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54

Q4

Original Articles

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ORIGINAL ARTICLE

Antibiotic Susceptibility Profile and Detection of Plasmid-Mediated Quinolone Resistant Genes Among Extended Spectrum β-Lactamases (ESBL) Producing Uropathogens in Women

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Abstract

Background/Aim: The most common bacterial diseases in women around the world are urinary tract infections. Aim of this study, was to evaluate the prevalence and current antibiotic resistance rate of uropathogens isolated from the female patients of a tertiary care hospital in Amritsar, Punjab, India.

Methods: Samples were collected from patients showing urinary tract infection (UTI) symptoms and analysed using microscopy, dipstick test and urine culturing followed by identification and characterisation of to identify the uropathogens. Antibiotic susceptibility test and MIC were performed.

Results: The results revealed that *E coli* (35.5 %) was the most prominent uropathogen followed by *Klebsiella spp* (21 %), *Enterobacter spp* (17 %), *Acinetobacter* (11 %), *Enterococcus spp* (6 %), *Pseudomonas spp* (4.5 %), coagulase negative *Staphylococci* (4 %), coagulase-positive *Staphylococci* (0.5 %) and *Corynebacterium aurimucosum* (0.5 %). The antibiotic susceptibility profile study reported eight isolates with multi-drug resistance properties. However, gentamicin, imipenem and meropenem were found to be the most effective antibiotics against the isolated uropathogens. All the extended spectrum β-lactamase (ESBL)-positive isolates possess the quinolone-resistant gene *qnr*B, while *qnr*A was absent.

Conclusion: The current study revealed that for appropriate treatment, it is crucial to be aware of the epidemiological data regarding the disease and to begin any empirical antibiotic treatment.

Key words: Urinary tract infection (UTI); Uropathogens; Antibiotic susceptibility; β-lactamase; Quinolone resistance; Multi-drug resistant.

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Introduction

Urinary tract infections (UTIs) are the most prevalent bacterial infections in women across the globe. These infections may be community-acquired or nosocomial that are acquired from hospital settings such as catheters.[1] UTI is particularly responsible for causing discomfort

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in elderly and immune-compromised patients constituting a risk of septic shock, bacteraemia, respiratory distress syndrome and even death.[2] Patients having UTIs are usually treated by empirical antibiotic treatment.[3] Therefore, to begin with an appropriate treatment, it is crucial to be aware of the epidemiological data regarding the disease.[4] In the present era, where pathogens possess high antibiotic resistance rates, it is even more important to understand epidemiological information before starting the treatment. The main epidemiological factors that influence the type of UTI, causative agents and antimicrobial resistance rates are age group, sex, geographical location and hospital setting.[5]

Although there are many causative agents of UTIs, the members of the family *Enterobacteriaceae* accounts for most of the infections. *E coli* is the most common uropathogen involved in community-acquired UTIs because it belongs to the intestinal microflora of the human intestine and may easily colonise the urinary system. Several investigations on community cases revealed that the most common uropathogens are *E coli, Klebsiella spp, Enterococcus spp and Proteus spp.*[6]

Antibiotic resistance is one of the major growing concern today. The inappropriate use of antibiotics in human medicine and their misuse in the veterinary and agriculture field are the major contributing factors to antimicrobial resistance.[7] Further, the resistance of pathogenic microorganisms to commonly used antibiotics is a serious concern worldwide as it highly affects the treatment of infectious diseases like UTIs.[8, 9]

The World Health Organization published its first list of antibiotic-resistant "priority pathogens" - a database of 12 families of bacteria that represent the most serious threat to human health. Multidrug resistant bacteria are the most dangerous of all, posing a particular hazard in hospitals, nursing homes and among patients requiring devices such as ventilators and blood catheters. They include *Acinetobacter, Pseudomonas* and several members of *Enterobacteriaceae* family (including *Klebsiella, E coli, Serratia* and *Proteus*). They can cause severe and frequently fatal illnesses such as bacteraemia and pneumonia.[10]

Extended spectrum β-lactamases are a class of genetic alterations that confer resistance by hydrolysing penicillins, 1st, 2nd and 3rd generation cephalosporins and aztreonam. β-lactamase inhibitors can prevent them from growing. Three primary sets of genes encode extended spectrum β-lactamases (ESBLs) ie TEM, SHV and CTX-M and these enzymes are frequently detected in *E coli* and *K pneumoniae*.[11] ESBLs are produced by a variety of bacteria and were initially connected with hospital-acquired infections, but are now increasingly linked with community-acquired illnesses.[12]

Similarly, fluoroquinolones are used to treat UTIs induced by both gram-positive and gram-negative bacteria. The widespread use of these antibiotics has resulted in resistance, particularly among *Enterobacterales*.[13] Due to their potency, broad spectrum of activity, oral absorption and safety profile, cephalosporins and fluoroquinolones are frequently used to treat community-acquired UTIs in non-pregnant women. However, since antibiotic resistance spreads around the world, the efficacy of these antibiotic treatment alternatives may be jeopardised.[12]

Owing to the growing concern of antibiotic resistance UTIs cases in Amritsar (India), aim of this study was to analyse the prevalence of UTI causing bacteria and their antibiotic susceptibility pattern towards different antibiotics. Also, the study was aimed to uncover the presence of ESBL and plasmid-mediated quinolone resistance (PMQR) genes among isolated uropathogens.

Methods

Flow of the study is presented in Figure 1.

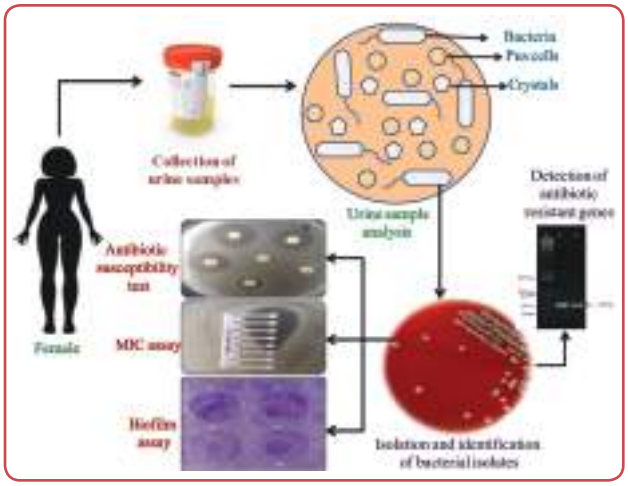

Figure 1: Flow of the study

Study area and population

The present study was carried out in the Amritsar city of Punjab, India. The urine samples were collected from Guru Nanak Dev Hospital, Amritsar. The study was carried out from April to September 2017. A total of 200 female patients with signs and symptoms of UTI who visited the outpatient department (OPD) of the hospital were selected for this study. The age of patients was between 21 to 60 years. The non-inclusion criteria for this study is the age of patients (< 21 years), patients who were on antibiotics, patients with a history of any implants and patients with a history of hospital admission a week before an OPD visit to rule out nosocomial infections. A questionnaire was prepared to have questions related to the signs and symptoms of the UTI, previous history of the UTI and medication or antibiotics taken.

The study was approved by the institutional ethical committee of Guru Nanak Dev University, Amritsar, Punjab (India) (Ref No: -659/HG; Date: 29-Mar-2016). The work was done according to the guidelines provided by Indian Council of Medical Research (ICMR). The purpose of the study was clearly stated and a written consent was taken from each patient involved in the study before sample collection.

Sample collection

The clean-catch midstream of freshly voided urine was collected in a sterile screw-capped container (50 mL). The instructions were given to the patients on how to collect the sample in the container. The collected samples were labelled and transported to the laboratory within 2 h of collection for analysis.

Urinalysis

Urine microscopy. In the preliminary urine analysis, 10 mL of well-mixed urine was taken in a centrifuge tube and centrifuged at 3000 rpm. The supernatant was discarded and the pellet/deposits left in the tube were smeared on the glass slide to observe under the light microscope for any cells, crystals and casts present in the urine.

Urine dip-stick test. The preliminary examination of the urine samples was done using 10 test strips coated with chemicals (*Orinasys, ARKRAY Healthcare Pvt Ltd*, Santacruz East, Mumbai, India). To perform the test, the manufacturer's methodology was followed and the results were also read accordingly. The chemical-coated strips were dipped in the un-centrifuged urine samples and taken out immediately on a blotting paper. The results were noted by observing the colour change on the patches.

Urine culture. The urine culture was done with the help of a calibrated loop. One µL of un-centrifuged, well-mixed urine sample was inoculated with the help of inoculating loop on the surface of MacConkey and blood agar plates (*HiMedia Laboratories*, Mumbai, India). For counting the bacterial colonies, a measured amount of urine (1 µL) was inoculated onto the nutrient agar plates with the help of calibrated inoculating loop. The colony-forming unit count was more or equal to 10^4 CFU/mL for a single potential pathogen interpreted as positive bacteriuria.

Identification of bacterial isolates

The isolated bacteria were identified using standard microbiological methods as stated in Bergey's Manual of Systematic Bacteriology.[14] Differential mediums were used such as mannitol salt agar and eosin methylene blue agar to identify bacteria. Different biochemical tests were performed for bacterial identification including IMViC, sugar fermentation (glucose, lactose, sucrose, mannitol), motility, oxidation/fermentation, oxidase, catalase, urease, coagulase and triple sugar iron. Gram staining was performed to differentiate between Gram-positive and Gram-negative bacteria. Few isolates were further confirmed by 16s rRNA gene sequence analysis. For molecular characterisation, each bacterium was grown on nutrient agar plates and DNA was isolated by following the protocol described by Kaur et al.[15] The 16s rRNA gene sequence of the bacterial samples was amplified using primers as described by Lane.[16] The primer sequences were: 27F (5'-CAGGCCTAACACATGCAAGTC-3') and 1492R (5'- GGGCGGWGTGTACAAGGC-3'). Polymerase chain reaction (PCR) was used to amplify the 16s rRNA gene in a 20 µL reaction mixture as described by Kaur et al.[15] The PCR was performed in a thermocycler (*Agilent Technologies*, Santa Clara, CA, USA). The PCR-amplified product was purified using a Gel Extraction Kit (*IBI Scientific*, Dubuque, IA, USA) following the instructions of the manufacturer. The purified PCR products were sequenced using the same primers provided by DNA sequencing services of 1st BASE, *Axil Scientific Pte Ltd*, Singapore. The sequences obtained were used for a gene similarity search against the National Center for Biotechnology Information (NCBI) database using the BLAST algorithm. The 16s rRNA gene sequences of the isolates were

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submitted to NCBI GenBank using *BankIt* (www.ncbi.nlm.nih.gov/Banklt/).

Antibiotic susceptibility pattern of the isolates

Antibiotic susceptibility test. The antibiotic susceptibility pattern of the bacterial isolates was evaluated by the disc diffusion method as given by Bauer and Kirby with few modifications.[17] The isolated bacteria were initially grown in Luria Bertani broth and the cell density was set equivalent to 0.5 McFarland standards by diluting the grown culture with fresh medium. The agar plates were prepared with Mueller-Hinton agar medium and the diluted bacterial culture (0.1 mL) was spread on each agar plate. A sterile forceps were used to place the antibiotic discs over the agar surface. The plates were incubated at 37 ºC in a BOD incubator for 24-48 h. A total of 24 antibiotics belonging to different groups based on their mode of action were procured from *HiMedia Laboratories* (Mumbai, India) and were tested. The antibiotic susceptibility of the isolates was determined by measuring the zone of inhibition around the discs and the results were interpreted according to the guidelines of CLSI.[18] *E coli* ATCC 25922 was used as the control strain.

Multiple antibiotic resistance (MAR) index. The MAR index was calculated by observing the antibiotic susceptibility pattern of the isolates. It was calculated by the method given by Krumperman.[19] Total number of antibiotics to which an isolate was resistant was divided by total number of antibiotics tested against it.

Minimum inhibitory concentration (MIC) assay. MIC of the selected bacterial strains was determined by using commercial *HiComb MIC™* strips that contain antibiotic concentrations gradient-wise (*HiMedia Laboratories* Mumbai, India). Briefly, it is based upon the diffusion of the antibiotic into the medium so that MIC values could be estimated directly using a single culture plate. Mueller Hinton agar plates were prepared and the *HiComb MIC™* strips were placed on them after inoculating them with desired bacterial culture (cell density equivalent to 0.5 McFarland standards). The MIC was recorded at the point of intersection of the clear zone with the point on the scale of the MIC test strip. In the present study, the sixteen antibiotics selected for the MIC test were: polymyxin B, ciprofloxacin, amoxicillin/clavulanic acid, gentamicin, levofloxacin, imipenem, tobramycin, nitrofurantoin, norfloxacin, nalidixic acid, piperacillin, aztreonam, meropenem, amikacin, cefepime and cefpirome were assessed using MIC strips (*Himedia*). *E coli* ATCC 25922 was used as the control strain.

Phenotypic detection of ESBL producers

Double-disc synergy test (DDST). The ESBL production of the isolated Gram-negative bacteria was tested by the modified double disc synergy test (MDDST) given by Kaur et al.[20] In this test, a disc of amoxicillin/clavulanic acid (20/10 µg) along with four cephalosporins; ceftriaxone, ceftazidime cefpodoxime (third generation cephalosporin) and cefepime (fourth generation cephalosporin) were used. A lawn culture of the isolate was made on a Mueller-Hinton agar plate as recommended by CLSI.[18] The amoxicillin/clavulanic acid disc (20/10 µg) was placed in the centre of the plate and the other discs of 3GC and 4GC were placed at 15 mm and 20 mm distance from centre to centre to that of the amoxicillin/clavulanic acid disc. Any distortion or increase in the inhibition zone towards the amoxicillin/clavulanic acid disc was considered positive for ESBL production. *Klebsiella pneumoniae* ATCC 700603 was used as a positive control strain.

Combination disc test (CDT). The test was performed according to the guidelines of CLSI in which the discs containing cephalosporin alone and with the clavulanic acid were used.[18] In this study, a ceftazidime (30 µg) disc alone and in combination with clavulanic acid (30 µg/10 µg) was used. The inhibition zone around the two discs was compared and an increase in the zone diameter ≥ 5 mm of the cephalosporin disc with clavulanic acid was interpreted as positive.

Detection of antibiotic resistance genes in MAR uropathogens. The Gram-negative isolates which were resistant to more than seven antibiotics were further explored for the presence of antibiotic-resistant genes. The plasmid-mediated β-lactamase genes (bla_{SHV}, bla_{CTX-M}, bla_{TEM}, bla_{AmpC}) and quinolone-resistant genes (*qnr*A and *qnr*B) were studied among two isolates of *E coli* RBRJ005 and RBRJ013 (Accession No: MN294475, MN294482), two isolates of *K pneumoniae* RBRJ019, RBRJ024 (Accession No: MN294488, MN294493), one isolate of *Acinetobacter baumannii* RBRJ027 (Accession No: MN294496) and one isolate of *Enterobacter cloacae* RBRJ017 (Accession No: MN294486) which were resistant to more than seven antibiotics.

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Plasmid DNA was isolated from these bacterial isolates using High-Speed Plasmid Mini Kit Cat. No IB47101 (*IBI, Scientific*, Dubuque, IA, USA). A single colony of each bacterium was grown in the Luria-Bertani broth for plasmid DNA isolation and the extracted DNA was used as a template for amplification. The multiplex PCR (*Agilent Technologies*, Santa Clara, CA, USA) was used to identify these plasmid-mediated ESBL and quinolone-resistant genes. PCR was performed in a thermocycler (*Agilent Technologies*, USA). The final reaction mixture was 20 µL containing various components.

