAb) and the patient's preferences. Information from the referral also included thyroid ultrasound examination after the surgery and histopathology. Please notice that the thyroid ultrasound showed only *stumps* of the *thyroid* lobes following thyroidectomy. The histopathology report described two lobes and isthmus of the thyroid and confirmed goiter hyperactivity. After surgery she received replacement levothyroxine (LT4) supplementation in a dose of 75 ug per day from a surgeon. Due to first symptoms of hyperthyroidism (six months after surgery) the endocrinologist reduced the dose of LT4 to 25 µg and next stopped the LT4 supplementation. The patient still presented symptoms of hyperthyroidism. Physical examination at the nuclear medicine outpatient clinic revealed inactive mild orbitopathy, increased sweating, regular heart rate of 90 beats/minute, normal blood pressure and an absence of pathology during palpation the neck area.

Ancillary investigations showed: suppressed serum levels of thyrotropin (TSH) 0.001 µIU/mL (0.55–4.78 µIU/mL), free thyroxine (fT4) 1.39 ng/dL (0.89–1.76 ng/dL), free triiodothyronine (fT3) 2.82 pg/ml (1.88-3.18 pg/ml), thyrotropin receptor antibody (TRAb) 34.0 IU/L (<1.5 IU/L). The thyroid ultrasound showed *stumps* of the *thyroid* lobes and the PL was not visualized. ^{131}I-iodide scintigraphy revealed two foci with excessive tracer accumulation. One of the foci in the middle of the neck corresponded to the PL (Fig. 1). The thyroid radioiodine uptake during the 24th hour was 39%. RAI therapy was administered after the patient's agreement. She was treated with 21.6 mCi of I 131 and developed symptoms of hypothyroidism.

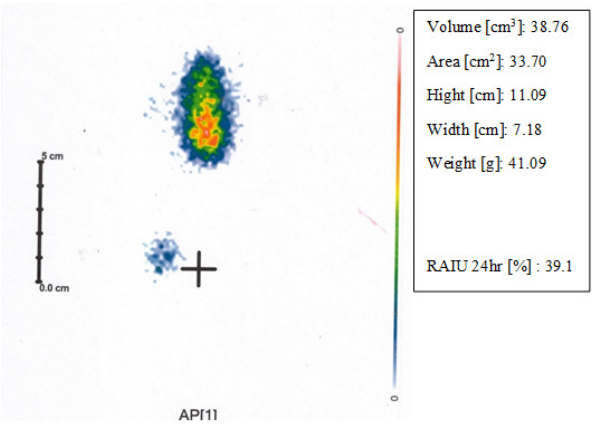

Fig. 1. Thyroid ^{131}I-iodide scintigraphy: foci with excessive accumulation of the tracer. In the middle of the neck, the pyramidal lobe was revealed

Currently, the patient is under observation in an endocrinology outpatient clinic. The patient is in a better condition without symptoms of hyperthyroidism, she takes LT4 (100 and 112 µg every other day, alternately). The thyroid hormone levels are normal.

Discussion

The pyramidal lobe could be a source of pitfalls in thyroidectomy, due to unreliable preoperative diagnostics. Imaging evaluation may influence the conduct and extent of thyroidectomy. The European Thyroid Association guidelines for the Management of Grave's hyperthyroidism recommend that if surgery is selected, total thyroidectomy is the procedure of choice. The initial thyroid imaging study is ultrasonography and should be performed in all patients before surgery. According to these guidelines scintigraphy of the thyroid is suggested when thyroid nodularity coexist with hyperthyroidism, and prior to RAI therapy.[5]

Total thyroidectomy rarely results in the removal of all thyroid tissue. The use of single-photon emission computed tomography to define specific anatomical sites of residual RAI uptake foci after total thyroidectomy shows uptake in 99% of patients, with 46 % in the pyramidal lobe. [6] When performing total thyroidectomy it's very important to look for identify and remove PL. Thyroid cells in the PL can become active after excision of the functioning thyroid tissue, so hyperthyroidism can appear in patients after total thyroidectomy due to GD.[7] The analysis of the available data presented by

Kim et al. revealed the sensitivity of preoperative sonographic detection of thyroid PL about 81%. There was no statistically significant difference in the sonographic detection rate of thyroid PL according to sex but the sonographic detection rate decreased with increasing age. In this study the number of false-negative cases of thyroid sonography were 11.4 %.[8]

Our patient had only undergone thyroid ultrasound examination before the surgery in which PL was not visualized. Undoubtedly, RAI ablation should be considered for hyperthyroidism recurrence after surgery, but the usefulness of thyroid scintigraphy before planning a surgery must be noted.