Biofilm assay. This assay was performed according to the method given by O'Toole with some modifications.[21] The cultures of the selected bacteria were grown overnight in the Luria broth and diluted in a ratio of 1:100 using a fresh medium. From the diluted medium, 100 µL was added to a sterile 96-well microtiter plate. The microtiter plate was covered and incubated for 24 and 48 h at 37 ºC in a BOD incubator. For the quantitative purpose, the assay was performed in the triplicate wells for each culture. After incubation of 24 and 48 h, the bacterial culture was dumped by flipping the microtiter plate and gently shaking to remove the liquid medium from the wells. The wells were washed twice with phosphate-buffered saline (1 x), thus removing the media components and planktonic cells if attached to the wells. About 125 µL 0.1 % crystal violet was used to stain the wells and after 10-15 min the stain was removed by flipping the plate and gently shaking. The autoclaved distilled water was used to wash the microtiter plate until all the excess stain was removed. After that, the plate was shaken and blotted vigorously on a stack of tissue paper and was then dried at room temperature overnight. Thirty three percent glacial acetic acid was added into each well to solubilise the dye for quantification purposes. The microtiter plate was incubated for 10-15 min at room temperature and the optical density (OD) was measured at 590 nm using a microtiter plate reader (*BioTek, Model Synergy HT*). Thirty three percent acetic acid was taken as blank. The biofilm mass and bacterial adhesion were expressed as OD590 nm values. Bacteria were classified according to the scheme of Stepanović et al on the basis of the cut-off OD (ODc) value as non-biofilm producer (OD < ODc), weak biofilm producer (ODc < OD ≤ 2 × ODc), moderate biofilm producer (2 × ODc < OD ≤ 4 × ODc), strong biofilm producer (OD > 4 × ODc).[22]

Results

The age distribution of the patients was between 21-60 years with mean age of 36.5 ± 11.87. The results of the present study revealed that patients of age group 21-30 and 31-40 years showed maximum symptoms of burning micturition, dysuria and pyuria (Table 1).

Table 1: Patients reporting UTI symptoms

Age (Years)	No of patients showing these symptoms (n = 200)				
	Burning micturition	Frequency/ Urgency	Dysuria	Haematuria	Pyuria
21-30	49	17	59	5	29
31-40	57	32	63	27	60
41-50	37	21	33	9	39
51-60	13	19	11	2	22

Patients gave multiple responses; UTI: urinary tract infection;

The maximum cases of pyuria were in the age group 31-40 years. In this study, the pyuria was confirmed by both the dipstick test and microscopic examination. The presence of pus cells in the sample was shown in Figure 2, as well as the presence of crystals in the urine samples.

Prevalence of uropathogens

The 16S rRNA PCR amplification gave approximately 1450 bp amplicons of different bacterial isolates. The alignment of partial 16S rRNA sequences against the NCBI database suggested that they belong to different bacterial species (Table 2). In the present study, the most frequently identified uropathogen using urine culture method was *E coli* (35.5 %) followed by *Klebsiella spp* (21 %), *Enterobacter spp* (17 %), *Acinetobacter spp* (11 %), *Enterococcus spp* (6 %), *Pseudomonas spp* (4.5 %) coagulase-negative *Staphylococci* (4 %), coagulase-positive *Staphylococci* (0.5 %) and *Corynebacterium aurimucosum* (0.5 %) (Table 2).

Antibiotic susceptibility pattern of uropathogens

All the isolated uropathogens were susceptible to gentamicin while, two isolates were resistant to amikacin and tobramycin (Table 3). Among the penicillins group, the only effective antibiotic was carbenicillin as only three isolates were resistant to it. In the cephalosporins group, most uropathogens were found to be resistant to third and fourth-generation antibiotics. About 8.5 % were resistant to ceftazidime, while 7.5 % were resistant to cefepime and 9.5 % to cefpirome. An

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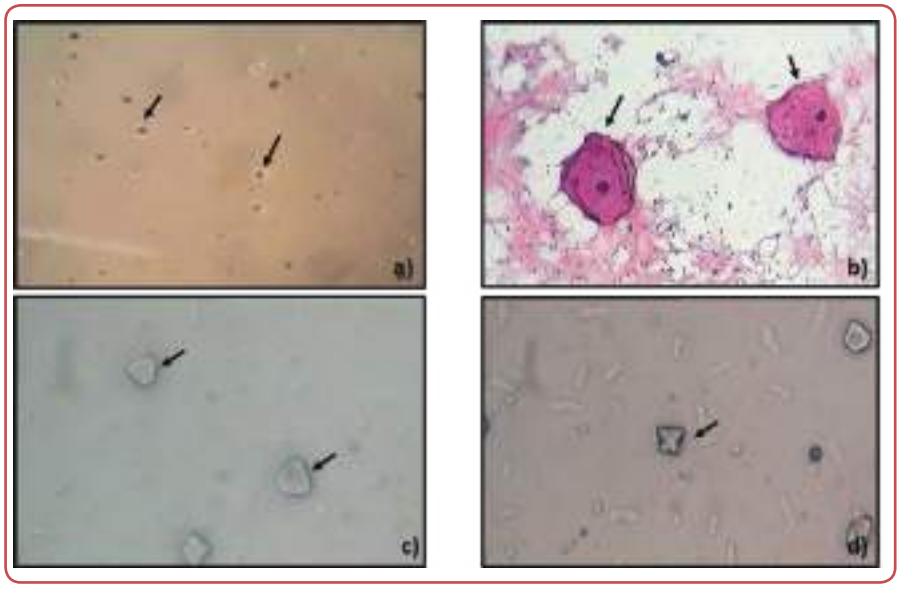

Figure 2: a) Pus cells, b) epithelial cells, c) cystine crystals and d) calcium oxalate crystals under light microscope (40x)

antibiotic belonging to group monobactams aztreonam proves to be ineffective against 11.5 % of the isolated urinary microbes. All the isolates were susceptible to carbapenems ie imipenem and meropenem, hence, can be used to treat recurrent UTIs. About 13.5 % isolates were resistant to nalidixic acid and ciprofloxacin was found to be ineffective against 7.5 % of the isolates. Polymyxin B was found to be ineffective against 3 % of the isolates. Trimethoprim/sulfamethoxazole was found to be ineffective against 12.5% of the uropathogens.

Table 2: a) Pus cells, b) epithelial cells, c) cystine crystals and d) calcium oxalate crystals under light microscope (40x)

Isolated bacteria	Number (n = 200)	Percentage
Escherichia coli	71	35.5
Klebsiella spp	42	21.0
Enterobacter spp	34	17.0
Pseudomonas spp	9	4.5
Enterococcus faecalis	12	6.0
Coagulase positive *Staphylococci*	1	0.5
Coagulase negative *Staphylococci*	8	4.0
Acinetobacter spp	22	11.0
Corynebacterium aurimucosum	1	0.5

Table 3: Antibiotic susceptibility of uropathogens towards different antibiotics

Mode of action of antibiotics	Antibiotic groups	Antibiotics	Antibiotic concentration (µg)	No of resistant isolates
Inhibitor of protein synthesis	Aminoglycosides	Amikacin	30	2 (1.0 %)
		Tobramycin	10	2 (1.0 %)
		Gentamicin	10	0 (0.0 %)
Inhibitor of cell wall synthesis	Beta-lactam antibiotics (Penicillins)	Amoxicillin/ clavulanic acid	30	19 (9.5 %)
		Ampicillin	10	26 (13 %)
		Carbenicillin	100	3 (1.5 %)
		Piperacillin	100	17 (8.5 %)
	Beta-lactam antibiotics (Cephalosporins)	Cefadroxil	30	9 (4.5 %)
		Cefuroxime	30	4 (2.0 %)
		Ceftriaxone	30	2 (1.0 %)
		Ceftazidime	30	17 (8.5 %)
		Cefepime	30	15 (7.5 %)
		Cefpirome	30	19 (9.5 %)
	Monobactams	Aztreonam	30	23 (11.5 %)
	Carbapenems	Imipenem	10	0 (0.0 %)
		Meropenem	10	0 (0.0 %)

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Inhibitor of nucleic acids	Quinolones	Nalidixic acid	30	27 (13.5 %)
	Fluroquinolones	Ciprofloxacin	5	4 (2.0 %)
		Levofloxacin	5	7 (3.5 %)
		Norfloxacin	10	15 (7.5 %)
	Furans	Nitrofurantoin	300	2 (1.0 %)
Inhibitor of membrane function	Polymyxins	Polymyxin B	300 U	6 3.0 %)
Inhibitor of metabolic processes	Sulphonamides	Trimethoprim/ sulfamethoxazole	25	25 (12.5 %)

Table 4: Multiple antibiotic resistance (MAR) and biofilm formation potential of the bacterial isolates

Bacterial isolates	MAR index calculation			Biofilm formation	
	Total number of antibiotics tested (y)	N of antibiotics to which the isolate was resistant (x)	MAR index (x/y)	24 h	48 h
E coli RBRJ005	24	7	0.29	+	++
E coli RBRJ013	24	8	0.33	++	+++
K pneumoniae RBRJ019	24	10	0.41	+	++
K pneumoniae RBRJ024	24	8	0.33	++	+++
Enterobacter cloacae RBRJ017	24	8	0.33	+	++
Acinetobacter baumannii RBRJ027	24	12	0.50	++	+++
Enterococcus faecalis RBRJ015	25	9	0.36	++	+++
Staphylococcus aureus RBRJ010	25	8	0.32	0	+

(0) non-biofilm producer; (+) weak biofilm producer; (++) moderate biofilm producer; (+++) strong biofilm producer;

Antibiotic susceptibility profile of multi-drug resistant uropathogens

Eight bacterial isolates were resistant to > 7 antibiotics and were considered multi-antibiotic resistant based on their antibiotic susceptibility profile. The bacterial isolates, namely *E coli* RBRJ005 and RBRJ013, *K pneumoniae* RBRJ019, RBRJ024, *Acinetobacter baumannii* RBRJ027, *Enterobacter cloacae* RBRJ017, *Enterococcus faecalis* RBRJ015 and *Staphylococcus aureus* RBRJ010 were resistant to > 7 antibiotics (Table 4).

Figure 3 shows the antibiotic resistance pattern, while Figure 4 shows the MIC values of the antibiotics against multi-drug resistant strains. The two *E coli* strains ie RBRJ005 and RBRJ013 were found to be resistant to antibiotics commonly used to treat UTIs. The antibiotic profile of the strain RBRJ005 showed that it was resistant to trimethoprim/sulfamethoxazole, ceftazidime, aztreonam, ampicillin, piperacillin, amoxicillin/clavulanic acid, nalidixic acid and cefepime, while the strain RBRJ013 was found to be resistant to antibiotics: trimethoprim/sulfamethoxazole, carbenicillin, ceftazidime, aztreonam, ampicillin, nalidixic acid, cefpirome and cefepime. The MIC assay revealed that both the strains were resistant to amoxicillin/clavulanic acid, nalidixic acid, piperacillin and aztreonam.

Similarly, the two strains of *K pneumoniae* RBRJ019 and RBRJ024 were found to be resistant to various antibiotics used to treat UTIs. The MIC assay revealed that the strain RBRJ019 was resistant to ciprofloxacin, amoxicillin/clavulanic acid and gentamicin, while the strain RBRJ024 was resistant to ciprofloxacin, amoxicillin/clavulanic acid, gentamicin, piperacillin, aztreonam and norfloxacin at the specified concentrations given by CLSI. The strain RBRJ017 of *Enterobacter cloacae* was also observed for antibiotic resistance pattern and found that it was resistant to cefuroxime, levofloxacin, cefadroxil, ampicillin, nitrofurantoin, norfloxacin, amoxicillin/clavulanic acid and polymyxin-B at the concentrations specified by CLSI. The other Gram negative bacteria *Acinetobacter baumannii* RBRJ027 of family *Moraxellaceae* was also explored for its antibiotic susceptibility profile and was found to be resistant to many antibiotics, namely amoxicillin/clavulanic acid, nitrofurantoin, piperacillin, aztreonam and cefepime.

The two Gram positive bacteria *Enterococcus faecalis* RBRJ015 and *Staphylococcus aureus* RBRJ010 were investigated for their susceptibility towards different antibiotics including methicillin. The strain RBRJ015 was resistant to ceftazidime, aztreonam, amikacin, nalidixic acid,

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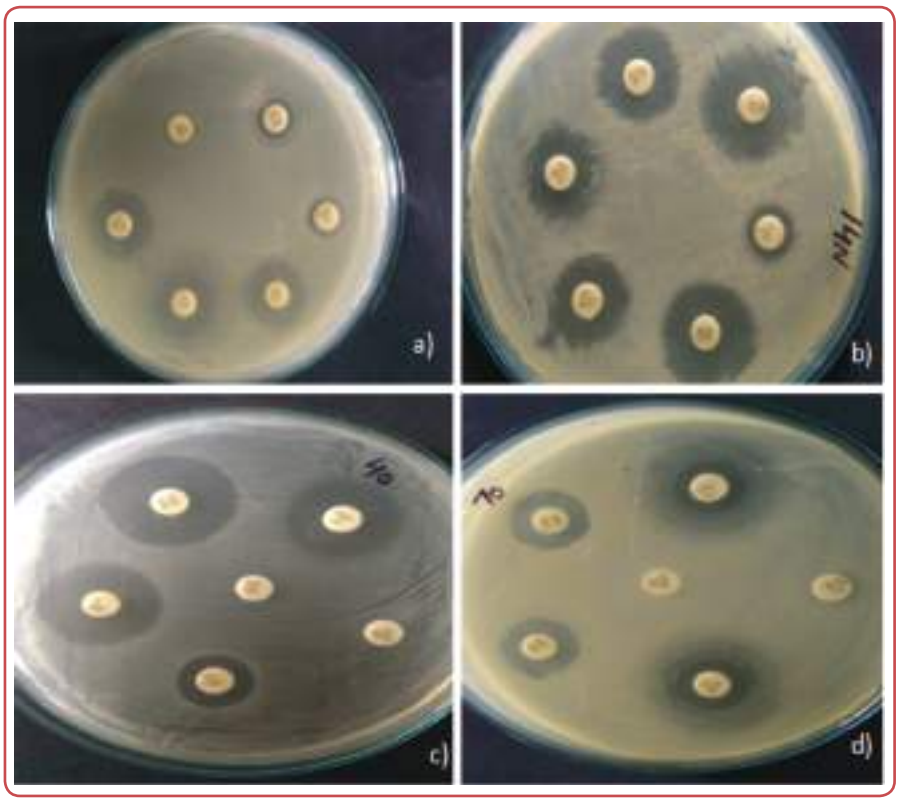

Figure 3: Antibiotic susceptibility of a) Enterobacter cloacae b) Escherichia coli c) Enterococcus faecalis d) Acinetobacter baumannii towards different antibiotics