In many cases scintigraphy provides considerably more functioning and anatomic details than ultrasound.[9] According to the surgeon's guidelines, thyroid scintigraphy is not necessary before surgery.[10] Some reports have indicated that preoperative diagnosis of the PL based on scintigraphic images is unreliable, while other reports have suggested that thyroid scintigraphy is recommended in every patient before surgery.[3,7]

According to reports Braun et al. it is not reliably diagnosed the presence of PL by scintigraphy imaging because it can only give functional information. The authors note that the anterior cervical region has to be investigated very carefully during operation in order not to leave residual thyroid tissue in total thyroidectomy.[3] In a study presented by Cengiz et al. the prevalence of PL visualization using thyroid scintigraphy can reach 18%. PL visualization rate in patients with diffuse goiter was found to be significantly higher compared to other patients.[7]

In thyroid scintigraphy, PL is observed in higher rates in patients with hyperthyroidism and large thyroid gland.[7,11] In the study by Siraj QH et al. a PL was visualized on thyroid scintigraphy in 85 (41%) of the 207 patients.[12] In another study, PL was observed at a rate up to 81% among Graves' patients.[4]

The thyroid scintigraphy is useful for evaluating size, location and ectopia of thyroid tissue. Visualization of radiotracer uptake within a thyroglossal duct remnant and PL is frequently seen in the Graves' disease.[13] Neck CT is also useful for detecting the presence, size, configuration, and location of the PL but due to cost currently is less common.[14]

PL often remains non-visualized during preoperative imaging studies so anterior compartment of the neck should be explored for variations of the PL and completely excised during thyroid surgery.

Conclusion

This case shows that the thyroid anatomy anomalies can lead to unnecessary implications for treatment. The knowledge about the presence of the PL of the thyroid is very important for surgeons to perform better resection of the thyroid tissue (especially in cases of thyroid cancer, but also in cases of GD). Identifying the PL preoperatively and removing it from patients requiring total thyroidectomy may decrease the recurrence rate of hyperthyroidism.

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CASUISTIC PAPER

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A rare and overlooked cause of massive gastrointestinal bleeding: Distal duodenal GIST

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ABSTRACT

Introduction. Gastrointestinal stromal tumors (GIST) are tumors of mesenchymal origin which originate from the walls of gastrointestinal system (GIS) organs.

Aim. In this case report we aim to discuss the clinical, labaratory and radiological presentation of distal duodenal GIST as a rare and overlooked cause of life-threatining GIS bleeding.

Description of the case. A 76-year-old male patient was presented to the emergency department with massive gastrointestinal bleeding. Computerized tomography revealed a mass soft tissue density of 4x4cm at the level of the 3-4th segment of the duodenum. At the endoscopy, there was a deep ulcer in the proximal part of the 3rd segment of the duodenum with a diameter of 2 cm with a bleeding vessel protruding into the lumen. After endoscopic treatments, biopsies were taken from the edges of the ulcer. Histopathological examination revealed a sheet-like infiltration composed of mildly pleomorphic cells with oval-spindle nuclei and abundant eosinophilic cytoplasm in the duodenal lamina propria, as the patient was diagnosed of GIST.

Conclusion. GIST and its clinical, labaratory and radiological presentation should be kept in mind in the approach to massive duodenal GIS bleeding.

Keywords. duodenum, gastrointestinal stromal tumor, GIST, massive gastrointestinal bleeding

Introduction

Gastrointestinal stromal tumors (GIST) are rarely encountered tumors of mesenchymal tissue, often localized in the gastrointestinal system (GIS). Stomach is the GIS segment where tumors are most frequently seen with a rate of 50-70%.[1] GISTs constitute up to 1% of all GIS tumors and are most frequently observed in the small intestine after the stomach.[2] These tumors, which are thought to originate from interstitial Cajal cells, were classified as leiomyoma, leiomyosarcoma and leiomyoblastoma, considering that they were smooth muscle cell-derived tumors due to their similar appearance in light microscopy.[3] However, with immunohistochemical methods, GISTs are now accepted as a completely different disease. Although the clinical symptoms often vary depending on the location of the GIS where the tu-

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mor is located, patients may apply to these areas with non-specific complaints.[4,5] Although GISTs may cause some non-specific symptoms, their detection is usually coincidental.