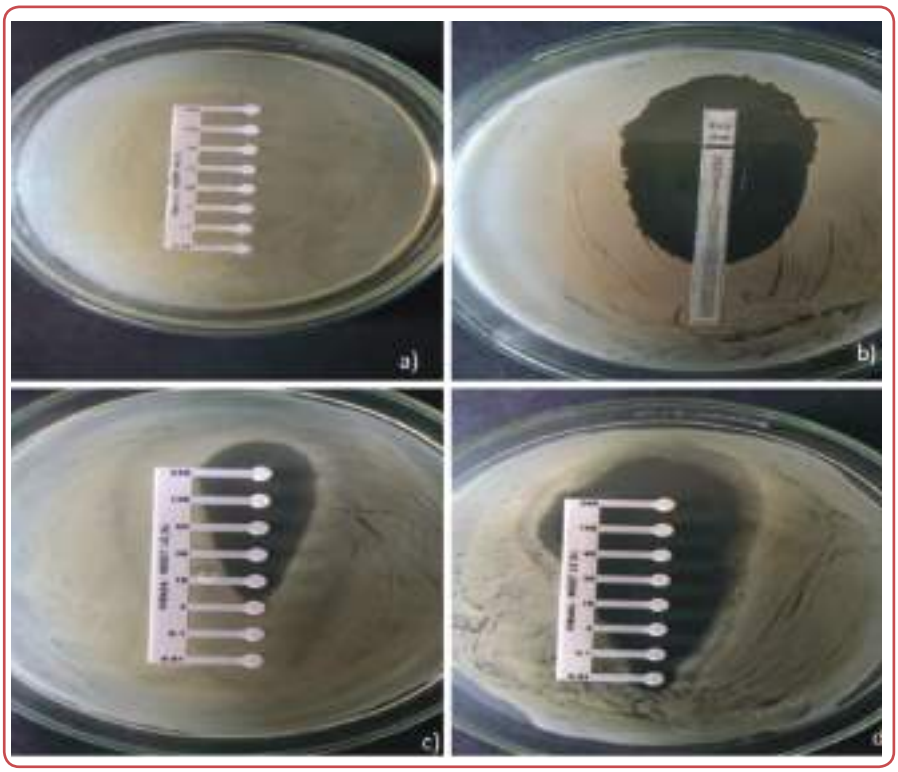

Figure 4: Minimum inhibitory concentration (MIC) of the antibiotics towards different bacterial isolates; a) Enterococcus faecalis b) E coli c) Enterobacter cloacae d) Acinetobacter baumannii

Table 5: Antibiotic resistant genes present in bacterial isolates

Type of gene	Bacterial isolates					
	E coli RBRJ005	*E coli* RBRJ013	*K pneumoniae* RBRJ019	*K pneumoniae* RBRJ024	*Enterobacter cloacae* RBRJ019	*Acinetobacter baumannii* RBRJ027
bla_{SHV}	+	+	+	+	+	+
bla_{TEM}	+	+	+	+	+	+
bla_{CTX-M}	-	+	+	+	+	+
bla_{AmpC}	+	+	+	+	+	+
qnr_A	-	-	-	-	-	-
qnr_B	+	+	+	+	+	+

Gene: '+' present; '-'absent;

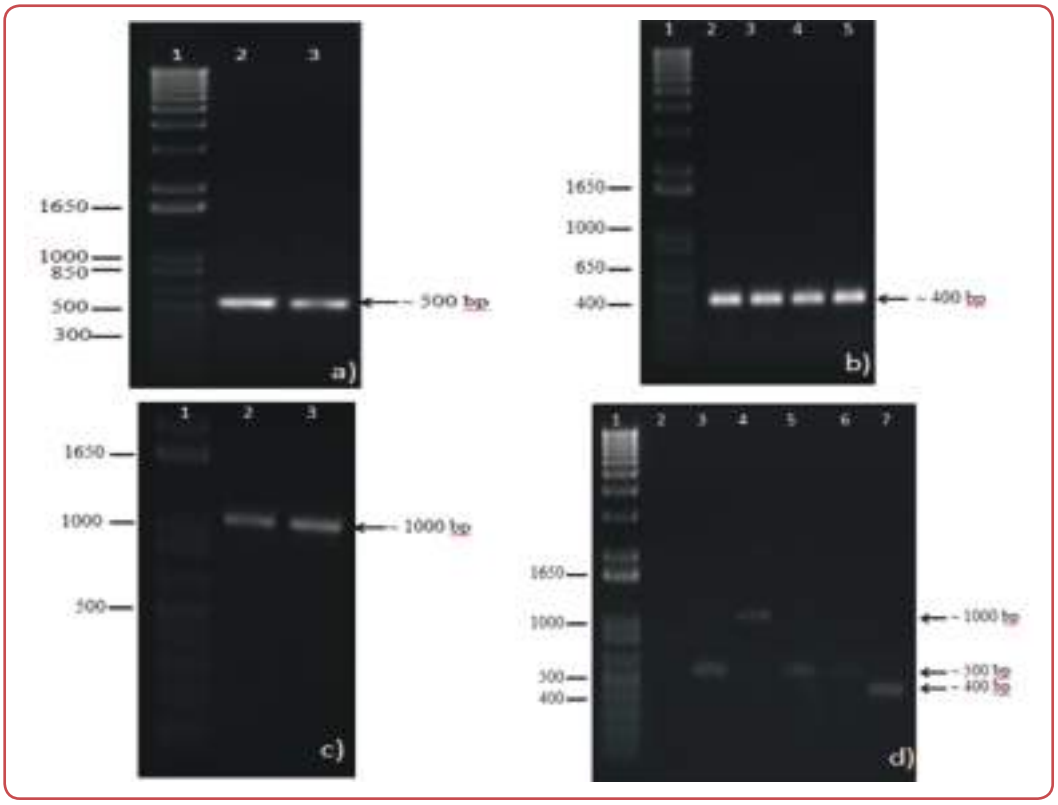

Figure 5: Gel electrophoresis of the PCR products

a) Gel electrophoresis of the PCR products of bla_{CTX-M} gene; lane 1: DNA Ladder (1 Kb), lane 2-3: 500 bp band of PCR product in K pneumoniae RBRJ019 and Acinetobacter baumannii RBRJ027;
b) Gel electrophoresis of the PCR products of qnr_B gene; lane 1: DNA Ladder (1 Kb), lane 2-5: 400 bp band of PCR product in E coli RBRJ013, K pneumoniae RBRJ019, K pneumoniae, RBRJ024, Enterobacter cloacae RBRJ017;
c) Gel electrophoresis of the PCR products of bla_{SHV} and bla_{TEM} gene in E coli RBRJ005; lane 1: DNA Ladder (1 Kb), lane 2-3: 1000 bp band of PCR product;
d) Gel electrophoresis of the PCR products of bla_{TEM} (lane 4) in K pneumoniae RBRJ024 (1000 bp); bla_{AmpC} (lane 3, 5, 6) in E coli RBRJ013, K pneumoniae RBRJ019 and Enterobacter cloacae RBRJ017 (500 bp); qnr_B (lane 7) in Acinetobacter baumannii RBRJ027 (400 bp); lane 1: DNA Ladder (1 Kb); lane 2: negative result.

polymyxin-B, cefpirome and cefepime, while the strain RBRJ010 was resistant to trimethoprim/sulfamethoxazole, aztreonam, ampicillin, piperacillin, amoxicillin/clavulanic acid, nalidixic acid, polymyxin-B and cefepime antibiotics. The strain RBRJ010 was resistant to polymyxin-B, amoxicillin/clavulanic acid, nalidixic acid, piperacillin, aztreonam and cefepime, while the antibiotics polymyxin-B, nalidixic acid, aztreonam, amikacin and cefepime were proved ineffective against the strain RBRJ015. The bacterium *Staphylococcus aureus* RBRJ010 was susceptible to antibiotic methicillin giving inhibition zone of 18 mm, while the bacterium *Enterococcus faecalis* RBRJ015 was resistant to it.

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Detection of antibiotic resistant genes

All the multi-antibiotic resistant Gram-negative bacterial strains ie RBRJ005, RBRJ013, RBRJ019, RBRJ024, RBRJ017 and RBRJ027 were ESBL producers.

The presence of the β-lactamase genes *viz* $bla_{SHV,}$ $bla_{CTX-M,}$ bla_{TEM}, bla_{ampC} and quinolone-resistant genes *viz qnr*A and *qnr*B among bacterial isolates is given in Table 5. bla_{SHV}, bla_{TEM} and bla_{ampC} were present in all the members of the family *Enterobacteriaceae*, while bla_{CTX-M} was present in RBRJ013, RBRJ019, RBRJ024 strains. The strain RBRJ027 was found to be positive for bla_{TEM}, bla_{CTX-M} and bla_{ampC}. The quinolone genes *viz qnr*A was absent in all the MAR isolates, while *qnr*B was present in all the isolates. The bands in the agarose gel showed the presence of various genes in extra-chromosomal DNA of the bacterial isolates (Figure 5).

Biofilm assay

The isolates, *E coli* RBRJ013, *K pneumonia*e RBRJ024, *Acinetobacter baumannii* RBRJ027 and *Enterococcus faecalis* RBRJ015 were moderate biofilm producers within 24 h and strong biofilm producers within 48 h (Table 4). On the other hand, the strain RBRJ010 of *Staphylococcus aureus* was unable to form biofilm within 24 h. The study results showed that most isolates possess strong adhesion potential to form biofilms.

Discussion

In the preliminary analysis of the urine samples, the results of the microscopic study and dip sticks observations were recorded. The UTI symptoms like burning micturition, dysuria, frequency/urgency, pyuria and haematuria were recorded from the questionnaires filled by the patients and by direct and microscopic observations of the samples. Only those samples were selected for the study which showed these symptoms and gave positive culture results. The females were selected for this study as they are more prone to UTI's. The main reasons for UTI to be more common in females is due to their shorter urethra as compared to males, reduction in normal microflora ie *Lactobacilli*, less acidic pH of the vagina, poor hygiene and sanitation conditions.[23, 24]

The patients of all age groups showed the condition of dysuria with other symptoms like urine urgency, frequency, painful micturition, nocturia and bladder discomfort. These all symptoms and conditions among adult females were also reported by Wrenn (1990).[25] The main cause of dysuria is stones in the urinary tract or inflammation of the bladder (cystitis), inflammation of the kidney (pyelonephritis) and inflammation of the urethra (urethritis).[26] According to Kurowski, in adult women, the pain felt due to the passage of urine over the inflamed vaginal labia indicates external dysuria which may be due to vaginal infection or inflammation, while the pain felt inside the body is due to internal dysuria which may be due to bacterial cystitis or urethritis.[27]

The urinary urgency and frequency were more observed in the age group 31-40 years followed by 41-50 years. Urinary urgency was mainly caused by trigonal or posterior urethral irritation which may be due to the presence of stones, inflammation or tumours. The discharge from the urethra was mainly linked with the condition of urethritis.[28] The history of the frequency of normal urination is somewhat difficult to obtain as it is different for every individual depending upon their bladder capacity and fluid intake. The urgency to urinate may arise with or without urination and is highest in incontinence. The urge to urinate may become constant in lower urinary tract inflammation by eliminating only a few millilitres of urine during each voiding.[25, 29]

The increased number of pus cells or leukocytes (WBCs) (≥ 10 WBC/high power field) in microscopic examination or the positive leukocyte esterase test of the urine samples indicates pyuria and evidence for the inflammation of the genitourinary tract.[30]

The formation of kidney stones is due to the accumulation of dissolved minerals on the kidney's inner lining. These minerals in the urine lead to the formation of crystals. Most of the stones are composed of calcium followed by uric acid, struvite and cystine. These are mainly present in infected urine so also called infection stones. Inside the kidneys, the urine backs up in the tubes when the urinary tract is blocked by these stones. The bacteria that may trap in the urine due to blockage cause UTI; also the excessive pressure on the kidneys results in swelling (hydronephrosis) and kidney damage.[31]

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The alignment of partial 16S rRNA sequences against the NCBI database suggested that bacterial isolates belong to different bacterial species. The results of the present study are in accordance with the other researchers who reported *E coli* as the prime aetiological agent in causing UTIs among females.[32-37] The presence of bacteria in the urine indicates UTI or bacteriuria. The presence of at least 10^4 bacteria/mL in a freshly voided midstream urine sample indicates significant bacteriuria. Bacteria invade the urinary tract by ascending or descending invasion causing UTIs. The ascending pathway is the more common mode where the normal faecal microbiota gets access to the urinary tract by colonising the urethra. The bacterium mainly involved in UTIs is the bowel microbiota ie *E coli* in most cases causing ascending infection.[38, 39]

The symptomatic infections are linked with the virulence of the causing organism which competes with the innate defence system of the host and the inflammation or injury is due to the host's immune response not because of bacterium.[40] The bacteria colonise the urethral opening often called microbiota is routinely present in the urine in both men and women. But, the bacteria present in the urine in the urethra are often flushed out during micturition. In women, the shorter distance to the bladder makes it easy for the uropathogens to access and colonise the bladder easily before being removed by urination. Also, the closer proximity of the urethral opening, vaginal cavity and rectum make it easier for the bacterial colonisers to get easy access to the bladder.[6, 34] Sexual activity may also directly transfers bacteria from the vaginal cavity to the urethra or indirectly through oral sex. Mostly uncomplicated UTIs are associated with sexual activities and are more common among the females of the age group 18-29 years.[6, 41]

Resistance to commonly used antibiotics used against various infections is now a serious global problem. The current study revealed the antibiotic resistance among isolated uropathogens against commonly prescribed antibiotics in UTI's. Despite the large availability of antibiotics, UTIs are still the most common among females.[42] Antibiotic course during UTI affects the normal vaginal and gastrointestinal flora to great extents.[43] Antimicrobial resistance among uropathogens varies from one region to another depending upon many factors. The most common one is prescription of antibiotics by physicians without any culture sensitivity testing and their haphazard use by laypeople leads to increasing resistance among bacteria. Also, the improper dose, duration and leaving the antibiotic course in between make the uropathogens more resistant.[44] Besides this, the use of antibiotics in fish farms and the animal farming sector makes animals and poultry resistant thus, transferring resistant strains to humans.[45] Another main reason for antibiotic resistance among uropathogens is mainly due to horizontal antibiotic gene transfer.[46] It also leads from one bacterium to other and through this process, bacteria become resistant to more than one antibiotic at once. The bacterial plasmid DNA possesses multidrug resistance genes which they transfer to other enterobacterial species.[47, 48]

The treatment of UTIs is increasingly getting complicated because bacteria develop resistance to various antibiotics. The increasing antibiotic resistance among bacteria often leads to treatment failures which have serious effects on critically ill patients.[49] The resistant bacteria, particularly *E coli*, *Klebsiella spp*, *Pseudomonas spp*, *Enterobacter spp*, *Staphylococcus spp* and *Enterococcus spp* are more commonly emerging in community-acquired as well as in nosocomial infections.[50] The susceptible bacterial population may acquire resistance to antimicrobial agents through mutation and selection or through genetic information from other bacteria that encodes resistance involving different mechanisms such as conjugation, transformation and transduction.[46] Eight bacterial isolates were resistant to > 7 antibiotics and were considered multi-antibiotic resistant based on their antibiotic susceptibility profile. The bacterial isolates, namely *E coli*, *K pneumoniae*, *Acinetobacter baumannii*, *Enterobacter cloacae*, *Enterococcus faecalis* and *Staphylococcus aureus* were resistant to > 7 antibiotics. The results are similar with the studies published from other regions of the country.[51-54]

All the multi-antibiotic resistant Gram-negative bacterial strains ie RBRJ005, RBRJ013, RBRJ019, RBRJ024, RBRJ017 and RBRJ027 were ESBL producers. The frequency of ESBL-producing members of the family *Enterobacteriaceae* isolated from urine samples varies in different regions of the country and was studied by many authors.[37, 55]

UTIs are the most common bacterial infections in women and *E coli* is the primary pathogenic agent in these infections. There were many reports across the country on antibiotic resistance

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and ESBL-producing Gram-negative bacilli isolated from urine samples. Gajamer et al investigated the major ESBL-producing uropathogens in female patients of Sikkim and Darjeeling.[37] They found that the $bla_{CTX\text{-}M\text{-}15}$ group was more predominant in the isolates than all other ESBL genes. In a similar study, Ojdana et al observed the prevalence of $bla_{CTX\text{-}M}$, bla_{SHV} and bla_{TEM} genes in *K pneumoniae*, *E coli* and *Proteus mirabilis* strains.[56] They revealed that thirty-six of the tested strains exhibited $bla_{CTX\text{-}M}$ genes, twelve strains harboured bla_{SHV} genes and twenty-five strains showed the presence of bla_{TEM} gene respectively. Gajamer et al studied the occurrence of Extended Spectrum β lactamase genes coexisting with carbapenemase, AmpC and aminoglycoside resistance gene in uropathogens and reported the high prevalence of carbapenemase resistance among ESBL positive isolates.[57]

Similarly, the prevalence of quinolone resistance genes in uropathogenic *E coli* was observed by Malekzadegan et al and revealed that 33.1 % of the isolates were positive for *qnr*S gene and 12.4 % of the isolates were positive for *qnr*B genes respectively, while, none were found to be positive for *qnr*A gene.[58] The present study also reported similar findings. Tayebi et al investigated the plasmid-mediated quinolone resistance genes in ESBL-producing *E coli* isolated from UTIs and found that the widespread presence of plasmid-mediated quinolone resistance genes in ESBL-positive isolates is increasing at an alarming rate.[59] Presented study findings were also concordant with these as all the Gram-negative *Bacilli* possess *qnr*B gene. The co-dissemination of these genes among bacterial isolates is a major threat to public health.

There were many reports on uropathogenic *E coli* (UPEC) which is the primary causal agent of UTIs, forming biofilms on different sources. In a recent study, Eberly et al reported that *E coli* forms biofilms on catheters as well as on and within urinary bladder epithelial cells.[60] Biofilms mainly protects these isolates from antibacterial agents, environmental conditions and the host's immune system. In another study, Zheng et al characterised the biofilm formation by *Enterococcus faecalis* isolates derived from UTIs in China.[61] Alves et al, Taya et al and Karigoudar et al have seen a significant association between the antibiotic resistance pattern and biofilm formation among clinical isolates from UTIs.[62-64]

Conclusion

The current study revealed that the incidence of UTIs is more prominent in the age groups 21-30 and 31-40 years. The menacing state of drug resistance among Gram-negative bacilli in this geographical region is revealed. The effective group of antibiotics against these isolates are aminoglycosides (gentamicin) and carbapenems (imipenem and meropenem). All the Gram-negative multidrug-resistant isolates were ESBL producers which also possess *qnr*B gene. Furthermore, it is extremely crucial to design a strict antibiotics prescription policy and judicious use of antibiotics should be encouraged.

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Conflict of interest

None.

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ORIGINAL ARTICLE

The Acceptance and Commitment Therapy (ACT) Reduce Stress in Patients With Type 2 Diabetes Mellitus

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Abstract

Background/Aim: Diabetes mellitus patients may be more vulnerable to stress due to the requirement for lifestyle changes and blood sugar management. Stress occurs when people sense a mismatch between their available resources and the expectations put on them, especially when the expectations are believed to be excessive in comparison to the preceding circumstance. The purpose of this study was to look at the effectiveness of the acceptance and commitment therapy (ACT) as a stress-reduction intervention in patients with type 2 diabetes mellitus (T2DM) by enhancing their psychological well-being.
Methods: A total of 40 people were allocated to either the intervention or control groups at random. The intervention group got counselling on the pillars of diabetes control, followed by a stress level pre-test. Following that, the ACT intervention was carried out. After completing the post-test, the control group got the same intervention. The ACT module and a questionnaire to evaluate stress levels in T2DM patients were used in this study.
Results: Following the ACT session, the measuring findings for stress levels in the intervention group showed a reduction. Both the intervention and control groups saw stress reduction; however, the intervention group had a greater reduction.
Conclusions: The ACT intervention reduced stress levels in the intervention group. ACT appeared as the variable having the largest effect on lowering stress levels after taking into account variables such as duration of suffering, education, sex and age-related to T2DM.

Key words: Stress levels; Type 2 diabetes mellitus; Acceptance and commitment therapy.

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Introduction

Diabetes mellitus (DM) with severe consequences is one of the national issues that need greater attention.[1-3] According to Basic Health Research statistics in Indonesia, there are 10 million diabetics and 17.9 million persons at risk of developing the condition. Meanwhile, East Java Province ranks eighth in Indonesia in terms of diabetes prevalence, with a prevalence of 6.8 %. This result is one notch higher than DKI Jakarta, which is ranked tenth with a prevalence of 6.6 %. Meanwhile, North Maluku ranks first with a frequency of 11.1 %. While Surabaya has a greater incidence, it is ranked sixth.[4]

DM is a chronic condition with a long treatment period and high treatment expenses and the prevalence of DM is growing.[5-7] DM patients may be more vulnerable to stress since they must

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change their lives or maintain their blood sugar levels under control. One of the factors that causes stress in persons with type 2 DM (T2DM) is having unrealistic expectations. When expectations are judged to be excessive and distinct from the prior circumstance, stress occurs.[8, 9]

DM can be caused by a variety of variables, including heredity/genetic factors, obesity, lifestyle changes, poor food, medicines that impact blood sugar levels, a lack of physical exercise, the aging process, pregnancy, smoking and stress.[10-16] DM and stress have a very strong link, especially among city dwellers.[16-19] Stress and an unhealthy lifestyle, along with more rapid technology improvements and numerous ailments being endured, cause a reduction in a person's condition, triggering stress. DM patients who are stressed may have difficulty managing their blood sugar levels. Stress increases the excretion of catecholamines, glucagon, glucocorticoids, endorphins and growth hormones.[9, 20, 21]

Patients who were diagnosed with DM began to feel the emotional consequences of the circumstance and the disease remained for several months. Patients begin developing psychological problems including stress from the medical care they are receiving.[19, 22-25] Stress is the body's non-specific reaction to any disrupted bodily demands. Emotional stress can cause unpleasant or harmful sentiments toward oneself and others.[26, 27] Intellectual stress will impair one's perception and capacity to solve issues, while social stress will impair one's interpersonal interactions.[28-31]

The acceptance and commitment therapy (ACT) is a cognitive treatment that focuses on understanding self-emotions and actual behavioural interactions. Acceptance and care for individual values and behavioural principles are combined in ACT.[32-35] Acceptance focuses on the feelings, ideas and mental states experienced without avoiding or altering the circumstance. Behavioural concepts are utilised to attain goals by focusing on individual values.[36]

When compared to other therapies, ACT employs acceptance to increase psychological flexibility, behaviour change, acceptance and attentiveness. Anxiety, substance abuse, work stress, chronic pain and smoking among patients all decreased after the ACT intervention was provided. ACT differs from other cognitive behaviour treatments in that it focuses on the clarity of individual life objectives as well as the acceptance of value-emphasising beliefs.[37-43]

This study intended to fill a gap in prior research by investigating the use of the ACT in lowering stress in T2DM patients. Despite the fact that various prior studies have been conducted to assess the efficiency of ACT in diabetes control,[44-49] there have been no studies particularly investigating the use of ACT to alleviate stress in T2DM patients. Furthermore, the study varied from past research in that it compares the effectiveness of ACT with other mindfulness techniques.[49] Aim of this study was to give a better knowledge of the effectiveness of ACT in lowering stress in T2DM patients and lead to the development of more effective therapies to reduce diabetic patients' stress levels.

Methods

This research was conducted in June-July 2019 at the Islamic Hospital A Yani Surabaya, Indonesia. Before the start of the experiment, both the control and intervention groups were given an initial stress level questionnaire to determine their baseline stress levels. Participants in both groups were also given complete diabetes management information.

The intervention group participated in the ACT intervention program that consisted of four weekly sessions carried out over four weeks. Each class, which lasted around 30 to 45 minutes, was devoted to a certain topic. At the end of each session, participants were asked to reflect on their development and discoveries, which they wrote in a researcher-designed logbook. In the fourth week, a post-test was administered in conjunction with the control group, utilising a predefined questionnaire to assess stress levels. A simple random sampling procedure was used to determine the proper sample size, which might include up to 40 respondents. Furthermore, DM individuals with regular blood sugar level management and no substantial health issues were chosen using sequential sampling. Both the control and intervention groups had a maximum of 20 participants.

The inclusion criteria included a set of key criteria that persons participating in both the control

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and intervention groups must meet. Before the intervention, participants were asked to complete a stress level questionnaire to develop a basic understanding of their stress levels, which served as a baseline. Furthermore, before the intervention period, all people in both groups were exposed to complete diabetes management information. Participants assigned to the intervention group were required to actively participate in the ACT intervention. Attending four weekly sessions over four weeks was required for this intervention. Following that, participants in the intervention group were invited to participate in introspection, writing their own experiences and developmental insights in a notebook thoughtfully provided by the researchers after each session. As the fourth week approached, all individuals performed a post-test evaluation utilising a rigorously constructed questionnaire, which was administered concurrently with the control group, to determine stress levels.

The exclusion criteria included restrictions that guide the identification and exclusion of persons judged unfit for participation in the study. Individuals who did not reply to the first stress level questionnaire were mostly excluded from the analysis. Individuals who did not get comprehensive diabetes management information did not fulfil the study's inclusion criteria. Participants who refused or showed reluctance to participate in the ACT intervention were removed from the intervention group. As a result, people in the intervention group who were unwilling or unable to submit their thoughts within the offered logbook may have resulted in inadequate data collection. Individuals who did not complete the post-intervention evaluation in the fourth week were also excluded from the final analysis. Finally, DM individuals with significant health issues or irregular blood sugar level management were not eligible to participate in the study.

Instruments

The stress level questionnaire utilised in this study was adapted from the Post Traumatic Stress Disorder (PTSD) Checklist Civilian Version (PCL-C), a well-established instrument that has undergone rigorous validation and has been extensively employed in previous research endeavours.[51] The PCL-C serves as a comprehensive tool designed to assess stress levels and their ramifications on individuals.

This questionnaire comprises a series of inquiries intentionally crafted to discern the magnitude of stress experienced by participants. The exact number of items in this questionnaire was conformed to the structure and content of the original PCL-C, which underpins the questionnaire development for this study. Typically, the PCL-C consists of approximately 17 to 20 items, contingent upon the version and modifications adopted for the specific investigation. Each individual question item within the questionnaire pertained to symptoms or sentiments associated with stress levels.

The evaluation methodology of this questionnaire entailed participants responding to each question item based on their personal experiences and emotions. Each item presented response options that elucidate the extent to which participants have encountered the symptoms or emotions encapsulated within the posed questions. Generally, a Likert scale was employed as the response metric, encompassing a range of values such as "Not at all" to "Very strongly." Participants were prompted to select the response that most accurately aligns with their personal experiences and feelings. The cumulative values of the responses attributed to each question item were aggregated to compute a composite score. This cumulative score reflected the perceived stress level of participants. Higher scores denoted heightened levels of stress. Typically, the PCL-C employs predefined cut-off points that facilitate the categorisation of stress levels, such as "No stress," "Mild stress," "Moderate stress," and so forth.

The entire procedure was conducted to ascertain the baseline stress levels of subjects prior to the implementation of the intervention and to juxtapose alterations in stress levels subsequent to the intervention's execution. The data gleaned from this questionnaire were instrumental in discerning the impact of the intervention on stress levels, furnishing a foundational framework for subsequent analytical processes within the purview of this study.

The weekly therapy sessions in this study encompassed a diverse range of topics meticulously curated to address various dimensions of stress management and emotional well-being. These sessions served as comprehensive platforms for engaging participants in constructive discussions and experiential exercises. During the inaugural session, participants delved into an exploration

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of the fundamental concept of stress, gaining a comprehensive understanding of stressors, physiological responses and the intricate interplay between stress and emotional and physical health. The subsequent session focused on cultivating mindfulness and acceptance techniques, offering participants practical tools to enhance their present-moment awareness and reduce the impact of persistent rumination. Guiding participants towards a deeper level of self-awareness, the third session centred on values and commitment, encouraging participants to unearth their core values and correlate them with their life aspirations. This linkage between values and psychological well-being underscored the importance of alignment between one's actions and intrinsic values, ultimately nurturing emotional resilience during times of stress. The concluding session centred on fostering positivity and future-oriented thinking. Participants embarked on a journey of recognising the potency of positive self-affirmations in bolstering self-esteem and subsequently, in managing stress. Moreover, the session imparted techniques for formulating realistic goals and strategies to pave a proactive path for the future. Throughout this four-week intervention, each meticulously orchestrated session combined experiential exercises, guided discussions and reflective practices to equip participants with invaluable skills and insights. The holistic impact of these sequential sessions contributed to the overarching research objective, which was to assess the efficacy of the ACT intervention in alleviating stress levels among participants, thereby enriching their overall emotional well-being.

Data analysis

Multiple linear regression with a significance threshold of $p < 0.05$, as well as the statistical tests paired t-test and independent t-test, were employed to analyse the data.

Ethical consideration

The Islamic Hospital A Yani Surabaya's Ethical Review Board approved this study with permission No 017. EC.KEP.RSIAY.06.19. Prior to data collection, all participants provided informed consent, assuring their voluntary participation and the protection of their rights. Throughout the study, anonymity and secrecy were scrupulously observed. Personal information and identities of participants were kept with the utmost care and data were gathered and analysed in an aggregated and de-identified way. To guarantee fairness and eliminate prejudice, individuals were randomly assigned to intervention and control groups. It is worth emphasising that following the trial, there are plans to deliver the intervention program to the control group, providing fair benefits for all participants. These ethical issues were prioritised and presented in accordance with best standards, assuring the ethical integrity and protection of the participants throughout the study process.

Results

The research had 20 participants in the control group and 20 participants in the intervention group. Most respondents were in the 41-55 year range, female and with a high school education level. Finally, respondents with DM for less than 6 years made up the biggest group. There were no difference in the characteristics of respondents, such as age, gender, education and length of DM suffering, between the control and intervention groups (Table 1).

Table 1: Demographic characteristics of the respondents

Characteristics	Control group (n = 20)		Intervention group (n = 20)		p-value
	N	%	N	%	
Age (years)					
25-40	4	20	4	20	
41-55	13	65	12	60	0.715**
> 56	3	15	4	20	
Gender					
Male	6	30	4	20	0.520*
Female	14	70	16	80	
Last education					
No school	2	10	1	5	
Elementary school	4	20	3	15	1.000**
Secondary school	3	15	5	25	
High school	10	50	9	45	
Diploma/S1/S2	1	5	2	10	
Duration of DM					
< 6 years	14	70	13	65	0.117*
> 6 years	6	30	7	35	

** p < 0.05 based on the Pearson Chi-square test; **p < 0.05 based on the Chi-square Fisher's exact test; DM: diabetes mellitus;*

Before the intervention, stress levels in the control group were 25.93 ± 3.88, but after the intervention, they climbed to 26.93 ± 3.24 ($p < 0.001$) (Table 2). Meanwhile, baseline stress levels in the intervention group were 25.93 ±

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3.43, but after the intervention, they climbed to 30.73 ± 2.83 ($p < 0.001$). Prior to doing regression analysis, relevant assumptions such as normality, homoscedasticity and independence were investigated. All these assumptions have been satisfied, which validates the results.

Table 2: Changes in the respondent of stress level before and after being given the acceptance and commitment therapy (ACT)

Respondents	Stress level (mean ± SD)		95 % CI	T	p-value
	Before	After			
Control group	25.93 ± 3.88	25.93 ± 3.88	-1.37; -0.63	5.58	< 0.001
Intervention group	25.93 ± 3.43	25.93 ± 3.43	-5.54; -4.07	13.33	< 0.001

**p < 0.05 based on paired t-test; CI: Confidence interval;*

Table 3: The value of the difference in respondents' stress levels before and after being given the acceptance and commitment therapy (ACT)

Stress level	Control group	Intervention group	Mean difference	95% CI	p-value
Value difference	1.00 ± 0.98	4.80 ± 1.97	3.8	-4.61; -3.0	< 0.001*

**p < 0.05 based on independent t-test; CI: confidence interval;*

Table 3 shows the differences in stress levels before and after ACT delivery for both the control and intervention groups. The stress level in the control group was 1.00 ± 0.98 before the ACT intervention, however it jumped to 4.80 ± 1.97 after the session ($p < 0.001$). The mean difference between pre- and post-intervention stress levels was 3.80 ($p < 0.001$). Similarly, before the ACT session, the stress level in the intervention group was 1.00 ± 0.98, and after the intervention, it climbed to 4.80 ± 1.97. The mean difference in stress levels between pre- and post-intervention evaluations was 3.80 as well ($p < 0.001$).