Aim

In this case report, we aimed to discuss a case in the light of the literature who was consulted from the emergency department with a pre-diagnosis of massive upper GIS bleeding and was diagnosed with duodenal GIST as a result of the examinations performed.

Description of the case

A 76-year-old male patient was presented to the emergency department with complaints of melena, hematemesis and dizziness. In physical examination, his arterial blood pressure was 100/60 mmHg, pulse: 110/min and there was melena in the rectal examination. Labarotory tests were as hemoglobin 6.6 g/dL, hemotocrite 19.5%, urea 65 mg/dL, and creatinine 0.9 mg/dL.

At the endoscopy, there was a deep ulcer in the proximal part of the 3rd segment of the duodenum with a diameter of 2 cm with a bleeding vessel protruding into the lumen on the medial wall. Sclerotherapy of adrenaline was applied around the ulcer, and a clip was placed on the root of the vessel (Figure 1).

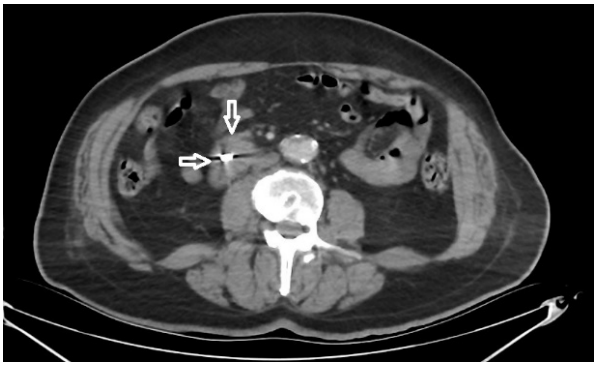

Fig. 1. Millimetric hyperdensity of hemostatic clip can be observed in the centre of the soft tissue density

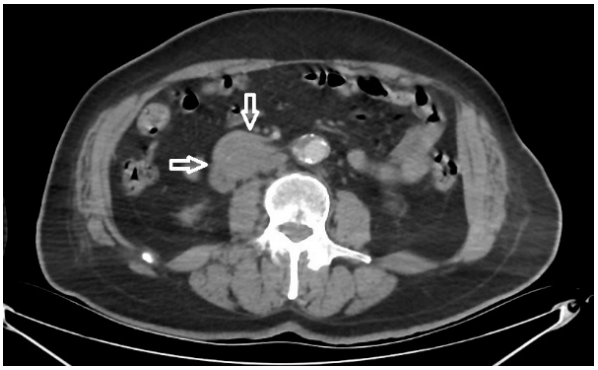

Fig. 2. CT of the abdomen with i.v. contrast, showing a mass soft tissue density of 4x4cm at the level of the 3-4th segment of the duodenum

The hemorrhage were controlled, but there were leakage from the ulcer floor in the upper part of the vessel. This area was coagulated with argon plasma and the hemorrhage stopped. After the endoscopy, computerized tomography revealed a mass soft tissue density of 4x4cm at the level of the 3-4th segment of the duodenum (Figure 2). In the control endoscopy, biopsies were taken from the edges of the ulcer (Figure 3).

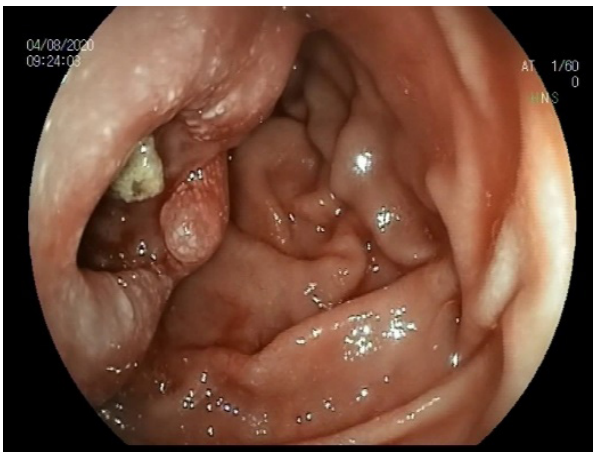

Fig. 3. Control endoscopy of deep ulcer in the proximal part of the 3rd segment of the duodenum which had 2 cm diameter with a bleeding vessel protruding into the lumen on the medial wall

On histopathological examination, a sheet-like infiltration composed of mildly pleomorphic cells with oval-spindle nuclei and abundant eosinophilic cytoplasm was observed in the duodenal lamina propria and the patient was diagnosed as gastrointestinal stromal tumor (GIST) of distal duodenum (Figure 4).