Table 4: Results of bivariate analysis of risk factors related to the stress level

Risk factors	p-value*
Age of respondent	0.666
Gender	0.497
Education	0.027
Duration of DM	0.802
ACT intervention	0.000

**p < 0.25; DM: diabetes mellitus, ACT: acceptance and commitment therapy;*

According to bivariate data analysis results, ACT ($p < 0.001$) and education ($p = 0.027$), had a significant influence on the stress level (Table 4). This variable were subjected to multiple linear regression analysis (Table 5).

Table 5: Results of linear regression analysis of the acceptance and commitment therapy (ACT) intervention on stress levels

Risk factor	B	β	p-value*
Constant	25.82		< 0.001
ACT intervention	3.69	0.52	< 0.001
Education	0.79	0.25	0.021

**p < 0.05 based on linear regression;*

The constant value for stress levels reflecting a change in stress without any input from other factors is 25.82, as shown in Table 5. The findings of the linear regression analysis revealed that the ACT intervention had the greatest effect on the reduction in stress levels. The stress level is assessed to be 25.82 using the linear regression approach. Stress decreases by 3.69 for every gradual frequency of ACT session. Every extra degree of education reduces stress by 0.79.

Discussion

The homogeneity test performed in this study revealed no significant differences between respondents in the intervention and control groups. DM was found to be prevalent in those aged 41 to 55. Age is one element that increases

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stress levels in relation to DM features. The results of this study are in accordance with previous studies which stated that the productive age of 30-50 years is old adulthood, when a person prefers to work more, increasing the potential of stress, however at the age of > 50 years, one merely adjusts to what has been achieved in old age.[52-56]

This condition can affect both male and female diabetes individuals who are stressed. Gender is a risk factor for stress; women are more prone to stress than males in terms of blood sugar regulation. Women are more easily agitated when it comes to adhering to a DM intervention program. Overall, women tend to be more affected by psychological stress than males. Furthermore, women in older age have a higher risk of cardiovascular disease, myocardial infarction and stroke death than males.[57-60]

The outcomes of research at the highest levels of education indicated that high school had the most data in the intervention group. Some studies that support this research mention that the more information a person gains, the more competent they are to manage with stress than individuals with a lower degree of education. A lack of understanding in dealing with challenges at work might lead to stress.[61-69]

According to the findings, the majority of respondents had DM for at least 6 years. Those with DM who have had the condition for a longer period of time have less stress as they become accustomed to the treatment regimen.[8, 70-72]

Age, current education and duration of DM can all impact stress levels. However, how everyone controls the stress that arises in his body can also alter stress levels. Stress management is the ability to use (human) resources effectively to overcome mental and emotional disorders or disorders caused by responses, such as identifying habits or things that cause stress, changing habits that can cause stress/coping with unhealthy stress, socialising with others, making time to relax and living a healthy lifestyle.[9, 57, 73, 74]

The findings of evaluating variable stress levels in T2DM patients in the intervention group revealed that stress levels decreased following the ACT intervention. Prior to receiving ACT, respondents reported feeling stressed. The majority of responders were between the ages of 41 and 55. Indeed, in some studies it has been mentioned that in terms of stress levels, the elderly prefer to heed the advice of health practitioners and are more responsible, orderly and thorough in controlling their DMT2 condition. However, there are other reasons why the elderly are unable to handle stress when coping with diabetes, including restricted access and availability of psychiatric therapies, patients' limited time, money and mobility and fear of stigmatisation. The failure of health personnel to recognise and treat DM is thus a barrier to stress management.[75-79]

Female T2DM patients are better able to manage their diabetes following therapy because males engage in greater physical activity outside the home than women and consume more calories due to erroneous food composition.[80-82] This contradicts study by Anne et al, which demonstrates that males are more engaged in enforcing certain regulations than women. Furthermore, males often consume everything given by their family, unless their family has prepared an appropriate dinner for them.[83]

According to previous study, DM patients who have received information on the disease would have a reasonable degree of awareness would adhere to treatment and prevention more successfully.[8, 84-86] Although T2DM patients had a high degree of awareness of infection transmission, there is potential for improvement. It is usually assumed that communities that are more informed about this disease would adhere to preventative and treatment measures more efficiently.[87-90]

The severity of diabetes is a further variable that impacts stress levels in the management of T2DM. According to Hilliard et al research, the longer a person suffers from diabetes, the lower the degree of stress experienced since they are acclimated to the treatment procedure, however for someone who has just been diagnosed with diabetes, the stress level is considerably greater.[91]

Stress can be avoided in T2DM patients by following the DM management pillars of meal planning or diet control, physical activity, hyperglycaemic therapy and health education. However, the most crucial aspect of diabetes control is meal planning or diet management.[81, 92-96] Patient education is a key pillar in DM management for optimising interventional treatment. If education is delivered successfully, it can increase patient adherence and illness self-management, allowing patients to avoid stress.[97]

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ACT is a cognitive behavioural therapy that examines a person's clinical behaviour and experiences. The aim of ACT therapies are to improve well-being and reduce individual suffering. Unpleasant experiences is altered in accordance with the ACT philosophical thought. These experiences include increasing impairment as well as felt tension, stress and chronic discomfort. Acceptance and awareness of both good and unpleasant events, as well as supportive ideals are all part of the ACT intervention.[42, 98]

The ACT intervention has a considerable impact on the applicability of activities given by health staff to patients with T2DM in disease management.[99-104] ACT is a variant of cognitive behavioural therapy (CBT) that focuses on improving persons with T2DM's perspectives on illness acceptance and psychological well-being.[105-109] The purpose of the ACT intervention, which also involves families in its execution, is stress management in patients with T2DM. Making contracts with the patient's family to manage the stress of T2DM patients and to be more positive is the first stage in the ACT intervention.[8, 57, 110-112]

ACT intervention, according to the results of multivariate linear regression analysis, was a process-oriented treatment used to enhance psychological components in the individual's efforts to regulate himself and prevent undesired thoughts and sensations as a barrier to valued and successful action.[112-114] ACT was the variable with the greatest impact on stress reduction. T2DM patients' stress is caused by unstable blood sugar levels as a result of irregular control and limits in exams with health services.[115-117]

The Meleis transition theory method can promote adaptive coping and client responsiveness by enhancing the psychology and management of T2DM patients in coping with changes in their health.[118] ACT interventions are used to help people reach their health goals. The health counselling technique is carried out continually by the challenges encountered by patients for diabetics to become self-sufficient. The advantage of this strategy is that T2DM patients can actively engage in the decision-making process by following the prescribed treatment guidelines with or without the assistance of a health practitioner.[119-127]

The ACT method emphasises assisting people with T2DM to speak about what bothers them the most about their condition, what they want to change the most, the support they can gain for change and the hurdles or challenges that must be reduced to encourage healthy habits.[85, 128, 129] The primary purpose of the health coach is not to teach or offer counsel to patients, but rather to focus on specific concerns and challenges that are unique to each T2DM patient based on the context of the patient's life.[130-132]

Education level is another element that impacts the stress faced by DM patients when treating their DMT2 condition. The majority of patients have a high school diploma. The amount of formal education forms the foundation for patients to accomplish anything, to comprehend and comprehend something more, or to accept and reject something.[133] A well-educated individual is more mature in the process of self-change, making it simpler to absorb external influences that are positive, objective and open to learning about many health topic.[29, 67, 134, 135]

The development of constructive coping skills in the face of stresses is intended to give T2DM patients with a better knowledge.[61, 93, 136] Patients with T2DM who have undergone ACT therapies have a positive attitude and participate in necessary activities to reduce stress levels.[45, 47, 57, 137]

Through the deployment of successful DM management pillars to achieve stable blood sugar levels so that patients may minimize stress, ACT intervention can improve the positive view on DM management. The ACT intervention begins with an assessment of the patient's condition and then defines goals that address the problem of physical changes in the form of unstable blood sugar levels. T2DM patients' have psychological alterations due to stress caused by their DM condition. The following stage is to examine the problem, decide on potential possibilities and effect change by recognising and determining the commitment to action. This level is attained when T2DM patients comprehend the benefits of stress management in T2DM therapy.[8]
ACT treatments are psychological interventions that involve behaviour modification, acceptance and attention and acceptance to increase psychological flexibility and are more successful than other therapies. Anxiety, substance usage, stopping smoking, work stress and chronic pain all improved following the ACT session. ACT differs from other cognitive behavioural

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treatments in that it focuses entirely on the frequency of cognition and individual content.[38, 40, 108, 113, 138, 139]

The advantage of ACT therapies is that T2DM patients may participate more actively in what has been generated by following the specified intervention guidelines, with or without the assistance of health experts.[44, 119, 140-146]

Conclusion

The study's findings show that stress levels in the intervention group dropped when ACT was implemented. Furthermore, when evaluating characteristics related to DM such as length of suffering, education, gender and age, the ACT intervention appeared as the element having the highest impact in lowering stress levels. Based on these findings, it is critical for healthcare practitioners to emphasise the importance of T2DM patients evaluating their capacity to engage in health-promoting behaviours, such as stress management, as a method of effectively managing their medical condition. Incorporating ACT as a regular component in the therapy of T2DM patients can aid in the immediate and continual resolution of patient concerns. By applying these guidelines, healthcare practitioners can better help T2DM patients on their path to better health. Furthermore, policymakers and healthcare system stakeholders should explore incorporating ACT therapies into routine care for T2DM patients in order to improve patient outcomes and overall diabetic management.

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Conflict of interest

None.

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ORIGINAL ARTICLE

Artificial Intelligence (AI) Integration in Medical Education: A Pan-India Cross-Sectional Observation of Acceptance and Understanding Among Students

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Abstract

Background/Aim: From accurate diagnostics to personalised treatment plans, artificial intelligence (AI) has the potential to revolutionise healthcare. The abundance of medical data has led to AI being employed for accurate diagnoses, treatment protocols and patient care. Students' perception of AI integration in medical education is crucial for its successful implementation. This study aimed to assess the acceptance and understanding of AI integration among students in medical education across different regions of India through a cross-sectional observation.

Methods: A pan-India survey was conducted among medical students between 1 August 2023 to 20 August 2023 with a pre-validated questionnaire covering AI awareness and understanding through Google Form, circulated via WhatsApp.

Results: A total of 730 medical students completed the survey of which 58.6 % were males and 41.4 % were females. Most students (80.7 %) knew about AI, but 53.6 % had limited awareness of AI in medicine. Opinions on AI integration was diverse, with 46.8 % in favour. Workshops (45.2 %) and lectures (31.1 %) were preferred learning formats. Students were interested in various AI topics and expect AI to positively impact medicine (45.9 %). Radiology, surgery and general medicine were predicted to be most influenced by AI. Concerns about overreliance on AI (49.2 %) and lack of empathy (43.7 %) were highlighted.

Conclusions: Medical students in India display a keen interest in AI and its integration into medical education. To fully harness AI's potential in healthcare, comprehensive AI curricula and faculty training are needed. Students are aware of the challenges and opportunities, emphasising the importance of balanced AI adoption in medical practice and education.

Key words: Artificial intelligence; Medical education; Survey; pan-India; Medical students; Acceptance.

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Introduction

There is hardly anyone left in society who has never used man-made gadgets and devices, that have made everyday life easier. However, many inventors have tried to create machines with the intellect of a human brain. Today this is known as artificial intelligence (AI), a term attributed to Sir John McCarthy in the 1950s.[1]

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In today's world of modern medicine, AI is a life-changing force that drives humans to a new era, from the accuracy of diagnosing patients to personalised treatment plans. AI's integration into practicing medicine has the potential to be a revolutionising force. AI is capable of mimicking mental functions such as recognition of speech, solving problems and strategic reasoning. So, it could transform medicine by performing nearly accurate predictions in various tasks like diagnosing and treating a condition.[2,3] Presented article delves into the role of AI in college, exploring how it has the potential to revolutionise old-dated learning methods and shape the future of the healthcare education system.

Over time, medical data is becoming increasingly vast and unmanageable for human processing, for this purpose the concept of data feeding into AI has emerged which feeds on the large database and provides us with more accuracy. The greater amount of data leads to increased accuracy when it comes to diagnosis, establishing treatment protocol and providing patient care. AI is improving in assisting physicians to narrow down the probable diagnosis by translating the process of history taking, in which a doctor asks a series of questions and then combines them with symptoms reported, to arrive at a probable diagnosis.[3] Some of them are specialised in assessing the symptoms and guiding the patient to better advice on their diagnosis like Buoy Health, which was developed by Harvard medical school.[4,5] Other platforms like Linus Health focus on the early detection of cognitive function issues through neural pathway analysis.[6] Radiology has particularly benefited from AI, with improved diagnostic accuracy, faster image analysis and support in differential diagnosis research and data analysis, providing valuable tools for radiologists.[7,8]

Students should be introduced to AI right from the beginning of their medical education journey to help them become comfortable with its integration into the healthcare and medical education systems, but before that, it must looked into the acceptance of AI integration by students as well as their understanding of AI and evaluate what students think in terms of AI integration in the medical healthcare system or if they would appreciate and enjoy learning AI as a part of the formal education. For this purpose, the survey was conducted which allowed to take peek into the perception of students regarding AI in medical education.

For AI to be part of medical education various concerns have arisen, how education would be delivered, what are the topics to be taught, the medium by which they taught, whether is there a need to teach faculty first, is there any technical faculty required, should it be a part of a theory or combined with the practical curriculum.[9-11] Another dimension of concern comes with the ethical issues of AI in healthcare such as privacy and data compromise, informed consent, liability issues, algorithmic fairness and biases, which were main pillars that lead to legal issues.[12] Some of the challenges in medical education include difficulty in creating models, difficulty in assessing the effectiveness of AI and the lack of experienced and knowledgeable content specialists.[13]

Still, many questions are left unanswered but by combining the efforts of AI technology and humans together, which could surpass the issues faced by humans and AI working independently. There is no denying that many institutes have already taken the initiative to integrate AI to train their students in various medical practices.[14] National Yang-Ming Medical University in Taiwan is training interns to do sutures and ligatures via artificial skin which has a monitoring system [WKS-2RII] that asses the scoring of students' training thus improving their skills.[15]

Many administrators and educators, in the field of education are familiar with AI although they may not possess an understanding of its potential applications in medical education or the specific subjects that should be included in their curriculum, for students. Nevertheless, what AI will look like in the field of medical teaching and learning, how it could possibly affect learners and how to best utilise its benefits is still unclear.[16]

This study aimed to assess the acceptance and understanding of AI integration among students in medical education across different regions of India through a cross-sectional observation.

Methods

Design

This cross-sectional research study was designed to assess the knowledge of AI among medical college students in India and their attitudes toward integrating AI into the medical curriculum. It

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consisted of the following sections: consent, demographic details and a questionnaire consisting of 17 questions that have been pre-validated and tested for accuracy mainly based on topics covering AI awareness and understanding. The study was ethically approved by Swastic Ethics Committee (Decision No ECR/434/Inst./RJ/2013/RR-19, 15 July 2023).

Data collection

A simple random sampling method was used to collect data. Students from different years of study (eg first year, second year, third year and final year) have been considered as strata. Before the main data collection, the questionnaire was pilot-tested on a small group of medical students to identify any ambiguities, inconsistencies or difficulties in comprehension. The feedback from the pilot test has been used to refine the questionnaire.

Table 1: Questionnaire about knowledge and opinions about artificial intelligence

List of questions asked in the survey
1. Do you know what is artificial intelligence?
2. Are you aware of the uses of artificial intelligence in medicine?
3. Where have you learned about artificial intelligence in medicine?
4. Are you familiar with any application(s) currently using artificial intelligence to improve patient care, diagnostic accuracy and treatment planning?
5. Should artificial intelligence be a part of the medical college curriculum?
6. Your preferred format(s) of learning about artificial intelligence in medicine is/are:
7. What is/are the topic(s) in artificial intelligence that you would be most interested in?
8. What is the possible impact of artificial intelligence on medicine in the near future?
9. Which specialties will be most affected by artificial intelligence in the near future?
10. Artificial intelligence in medicine will be helpful as:
a) Supporting physicians in making a clinical diagnosis
b) Supporting physicians in the management protocol
c) Patients diagnosing and treating themselves with the help of artificial intelligence
d) It can improve the cost-effectiveness of medicine
11. Medical students who learn through artificial intelligence have the potential to become better physicians compared to their peers.
12. Possible disadvantage(s) of introducing artificial intelligence in medicine.
13. Possible challenge(s) in introducing artificial intelligence in therapeutics.

The questionnaire (Table 1) has been administered to participants through an online survey platform. The responses have been collected from 1 August 2023 to 20 August 2023. Participants have been provided with a clear explanation of the study's purpose and procedures and their informed consent has been obtained before participation.

Participant data were anonymised and kept confidential. Only aggregated data has been reported in the final article. The questionnaire was reviewed by a team of medical students and teachers.

Data analysis

The study utilised descriptive statistics like counts and percentages to summarise information that included details about their demographics (like age, gender, etc), understanding and attitude on the topic. To make sure that there were enough participants in the study for the results to be reliable, the following formula was used to figure out the right sample size for an infinite population[17] as mentioned below:

$$S = Z^2 \times P \times (1 - P) / M^2$$

Where: S = sample size for infinite population, Z^2 score, P-population proportion (assumed as 50 % or 0.5); M-margin of error, given: Z = 1.960, P = 0.5, M = 0.05 (Have taken the confidence level as 95 % and margin of error as 5 %). Thus, the sample size was calculated to be 384.16.

Result

The total number of responses received was n = 758 from the survey, out of which only n = 730 responses were valid responses. The survey was conducted online through a link generated via Google form and circulated all across medical colleges in India (Figure 1).

A 58.6 % of people participating in the survey were males. The majority of students in the study were in the age group 18 – 23 years old. A majority of participants demonstrated familiarity with AI, with 80.7 % acknowledging a fair understanding of AI, while 14.5 % were unsure and 4.8 % had no knowledge (Figure 2).

A 26.3 % of respondents were well-informed about the applications of AI in medicine, 53.6 % had a superficial understanding and the remaining 20.1 % lacked awareness (Figure 3).

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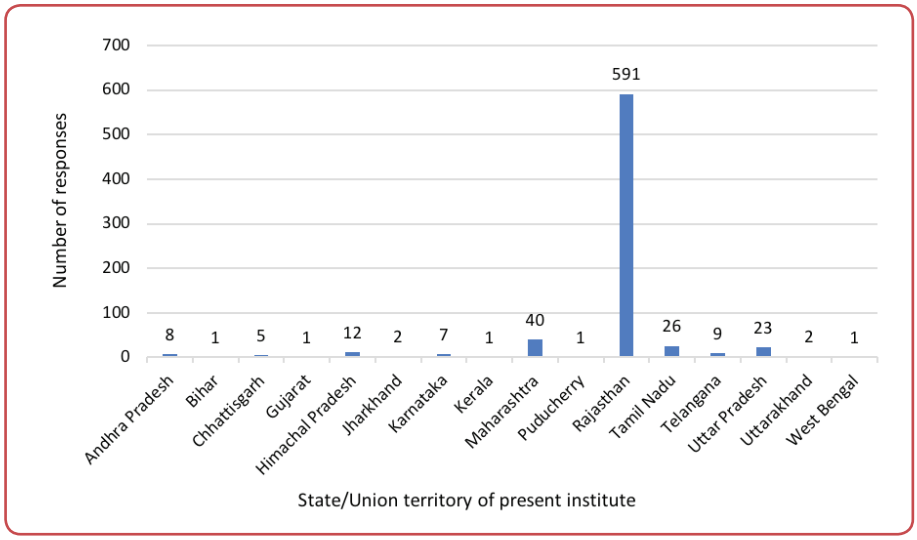

Figure 1: Number of responses from various state/union territory

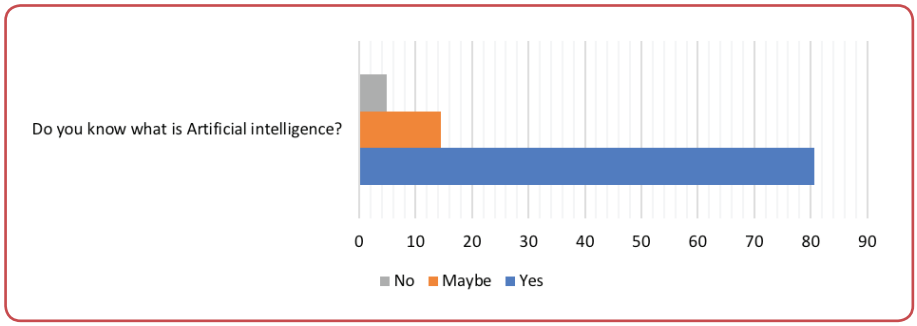

Figure 2: Survey on what is artificial intelligence (AI)

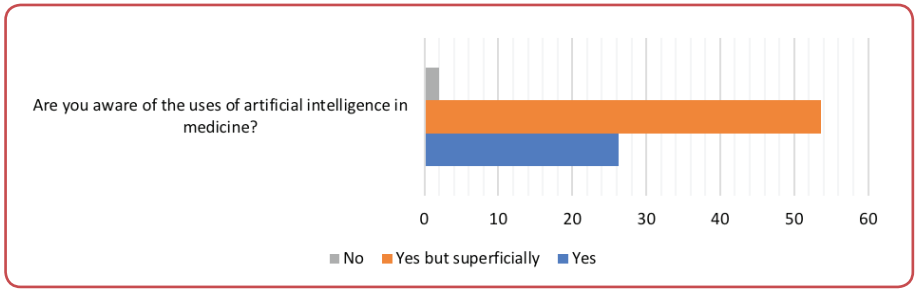

Figure 3: Survey on awareness of uses of artificial intelligence (AI) in medicine

Respondents believed that the main sources of their knowledge about AI in medicine were media (55.8 %), friends (28.1 %) and movies or TV series (34.2 %), which made them familiar with the advancements in newer technologies, while AI education in medical college (15.9 %) and online courses (8.2 %) also contributed to it (Figure 4).

Emerging AI-powered healthcare applications like PharmEasy, HealthifyMe and PathAI were recognised by respondents, highlighting the growing visibility of AI in medical practice (Figure 5).

Opinions were diverse regarding AI integration into medical education, as 46.8 % agreed that AI should be part of the curriculum, 25.8 % were neutral, 15.9 % strongly agreed and smaller percentages disagreed or strongly disagreed (Figure 6).

Workshops (45.2 %), lectures (31.1 %), online resources (33.7 %) and extracurricular activities (32.1 %) were preferred learning formats for AI in medicine (Figure 7).

Medical students demonstrated a keen interest in various AI topics, including its direct application in medical practice (56.4 %), contributions to medical research (49 %), recent innovations (34.9 %) and potential effects on the roles of physicians (37.7 %).

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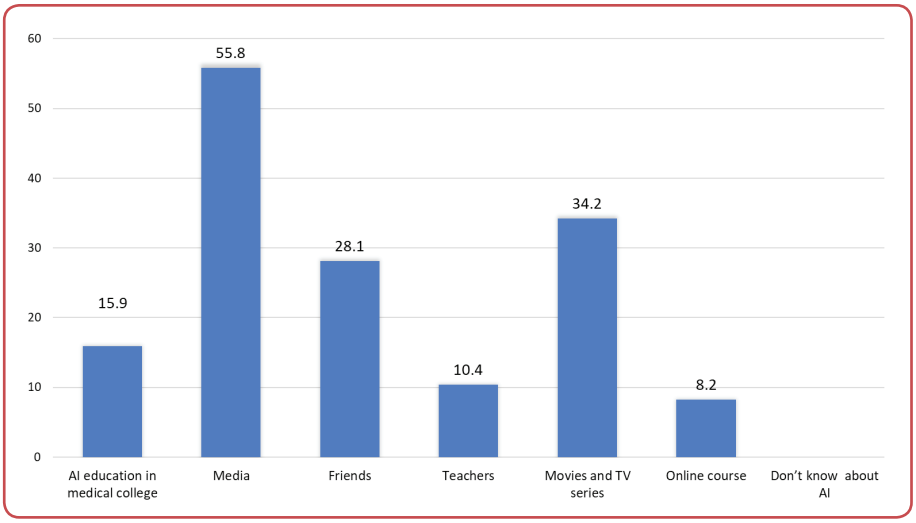

Figure 4: Survey on sources of knowledge about artificial intelligence (AI) in medicine

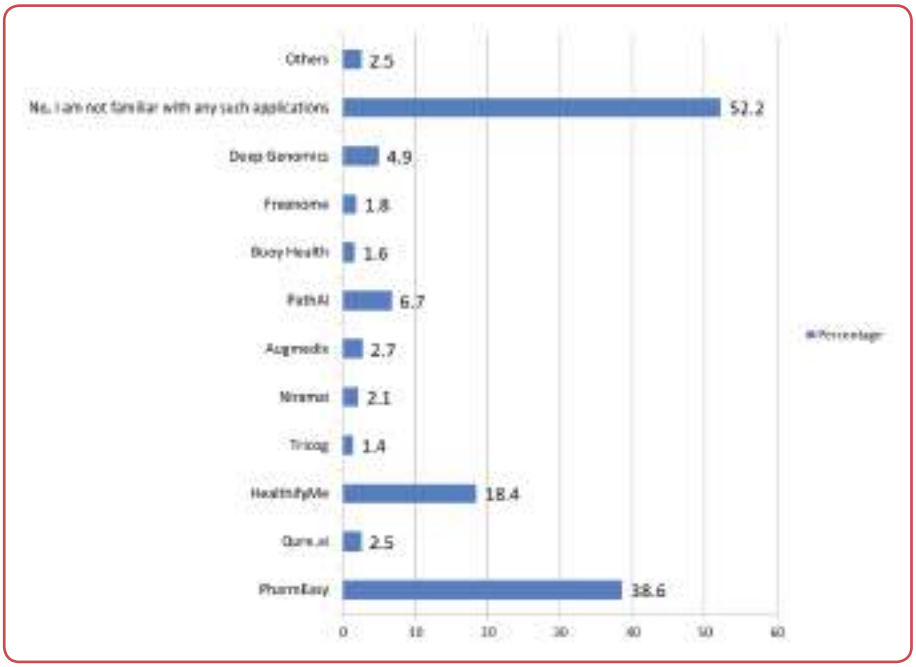

Figure 5: Healthcare applications recognised by respondents

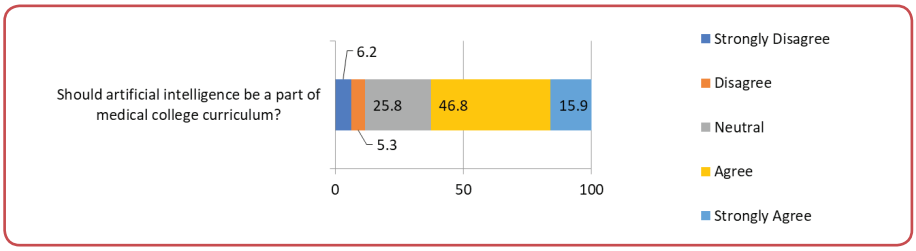

Figure 6: Views on artificial intelligence (AI) as a part of medical curriculum

Challenges to introducing AI in medical colleges encompassed resource availability (56 %), faculty expertise (45.8 %) and potential impact on student's clinical skill development (35.6 %) (Figure 8).

General medicine (42.7 %), surgery (42.5 %) and radiology (39.7 %) (Figure 9) emerged as the top specialties which were projected to be more significantly influenced by AI integration in the near future.

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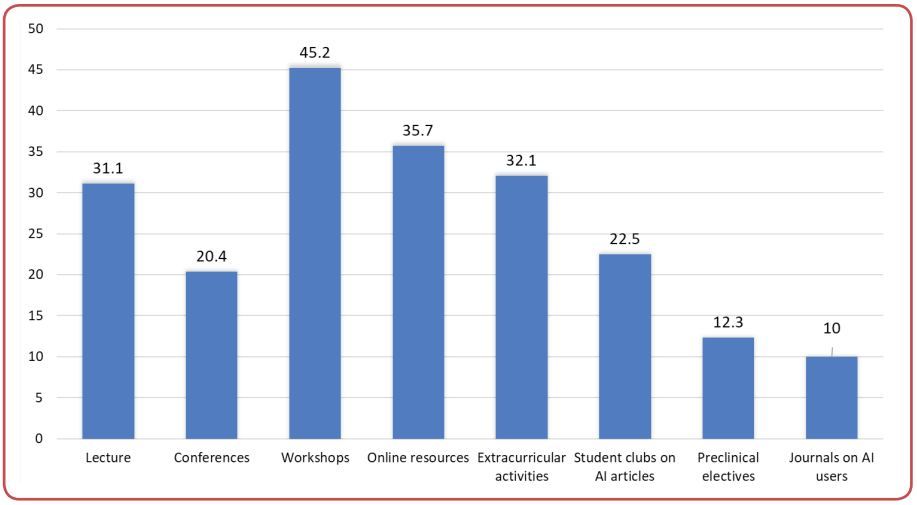

Figure 7: Survey on preferred format of learning about artificial intelligence (AI) in medicine

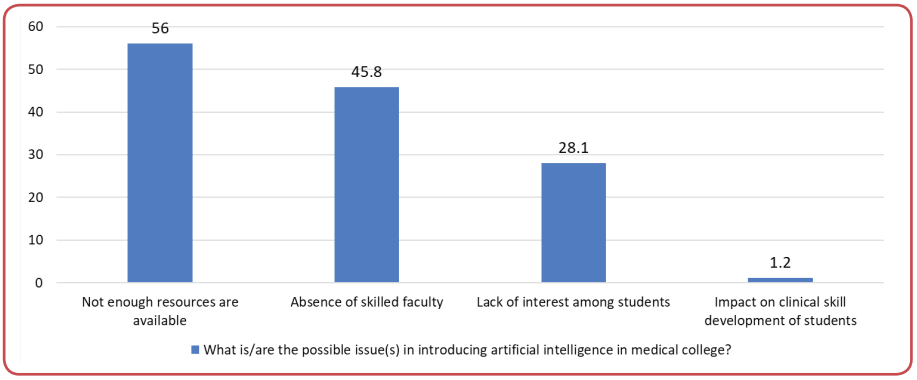

Figure 8: Survey on possible issues in introducing artificial intelligence (AI) in medical college

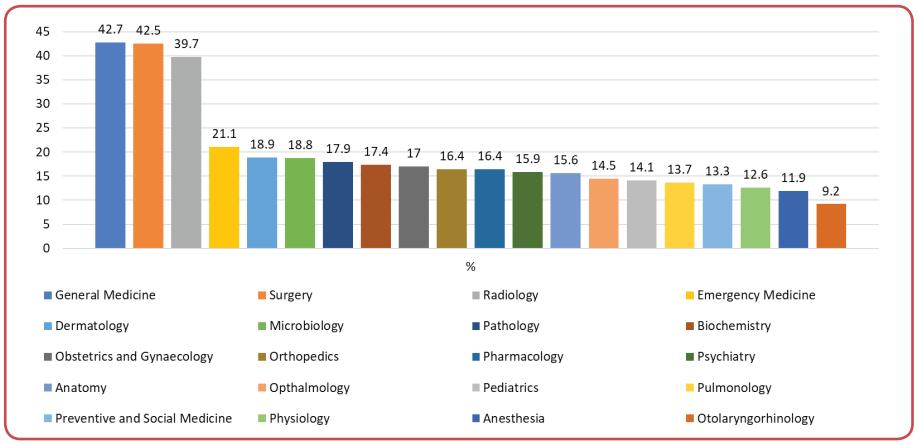

Figure 9: Survey on specialities presumed to be most affected by artificial intelligence (AI) in future

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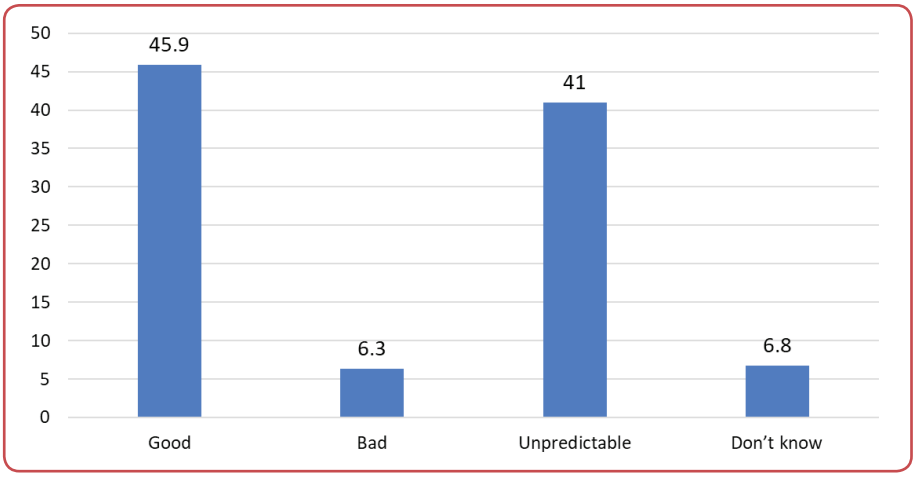

Figure 10: Survey on possible impact of artificial intelligence (AI) on medicine's future

Figure 11: Survey on artificial intelligence (AI)'s potential role in healthcare services

In terms of AI's impact on medicine's future, 45.9 % perceived it as positive, 41 % deemed it unpredictable and smaller proportions viewed it negatively or were uncertain (Figure 10).