Immunohistochemical analysis showed positive staining for CD117, DOG1 and SMA. Pan-Cytokeratin, S100 and CD34 were negative. The proliferation index (ki-67) was as 3%. Computed tomography revealed a mass of 4x4 cm diameter in the distal duodenum. Surgery has been planned for the patient.

Discussion

Gastrointestinal system tumors are rare mesenchymal tumors that arise from the walls of the GIS organs and whose behavior is unpredictable. Although GIS bleeding is one of the findings that can be observed in these patients, cases of duodenal GIST presenting with life-threatining GIS bleeding are rare. With this case report; we wanted to mention that duodenal GIST may be present in patients presenting with massive GIS bleeding. However, we wanted to draw attention to the fact that pre-surgical endoscopic intervention may be effective in case of emergency bleeding.

Although ultrasonography is partially helpful in determining the location and size of the tumor in di-

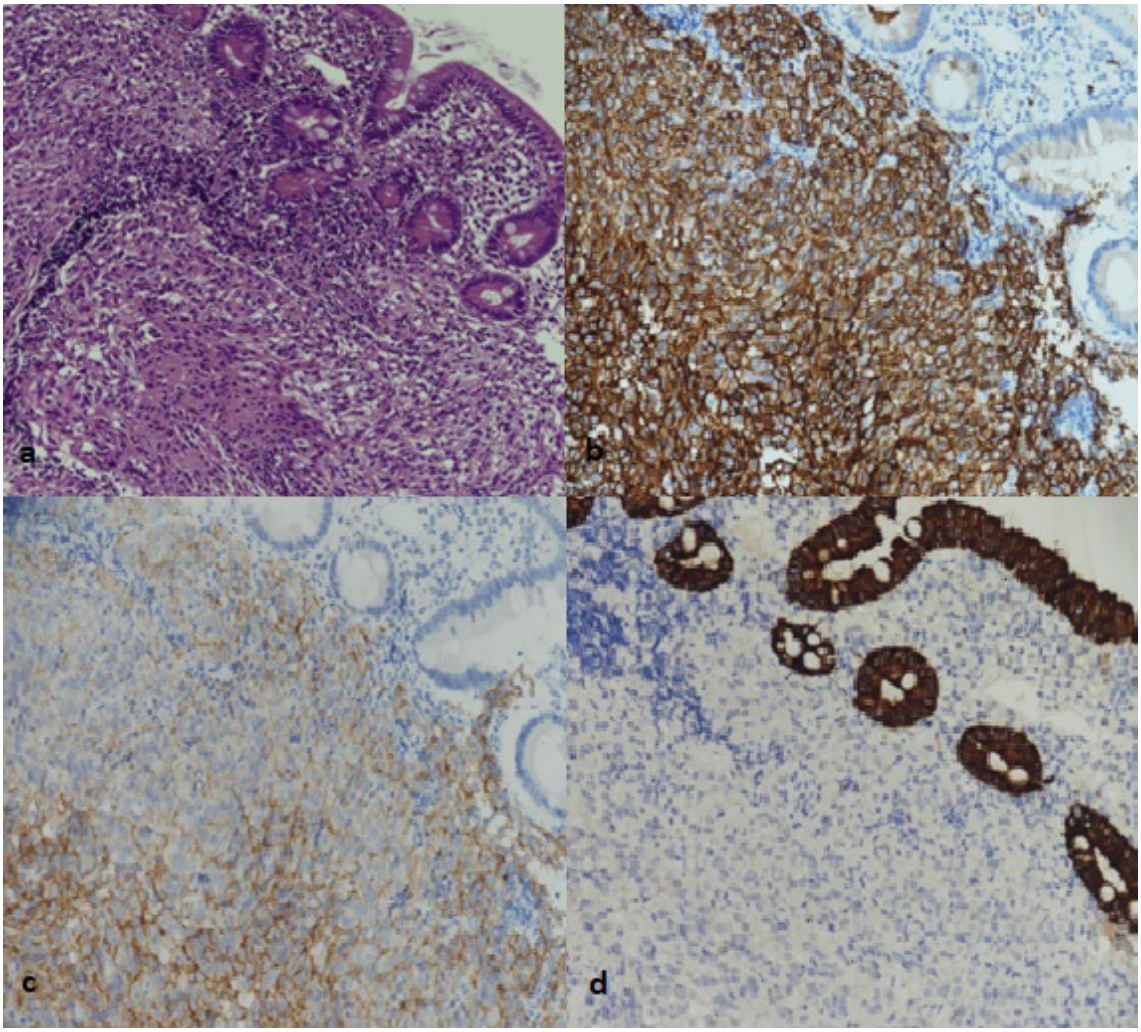

Fig. 4. Sheet-like infiltration composed of mildly pleomorphic cells with oval-spindle nuclei and abundant eosinophilic cytoplasm in the duodenal lamina propria. A. Duodenal lamina propria (H&Ex100), B. Diffuse membranous staining for CD117 (x200), C. Patchy membranous staining for DOG1 (x200), D. Negative pan-Cytokeratin staining (x200).

agnosis, today abdominal CT and magnetic resonance imaging (MRI) are considered the gold standard. It provides great benefit in diagnosis especially in patients with tumor diameter over 2 centimeters. However, these examinations have a special importance in ruling out the presence of metastasis. Another method for diagnosis is endoscopic examinations. Endosonographic examination can be performed if a lesion of different diameter and thought to be of submucosal origin is observed in the upper/lower GIS endoscopic evaluation. The diagnosis is confirmed by histopathological examination of the tissue sample.[6] Chemotherapy and radiotherapy do not have a significant place in treatment. Surgical removal of the mass is the most effective treatment method.[7] The most important point to be considered in surgery in terms of preventing recurrence is the removal of the tumor en-block at a distance of at least 2 cm from the surgical margin. Imatinib, a tyrosine kinase inhibitor, can be given lifelong at a dose of 400 mg/day in cases of unresectable, advanced stage, relapse or metastatic disease.[8] In tumor progression, the dose can be increased to 800 mg/day. If the patient cannot tolerate Imatinib, another tyrosine kinase inhibitor, Sunitinib, can be used. Surgical treatment was planned in the first place because no spread was detected in the abdominal CT of our patient. GISTs are tumors of mesenchymal origin which are rare and originate from the walls of GIS organs.[9-12] GIS bleeding is one of the findings in these patients, but distal duodenal GIST presenting with life-threatining GIS bleeding are much more rare.[13]

Conclusion

GIST and its clinical, labaratory and radiological presentation should be kept in mind in the approach to duodenal GIS bleeding. When there is no typical source of bleeding in typical site (cardia-Mallory Weiss, antrum, duodenal bulb-ulcer etc, the endoscopists should always try to go to the third part of duodenum, which is not assessed routinely on endoscopy.

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LETTER TO THE EDITOR

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Ethical issues surrounding false information about coronavirus disease 2019

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Dear Editor,

For many countries, the coronavirus disease 2019 (COVID-19) pandemic has undoubtedly been the worst health crisis in recent decades. As of January 23,2021, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread globally, infected more than 99,016,442 people, and caused more than 2,122,963 deaths worldwide.[1] This is the first time that humanity is witnessing the evolution of a global pandemic of such magnitude in real-time. As such, a staggering amount of information regarding COVID-19 is constantly circulating. The World Health Organization (WHO) expressed its fears that an pandemic of false news could generate confusion among the population, to the detriment of the dissemination of true information from official publications by health authorities based on the best available scientific knowledge.

The International Fact-Checking Network (IFCN) recently launched a collaborative project on COVID-19-related fake news. As of March 27 2020, this project, which includes more than 70 fact-checking media organizations from around the world, has already debunked more than 1,500 fake news stories circulating in 61 countries.[2] False information about coronaviruses is so abundant and widespread that journalists and fact-checkers can no longer meet the demand. The fact that this pandemic is marked by disinformation raises several ethical issues, including a crisis of confidence in institutions. False news affects the spread of the virus and is propagated by social media platforms, governments, and citizens

The confidence in public institutions

In a health crisis, confidence in public institutions is critical, as the success of most measures depends on the citizens' cooperation and, by extension, their confidence that the decisions of the authorities are justified. According to some, the climate of mistrust in political institutions is both a cause and a consequence of the widespread dissemination of false news. Some experts believe that the loss of public confidence in political authorities and traditional media is partly responsible for the circulation of false information on social networks. Conversely, false news and rumors may well increase distrust of governments, health officials, and reliable sources of information. The social media environment is deteriorating to the point where speculation, rumors, and conspiracy theories now dominate and overshadow factual information.