Medical students exhibited varying degrees of agreement on AI's roles, such as supporting clinical diagnosis (46.1 % agreement), aiding in management protocols (48.2 % agreement), potential for patient self-diagnosis and treatment (30.8 % agreement) and improving cost-effectiveness (39.3 % agreement) (Figure 11).

Respondents highlighted potential drawbacks to AI integration of which overreliance on AI (49.2 %) was the number one drawback followed by a perceived lack of empathy (43.7 %) and concerns about patient privacy (37 %). Regarding AI in therapeutics, respondents emphasised challenges such as resource scarcity (44.9 %), physicians' insufficient knowledge (37.5 %), high implementation costs (54 %) and the need for high-quality data (48.2 %).

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Discussion

The result of this study is in line with previous studies. The majority of students in the study are familiar with AI. A 53.6 % of respondents demonstrated only a limited understanding of AI's applications in medicine, which is quite close to 51.6 % in a study done by Pucchio et al.[18] Contrarily, in a study done by McLennan et al[19] 64.3% of participants expressed that they did not feel adequately informed about AI. This unfamiliarity is mainly due to a lack of education, awareness and interest among students. Even teachers are not well equipped with the knowledge of AI applications. Thus, it is important to prioritise training for teachers first so they can effectively convey the knowledge to the students.

In the study, 62.7 % of respondents agreed with the integration of AI in medical curricula and acknowledge its potential to revolutionise medicine in the near future. However, in studies conducted by Pucchio et al[18] and Ejaz et al,[20] higher percentages of 78 % and 92 %, respectively, shared this perspective.

A study by Ejaz et al[20] found radiology among the top specialties thought to be most affected by AI in the near future while in a study done in Georgia[11] also highlighted medical genetics along with radiology. However, medical students in this survey believe medicine, surgery and radiology will be most affected by AI.

Traditional healthcare methods have numerous advantages, particularly in terms of their interactive nature. However, access to healthcare services is unevenly distributed across the country, mainly affecting the rural areas. And there are many services that are not accessed by the whole population due to the high cost of treatment which is not affordable to the patients of low to lower-middle-class families. In this study, it was found that AI's assistance in clinical diagnoses yielded an agreement rate of 46.4 %, whereas the study conducted by McLennan et al[19] reported a higher agreement rate of 62.6 %. Likewise, in terms of supporting physicians in the development of management protocols, presented study showed an agreement rate of 48.2 %, while McLennan et al[19] observed a slightly higher agreement rate of 54.4 %.

German and Canadian studies have also revealed that AI will improve the cost-effectiveness of medicine. In this survey, 39.2 % of students agreed to it and a substantial proportion remained neutral as they could not judge because of lack of knowledge about the matter.

A study conducted by Gong et al[21] and Sit et al[22] showed participants' concern about the replacement of radiologists by AI in the future, thus causing anxiety in medical students considering radiology specialties. But in contrast, this study showed that students are not concerned about the replacement of physicians by AI which is also observed in other studies by Pucchio et al[18] and Ejaz et al.[20] What if the physicians lose touch with the use of excessive technologies or what if they over-rely on AI for their work, these are the critical questions along with the ethical issues that arise in the minds of students. These questions can only be answered with time as doctors start to work with AI. But for now, AI is being used for better purposes in the healthcare sector like robots in surgery, apps in medicine, electronic health records in hospitals, etc.

Many challenges have been observed that lie ahead but most medical professionals agree that AI promises a good impact in every field of our healthcare system. However, the widening knowledge gap could potentially hinder the effective utilisation of AI in the field of medicine which is why a well-organised AI curriculum should be planned in colleges that can equip all medical students with the knowledge needed to use these techniques competently to fully harness the potential of advancing technologies as shown in an article by Ötleş et al.[23] So far students have gained information through their peers, media or research papers. Therefore, the inclusion of a formal medical curriculum becomes necessary to enhance their comprehension and proficiency in this field.

Limitations

This survey only included the medical students, potentially excluding opinions from other healthcare professionals or educators, thus creating sampling bias. The survey was conducted online through Google Forms, which may introduce selection bias ie, people having easier access to the internet could have responded in large quantities. The form was delivered to nearly all states of the country but the students of only a few states responded to the survey which may not be sufficient to consider the opinion of the students of all medical colleges in the country. It is possible that only the students who were interested in the topic were the only ones participating in the survey. The survey was conducted in the English language, which may have limited the access to students who understand English potentially excluding non-English speakers.

Conclusion

The survey was designed to determine the level of interest among medical students regarding AI. This study revealed that students have a genuine interest in acquiring knowledge about AI and emerging technologies, as well as a strong inclination toward incorporating AI into their future medical careers. This signifies a positive outlook among students when it comes to integrating AI within the medical field. As AI keeps changing the way healthcare works, it is necessary to ensure that students are ready for these changes and can use AI effectively in their medical careers. To do this, it is important to make sure that students learn about AI properly. To create an awareness of AI and newer technologies, there is a demand for improvement in the current medical curriculum by introducing certain courses during their medical journey. Moreover, findings from this survey indicate that students are not only open to the benefits of AI but are also aware of the potential challenges and risks associated with its implementation. This perspective reflects a balanced understanding of the technology, acknowledging both its advantages and the need for caution.

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Conflict of interest

None.

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ORIGINAL ARTICLE

Changes in Lp-PLA$_2$ Are Associated With Elevated Alanine Aminotransferase Levels: A Nested Case-Control Study in a Three-Year Prospective Cohort

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Abstract

Background/Aim: Elevation in liver enzymes and hepatic fat may indicate a higher susceptibility to cardiovascular disease (CVD). This research sought to find anthropometric/biochemical variables significantly related to the alanine aminotransferase (ALT) increase in healthy populations.

Methods: Nine hundred healthy subjects were included in a 3-year prospective cohort study. The initial screening revealed that 538 were found to be nondiabetic (fasting glucose < 126 mg/dL) and had normal ALT levels. Among them, 79 individuals with slightly elevated ALT levels after three years were assigned to the elevated ALT group. Of the remaining 459 participants, 241 subjects matched to the increased ALT group were the control group.

Results: After three years of follow-up, individuals with elevated ALT showed notably higher aspartate aminotransferase (AST), ALT, gamma-glutamyltransferase (γ-GT), high sensitivity C-reactive protein (hs-CRP), lipoprotein-associated phospholipase A_2 (Lp-PLA$_2$) activity, oxidised low-density lipoprotein (ox-LDL), urinary 8-epi-prostaglandin $F_{2\alpha}$ (8-epi-PGF$_{2\alpha}$) levels and brachial-ankle pulse wave velocity (ba-PWV) in comparison to the control group. Changes (Δ) in ALT showed a positive correlation with Δ AST, Δ gamma-GT, Δ hs-CRP, Δ Lp-PLA$_2$ activity, Δ ox-LDL, Δ urinary 8-epi-PGF$_{2\alpha}$ and Δ ba-PWV. Furthermore, a direct positive link was observed between the Δ Lp-PLA$_2$ activity and Δ AST, Δ ox-LDL and Δ ba-PWV.

Conclusion: Increased Lp-PLA$_2$ activity and other CVD risk indicators were observed to have a pronounced association with elevated ALT levels. This mild ALT elevation could potentially contribute to chronic low-grade inflammation.

Key words: Alanine aminotransferase; Lp-PLA$_2$ activity; Inflammation; Arterial stiffness; ba-PW.

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Introduction

Non-alcoholic fatty liver disease (NAFLD) typically induces an asymptomatic increase in the levels of the liver enzymes, including gamma-glutamyltransferase (γ-GT), aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Among these enzymes, ALT is frequently utilised as a surrogate biomarker for NAFLD because of a positive correlation with the increase of hepatic fat.[1, 2] Elevated γ-GT was commonly observed in patients with NAFLD. However, the

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correlation between γ-GT and hepatic fat, as measured by proton magnetic resonance spectroscopy, is markedly weaker than that of ALT with hepatic fat.[1] Besides, increased γ-GT appears less frequently than changes in ALT in NAFLD,[3, 4] as γ-GT is also produced in other tissues.[5] Therefore, increased ALT is commonly utilised as an indicator for NAFLD in epidemiological research.

Recent epidemiological research has shown that the ALT level is related to the future risk of various metabolic diseases.[2, 6, 7] However, the evidence has still been controversial. Additionally, data are scarce regarding the association between the changes in liver enzyme tests (a crude indicator of NAFLD) and the activity of lipoprotein-associated phospholipase A_2 (Lp-PLA_2) (a distinctive feature of vascular inflammation).[8]

The primary goal of this research was to explore alterations in Lp-PLA_2 activity that were closely linked to increased ALT levels. To achieve the goal, Lp-PLA_2 activity was evaluated in an elevated ALT group (n = 79) defined as nondiabetic individuals with elevated who had high ALT levels (fasting ALT ≥ 30 IU/L for both genders) after 3 years of follow-up. The control group for comparison consisted of subjects who maintained normal ALT levels, with fasting ALT levels below 30 IU/L for both genders. Other cardiovascular disease (CVD) risk factors, including oxidative stress biomarkers [eg urinary 8-epi-prostaglandin $F_{2\alpha}$ (8-epi-$PGF_{2\alpha}$) and oxidised low-density lipoprotein (ox-LDL)], high sensitivity C-reactive protein (hs-CRP) and brachial-ankle pulse wave velocity (ba-PWV), which is a representative indicator of atherosclerotic vascular damage and CVD were also analysed.

Methods

Study subjects

The current study was based on 900 healthy individuals aged 30 to 65 who underwent triennial health examinations from January 2008 to January 2014 at the National Health Insurance Corporation (NHIC) Ilsan Hospital, Goyang, the Republic of Korea. Among a total of 900 subjects, only those who were nondiabetic, with fasting glucose levels below 126 mg/dL and exhibited ALT levels within the standard limits (fasting ALT < 30 IU/L for both genders) were included (n = 538).

Subsequent to the 3-year follow-up point, individuals with marginally increased fasting ALT levels (≥ 30 IU/L for both genders) were assigned to the elevated ALT group (n = 79). Otherwise, 459 subjects maintained ALT levels within the normal range throughout the 3-year follow-up period. Of these 459 subjects, 241 were allocated to the control group after matching with the elevated ALT group using gender, body mass index (BMI), age and fasting blood glucose.

The research protocol was authorised by the Institutional Review Boards of the NHIC Ilsan Hospital and Yonsei University and it was undertaken adhering to the principles of the Helsinki Declaration. All participants received a thorough explanation of the study before providing written informed consent.

Anthropometrics and biochemical assessments

The physical parameters of body weight, height and waist circumference were taken in the morning and after a usual expiration to determine BMI. An automatic blood pressure monitor was utilised to measure blood pressure (BP) levels twice on the left and the average measurement was used. An automated waveform analyser (model VP-1000; Nippon Colin Ltd, Komaki, Japan) was utilised to collect Ba-PWV readings.

For biochemical assessments, venous blood was obtained after 12 hours of fasting. The detailed methods for assessing each indicator were delineated in the previous study.[9, 10] Serum lipid profile analysis was performed as follows. Fasting triglycerides [reference value, 0–2.26 mmol/L], total cholesterol [0–6.22 mmol/L] and high-density lipoprotein (HDL)-cholesterol [1.04–1.16 mmol/L] were assessed using commercial kits (Daiichi, Tokyo, Japan), while the values of low-density lipoprotein (LDL)-cholesterol [0–3.37 mmol/L] were calculated by use of the Friedewald formula. Using the Hitachi 7600 Autoanalyzer (Hitachi Ltd, Tokyo, Japan), enzyme tests for free fatty acids (FFA) [172–586 uEq/L] were carried out. Commercial kits were also used for analysing serum glycaemic parameters: insulin [1.8–12.8 μIU/mL] kits from DIAsource ImmunoAssays S.A. (Louvain-la-Neuve, Belgium) and fasting glucose [3.88–5.55 mmol/L] kits from Siemens

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(Tarrytown, NY, USA). The colour reactions were observed using ADVIA 2400 (Siemens, Tarrytown, NY, USA) and SR-300 (Stratec, Birkenfeld, Germany) instruments. For the evaluation of insulin resistance (IR), the homeostasis-model assessment (HOMA) was utilised. Hitachi 7600 Autoanalyzer (Hitachi Ltd.) measured the enzymatical reaction of AST [0–40 IU/L], ALT [0–40 IU/L] and γ-GT [Male 10–17, Female 6–42 IU/L] for liver function testing. White blood cells (WBC) [4.00–10.00×10^3/μL] were counted using the HORIBA ABX diagnostic analyser (HORIBA ABXSAS, Parc Euromedicine, Montpellier, France). The hs-CRP level [0–5 mg/L] was assessed with CRPHS reagent kits (Roche, Mannheim, Germany) and a Cobas C502 (Roche, Mannheim, Germany). A urinary isoprostane ELISA kit was used (Oxford Biomedical Research Inc, Rochester Hills, MI) for analysing urinary 8-epi-$PGF_{2\alpha}$, thiobarbituric acid reactive substance assay kit (Zepto-Metrix Co, Buffalo, NY) for malondialdehyde (MDA) and ox-LDL enzyme immunoassay kit (Mercodia, Uppsala, Sweden) for ox-LDL. Lp-PLA_2 activity was measured by high-throughput radiometric activity assay.