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Public health and the common good

Maintaining the quality of information is essential to achieve the common good, especially when it has potentially disastrous consequences. Therefore, it is paramount that we are provided with the best available health information and knowledge to reduce the spread of SARS-CoV-2 and to protect people who may be more vulnerable to the disease. In recent years, the effects of false news on the proper function of political and electoral processes and financial markets have been demonstrated. However, their impact on public health has yet to be studied in-depth. Recently, researchers have been interested in the impact of disinformation on public health in a pandemic situation. They modeled the dissemination mechanisms of norovirus (gastroenteritis), influenza, and monkeypox according to the speed at which true and false information spreads on the social network "Twitter".[3] Their observation was unequivocal: false news accelerates the spread of infectious diseases. This research also showed that it is possible to fight back against false news, particularly in two ways: by disseminating quality information and providing education on disinformation in social media. These methods confer various responsibilities to social media platforms, public authorities, and citizens.

Ethical issues associated with media freedom during COVID-19

COVID-19 made everyone to start conversation about media profession, opportunities and issues of rights two-fold times. Globally, COVID-19 gave opportunities to governments to exploit the existed laws for criticizing the transmission of "counterfeit news," "misinformation," and "false news" to actualize original news.

The violations tracked by the International Press Institute range from restrictions on access to information to physical attacks and charges against journalists.[4] Access to information for journalists has been restricted before, now it is near impossible to get a response from government officials for journalists who do not work for pro-government media, regardless of whether the questions concern COVID-19 or not. Media organisations already in a fragile financial state are hit by an economic crisis and journalists are working under increasingly precarious conditions.[5]

In India, columnists publishing articles on the COVID-19 are brought to police station and asked to clear their accounts, including Peerzada Ashiq, a senior writer (The Hindu, Kashmir), and Siddharth Varadarajan, editorial manager (The Wire, Uttar Pradesh).[6] Moreover journalists reporting about human rights mistreats associated with the pandemic, and police abuses or prison conditions, were endangered, terrified, attacked and charged. Officials from many countries stopped paper publication and circulation to control the COVID-19 transmission and at some places, media controllers had blocked sites or evacuated articles.

The responsibility of social media platforms

False news and rumors are not new; however, their rapid expansion has been widely attributed to social media. Social media outlets have been accused of presenting information, regardless of its source and accuracy. According to some studies, social networks favor the circulation of fake news over verified information. In the face of mounting pressure and concerns from various organizations, governments, and users, the social media outlets Twitter and Facebook recently decided to take tougher measures and develop policies to ban and censor false information.[7] Twitter has adopted new measures to strengthen its content rules such that any information that may harm ethnic and national groups, go against the messages from official sources (such as health authorities), and contributes to the transmission of COVID-19 will be removed. This strengthening of content rules is not limited to regular citizens. It also applies to world leaders. Indeed, Twitter removed a video in which the President of Brazil, Jair Bolsonaro, who is known for his opposition to SARS-CoV-2 containment measures, praised the merits of treating COVID-19 with chloroquine, a drug for which the safety and effectiveness are presently not supported by the scientific community.[8] Facebook has also asked the Brazilian statesman to withdraw his video. ABC News telecasted a poll results of anxiety about the COVID-19 transmission is faster than the COVID-19, resulting in public panic worldwide, and social media is also a practical platform for the transmitting of information to the common people.[9,10]

Visual data is useful for people for easy and significant understanding most of the important information about COVID-19, just like Johns Hopkins University developed interactive data as per crowdsourcing information, which demonstrates data-driven visuals such as global cases map, critical data trends, and latest news about COVID-19 situation in all countries, enabling the citizens and scientists to access, understand and monitor the COVID-19 situation in 24 hours.[11,12] In China, WeChat (messenger app) introduced a location-based "Cases Nearby" feature as per confirmed cases surrounding the location by visual footprint for users by maintaining data privacy of all information and warns users to take safety measures in high-risk areas.[13] Additionally, Prince of Wales Hospital (Hong Kong) developed an infographic on the principles of airway management was introduced in 17 languages and shared in many online social networking sites and apps, helpful to medical hospitals to consider easily understandable prevention and control measures to decrease the spread of COVID-19.[14]

The responsibility of the government

Social media outlets must have procedures in place to remove news that were fake. However, they are unlikely to be able to remove all such contents, even when using artificial intelligence algorithms. The same fake news stories circulate the world and in different languages. In this age of social media, it is important for governments to ensure that quality information is released and to promote awareness of disinformation. Political figures and institutions have an important responsibility in this regard. An important concept to teach the public is cyber-citizenship, which involves the development of digital capabilities that improve the critical judgement of citizens concerning false information. To this end, the government of the United Kingdom launched an advertising campaign entitled "Don't feed the beast" that presents a list of criteria, the source of information is credible, the authenticity of images and facts, and the identification of errors.[15] Widespread human rights law confines censuring sham explanation, and this is a problem because of general prosperity. States should preferably expand their assurance to ensure that they transmit potential, verification based and solid information.

An individual's responsibilities

A study from Iraq showed that social media has a potential effect on developing panic about the COVID-19 outbreak, with a significant negative impact on psychological well-being and facebook has been widely used social media network for developing panic.[16] There is a significant positive statistical correlation between self-reported social media use, the development of panic about the COVID-19 (R=0.8701) and majority of participants aged 18-35 years are having psychological anxiety.[16]

In return, the citizens also have a responsibility for their use of social media. Our civic duty is accomplished, in particular, by cultivating one's critical sense, by checking the quality of our information sources, and by not sharing false news that did not meet the basic standards of journalism. However, this is sometimes easier said than done. Therefore, we have to be particularly vigilant. Research on cognitive development shows that much remains to be done to fully understand our cognitive biases, as well as our resistance to the intentional modification of our false or unverified beliefs.

Conclusion

Recent research on the subject shows that, regardless of its veracity or logic, we tend to prefer information that reinforces our own beliefs, attitudes, and aligns our beliefs with those of the people around us. Access to information for journalists should not be restricted by governments and in this age of social media, it is important for governments to ensure that quality information is released and to promote awareness of disinformation. Citizens and users should also be made accountable for the propagation of fake news in social media. Doctors should also initiate communication campaigns for restructuring routine behaviors by introducing healthy activities that can decrease loneliness of social distancing measures.

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LETTER TO THE EDITOR

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Coronavirus disease 2019: ethical and epidemiological issues with clinical trials

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Dear Editor,
In the current public health crisis arising from the coronavirus disease 2019 (COVID-19) pandemic, past experiences (with Acquired Immune Deficiency Syndrome (AIDS), Ebola, etc.) and a flexible framework can be used to quickly and effectively determine the best drug response. In clinical trials, the investigator or applicant should initially take care about rights protection of the subjects. Eventually, the subjects' rights of voluntary participation, the right to know, the right to privacy, the right to security, the right to timely treatment and other rights should be protected.[1,2] The balance between taking the necessary precautions and making the decision to administer an experimental treatment to a large proportion of the population is unique to each crisis.

Are any treatments showing encouraging signs?
Clinical trials of drugs are usually divided into four phases: phases I, II, III and IV. Each phase has different requirements and objectives, and the number of cases required is also different.[3] Some of the drugs currently registered are only effective *in vitro*, and the safety has not been proven.[4] This is the most pressing question, and the one that leads to all others. There are currently several hundred clinical trials[5] underway around the world to assess the effects of various treatments for COVID-19. For example, the Discovery trial,[6] carried out by European health centers on 3,200 patients, including 800 in France, is testing the effects of four treatments that have been used previously against other diseases. The four treatments that will be compared to standard protocol are as follows: Remdesivir (used to treat Ebola), lopinavir/ritonavir (a drug combination used to treat AIDS), interferon beta-1A (interferon added to stimulate immune defenses), and hydroxychloroquine (used to treat rheumatoid arthritis and lupus). These treatments have been included in this article because they have previously demonstrated potent antiviral actions against

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

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viruses and *in vitro* actions against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). They have also been the subject of preliminary studies; however, to date, the effects described in both publications and pre-publications have not been conclusive. A small fraction of these publications comprised reports of prospective clinical trials (0.25%), and many of these trials have imparted conflicting conclusions, leading to confusion among the public and the scientific community.[7]

Drug repurposing is also an impressive idea for treating COVID-19 as it involves low cost and rapidly available in the pharmacies. This idea alleviates some steps of clinical trials, especially those concerning the strenuous diligence and time needed for phase 1 and 2 trials.[8]

Where is the knowledge deficit currently?

Although the pressure and urgency for conducting COVID-19 research abounds during this worldwide crisis, this should not preclude scientific principles and ethics.[9] Pandemics create issues regarding scientific and ethical questions for research and understanding what ethical concerns remain the same and differs is essential for conducting clinical trials. There have been promising results reported in studies on treatments for COVID-19. If one study found a treatment which was effective in vitro against viruses that showed similarities to SARS-CoV-2 in terms of their function. This indicates that preliminary studies are headed in the right direction. Unfortunately, a few encouraging results does not guarantee with any certainty the efficiency and safety of these treatments. The questions that arise are related to the effectiveness of each treatment and their side effects when used in patients who may be in respiratory distress or have other diseases. Additionally, the use of an appropriate protocol is equally vital: the dosage, when to administer treatment, and in which patients (age, sex, comorbidity factors, undergoing other treatments). However, there is also an important issue in the comparative evaluation of these treatments from the point of view of their risks and benefits. Indeed, even if a promising and effective treatment is found with moderate risks, the research environment arising from the current pandemic is strongly encouraging the scientific community to initiate studies to determine which of these treatments maximizes the benefits and minimizes the risks. This is due to the fact that this pandemic is calling for the treatment of hundreds of thousands of individuals globally. Any approximation in this regard could otherwise result in a significant number of victims, who would have reacted better with treatments that were more effective or better tolerated. Therefore, the current knowledge deficit concerns the absolute efficacy and safety of each treatment, as well as the comparison among treatments. The interventions selected for testing should consist of the most promising therapies, as determined by existing data. The value of clinical trials depends on the quality of information produced and the relevance of the data to address public health needs.[10]

Should the knowledge deficit be set aside given the urgency of the situation?

This point of tension arises directly from the uncertainty regarding the treatments being tested during an ongoing crisis in which many lives are at stake. Hydroxychloroquine is at the center of a controversy in France and several other countries, where many are wondering about the benefits of systematically treating patients with hydroxychloroquine, even if this means postponing scientific certainty. However, even in a case where a patient's condition deteriorates, and an uncertain treatment appears to be more acceptable (so called "compassionate use"), the administration of this treatment to all those affected remains questionable. This holds true if the implemented protocol targets patients at an early stage, thus calling for the systematic treatment of any person that tests positive or presents moderate symptoms. Since the overwhelming majority of COVID-19 patients do not progress to severe forms, treating them with hydroxychloroquine or other treatments with side effects or potentially dangerous effects poses a serious public health risk.[11]

What are the general provisions, and how are they adapted to the circumstances?

Clinical trials are not only subject to methodology but also to legislation, which guarantees respect for individuals, non-maleficence, and justice.[12-15] However, the balance between taking the necessary precautions, which are typically already in use, and the decision to administer an experimental treatment to a large proportion of the population is unique to each crisis. The mortality rate of AIDS was extremely high during the initial years of the epidemic, similar to that of Ebola. Thus, the lethality of these diseases justified the administration of treatments in the absence of proof of their superiority and without a placebo group. Compared to AIDS and Ebola, the lethality of COVID-19 appears to be lower, but is more difficult to establish since the total number of cases is not yet known, and lethality is dependent on age and the healthcare system. Therefore, the administration of non-validated treatments remains problematic, especially since a large majority of the total cases are asymptomatic or have more mild forms of the infection. Despite these challenges, several factors have already helped to adapt the clinical standards to the circumstances in this pandemic.

First, clinical trials have been set up in record time and making agreements among several healthcare centers that have the capacity to analyze large amounts of

samples rapidly.[6,16] Second, these trials are making use of relatively recent methodologies with several arms, allowing for the observation of the effects of several treatments simultaneously and adapting them accordingly. This last point allows researchers to stop one of the arms quickly if it demonstrates ineffectiveness and to continue only with those treatments that show promise.[17]

Conclusion

In terms of study design, patients' rights should be primarily considered, followed by scientific value and commercial interest. Multidisciplinary international cooperation should be conducted to reduce the harm to patients' rights and interests. The medical and research professions have the means to reconcile care and implement rigorous testing for COVID-19, with the possibility of making initial results available in a few days. During the situation of COVID-19, the review standards for clinical studies should not be lowered.

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