Statistical analyses

For all statistical analyses, SPSS v.23.0 (IBM, Chicago, IL, USA) was utilised. A p-value < 0.05 (two-tailed) was used to identify the level of statistical significance. Skewed variables were transformed into logarithmic values. The Chi-squared test analysed categorical variables. An independent t-test examined differences in continuous parameters between the two groups at both the first assessment and follow-up point. When comparing changes in the variables, a general linear model was employed, which corrected for initial levels. Differences between values at the first assessment and the follow-up point within each group were evaluated using paired t-test. The associations between the variables were investigated using Pearson's correlation coefficients and a heat map was constructed to represent these associations visually.

Results

Clinical characteristics and liver enzymes

In all initial characteristics except AST and ALT there were no statistical difference between the elevated ALT group (n = 79) and the control group (normal ALT, n = 241) (Table 1). The elevated ALT group consisted of 59.5 % males and 40.5 % females, compared to 51.5 % males and 48.5 % females in the control group. The average age of the control group was 46.9 ± 0.55 years, while that of the elevated ALT group was 47.2 ± 1.06 years (data not shown).

After the 3-year period, considerable increases were observed in the control subject's diastolic BP, total- and LDL-cholesterol, AST, ALT and MDA levels. Besides, the subjects in the control group showed notable reductions in waist circumference, insulin, HOMA-IR, FFA, γ-GT and hs-CRP levels at the follow-up point. On the other hand, individuals with elevated ALT displayed substantial rises in waist circumference, diastolic BP, total- and LDL-cholesterol, AST, ALT, hs-CRP and MDA levels after 3 years compared with the initial values. Furthermore, the elevated ALT group had notably higher levels of FFA, AST, ALT, γ-GT and hs-CRP at the follow-up evaluation after 3 years than the control group. Changed values of waist circumference, FFA, AST, ALT, γ-GT and hs-CRP were more significant in the elevated ALT subjects than in the control subjects (Table 1).

Lp-PLA_2 activity, oxidative stress markers and ba-PWV

There were no remarkable differences in the initial traits, including Lp-PLA_2 activity, oxidative stress indicators (ox-LDL and 8-epi-$PGF_{2\alpha}$) and ba-PWV, between the control and elevated ALT subjects (Figure 1). Regarding changed values, the participants in the control group demonstrated a noteworthy decrease in Lp-PLA_2 activity, while ox-LDL levels substantially increased. Conversely, the elevated ALT group exhibited substantial increases in Lp-PLA_2 activity, ox-LDL, urinary 8-epi-$PGF_{2\alpha}$ and ba-PWV. Moreover, the observed changes were more prominent in the elevated ALT subjects, in contrast to the normal control subjects, after accounting for initial levels. Upon reaching the 3-year point of the follow-up, individuals with elevated levels of serum ALT displayed considerably higher levels of Lp-PLA_2

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activity, ox-LDL, urinary levels of 8-epi-$PGF_{2\alpha}$ and ba-PWV when compared to individuals in the control group (Figure 1).

Relationship between the changes in ALT and waist circumference, biochemical parameters and ba-PWV

Figure 2 presents the correlation matrix between alterations in liver enzymes, waist circumference, biochemical parameters and ba-PWV within the entire study cohort (n = 320). The difference in ALT exhibited a positive correlation with variations in waist circumference (r = 0.194, p < 0.001), triacylglycerol (r = 0.206, p < 0.001), FFA (r = 0.178, p = 0.001), AST (r = 0.706, p < 0.001), γ-GT (r = 0.344, p < 0.001), hs-CRP (r = 0.123, p = 0.031), urinary 8-epi-$PGF_{2\alpha}$ (r = 0.141, p = 0.017), ba-PWV (r = 0.208, p < 0.001), ox-LDL (r = 0.133, p = 0.025) (Figure 3) and Lp-PLA_2 activity (r = 0.479, p < 0.001) (Figure 3). Furthermore, FFA (r = 0.221, p < 0.001), AST (r = 0.362, p < 0.001), ba-PWV (r = 0.170, p = 0.003) and ox-LDL (r = 0.198, p = 0.001) alterations had a positive association with changes in Lp-PLA_2 activity (Figure 3). Additionally, the difference in ba-PWV showed positive correlations with changes in waist circumference (r = 0.149, p = 0.008), AST (r = 0.210, p < 0.001), γ-GT (r = 0.141, p = 0.029) and hs-CRP (r = 0.142, p = 0.013).

Table 1: Clinical and biochemical characteristics of the control and elevated ALT groups

Parameter	Total subjects (n = 320)				P^a	P^b	P^c	P^d
	Control (n = 241)		Elevated ALT (n = 79)					
	Initial	Follow-up	Initial	Follow-up				
BMI (kg/m²)	23.3 ± 0.17	23.4 ± 0.17	23.4 ± 0.30	23.7 ± 0.34	0.903	0.441		
Waist (cm)	83 ± 0.44	83.6 ± 0.49	81.9 ± 0.77	84.6 ± 0.92**	0.226	0.306		
Change	**0.59 ± 0.38**		**2.7 ± 0.76**				0.008	0.017
Waist to hip ratio	0.89 ± 0.00	0.88 ± 0.00***	0.89 ± 0.01	0.88 ± 0.01	0.355	0.653		
Systolic BP (mmHg)	117.2 ± 0.85	118.5 ± 0.95	117.8 ± 1.42	119.6 ± 1.70	0.714	0.522		
Diastolic BP (mmHg)	72 ± 0.66	73.5 ± 0.69*	72 ± 1.18	74.8 ± 1.13*	1	0.355		
Triglycerides (mmol/L)$^{\$}$	1.16 ± 0.05	1.24 ± 0.05	1.11 ± 0.06	1.41 ± 0.12	0.834	0.346		
Total-cholesterol (mmol/L)$^{\$}$	4.98 ± 0.05	5.17 ± 0.05***	4.76 ± 0.08	5.1 ± 0.10**	0.052	0.48		
HDL-cholesterol (mmol/L)$^{\$}$	1.37 ± 0.02	1.35 ± 0.02	1.31 ± 0.04	1.29 ± 0.03	0.186	0.25		
LDL-cholesterol (mmol/L)$^{\$}$	3.08 ± 0.05	3.26 ± 0.05***	2.93 ± 0.08	3.19 ± 0.09*	0.222	0.395		
Glucose (mmol/L)$^{\$}$	5.08 ± 0.03	5.11 ± 0.03	5.07 ± 0.06	5.14 ± 0.06	0.816	0.751		
Insulin (μIU/mL)$^{\$}$	8.32 ± 0.20	7.6 ± 0.21***	8.3 ± 0.38	7.91 ± 0.44	0.83	0.684		
HOMA-IR$^{\$}$	1.88 ± 0.05	1.73 ± 0.05**	1.87 ± 0.09	1.81 ± 0.11	0.845	0.606		
Free fatty acid (uEq/L)$^{\$}$	534.8 ± 15.3	474.4 ± 15.4***	506.3 ± 24.9	563.6 ± 29.7	0.349	0.002		
Change	**-60.4 ± 19.1**		**57.3 ± 32.5**				0.002	0.002
AST (IU/L)$^{\$}$	20.7 ± 0.23	21.3 ± 0.23**	22.5 ± 0.42	33.6 ± 1.13***	< 0.001	< 0.001		
Change	**0.6 ± 0.23**		**11.1 ± 1.17**				< 0.001	< 0.001
ALT (IU/L)$^{\$}$	17 ± 0.33	17.7 ± 0.31**	19.6 ± 0.55	37.1 ± 1.18***	< 0.001	< 0.001		
Change	**0.73 ± 0.31**		**17.5 ± 1.21**				< 0.001	< 0.001
Gamma-GT (U/L)$^{\$}$	23.2 ± 1.98	21.4 ± 1.77***	24.1 ± 1.63	26.5 ± 2.19	0.092	0.002		
Change	**-1.84 ± 0.68**		**2.47 ± 1.72**				0.023	0.002
hs-CRP (mg/L)$^{\$}$	1.41 ± 0.25	0.72 ± 0.05**	1.11 ± 0.22	1.88 ± 0.65*	0.538	< 0.001		
Change	**0.72 ± 0.25**		**0.81 ± 0.72**				0.01	0.002
WBCs (x10³/μL)$^{\$}$	5.34 ± 0.12	5.16 ± 0.09	5.5 ± 0.19	5.38 ± 0.16	0.421	0.259		
Malondialdehyde (nmol/mL)$^{\$}$	9.49 ± 0.17	10.4 ± 0.20***	9.22 ± 0.27	10.6 ± 0.37**	0.539	0.761		

BMI: body mass index; BP: blood pressure; HDL: high-density lipoprotein; LDL: low-density lipoprotein; HOMA-IR: homeostatic model assessment for insulin resistance; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GT: glutamyl transferase; WBC: white blood cells;

*Mean ± SE. $^{\$}$The data were subjected to logarithmic transformation for testing. Pa-values were obtained from an independent t-test at the initial assessment. Pb-values were obtained from an independent t-test at follow-up assessment. Pc-values were obtained from an independent t-test of changed values. Pd-values adjust changed value with initial levels. *P < 0.05, **P < 0.01, ***P < 0.001 were assessed ased on the results of a paired t-test.*

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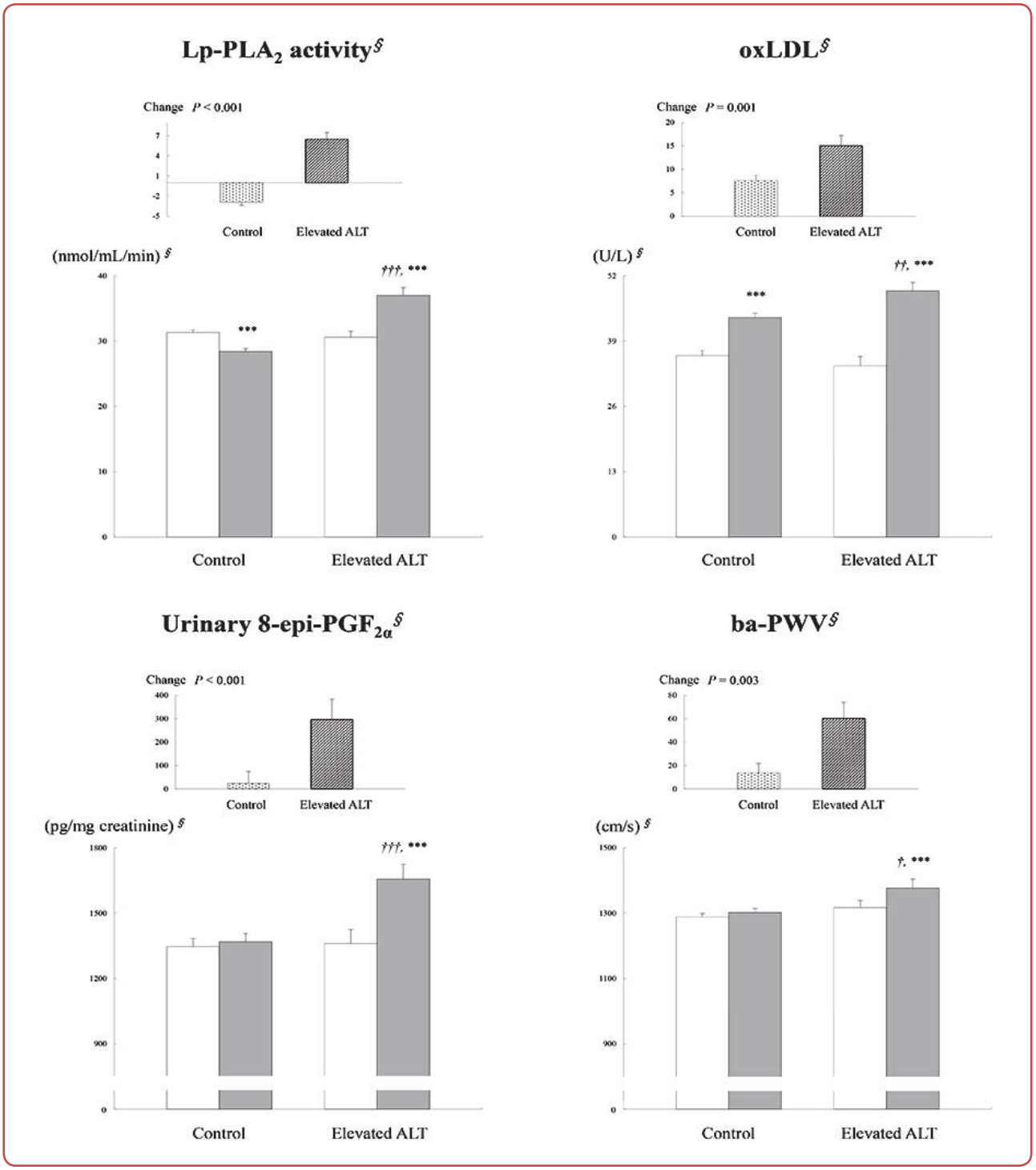

Figure 1: Lp-PLA$_2$ activity, ox-LDL, urinary 8-epi-PGF$_{2\alpha}$ and ba-PWV in control and elevated ALT subjects at initial (□) and follow-up (■).

*Mean ± SE. §The data were subjected to logarithmic transformation for testing. †P < 0.05, ††P < 0.01, †††P < 0.001 were obtained using an independent t-test comparing the groups at both initial and follow-up assessments. *P < 0.05, **P < 0.01, ***P < 0.001 were obtained from a paired t-test within each group.*

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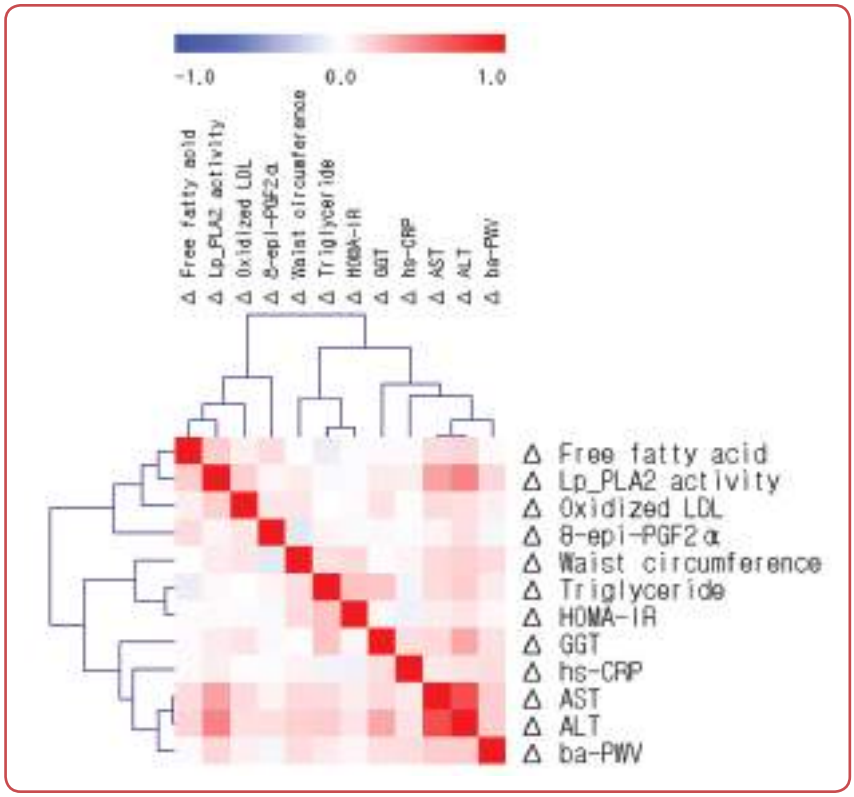

Figure 2: Correlation matrix of changes (Δ) in liver enzymes, waist circumference, biochemical parameters and ba-PWV across all study participants.0.001 were obtained from a paired t-test within each group.

Correlations between variables were determined using Pearson's correlation coefficient. Positive correlations are indicated by the colour Red, while negative correlations are denoted by the colour Blue in the correlation matrix.

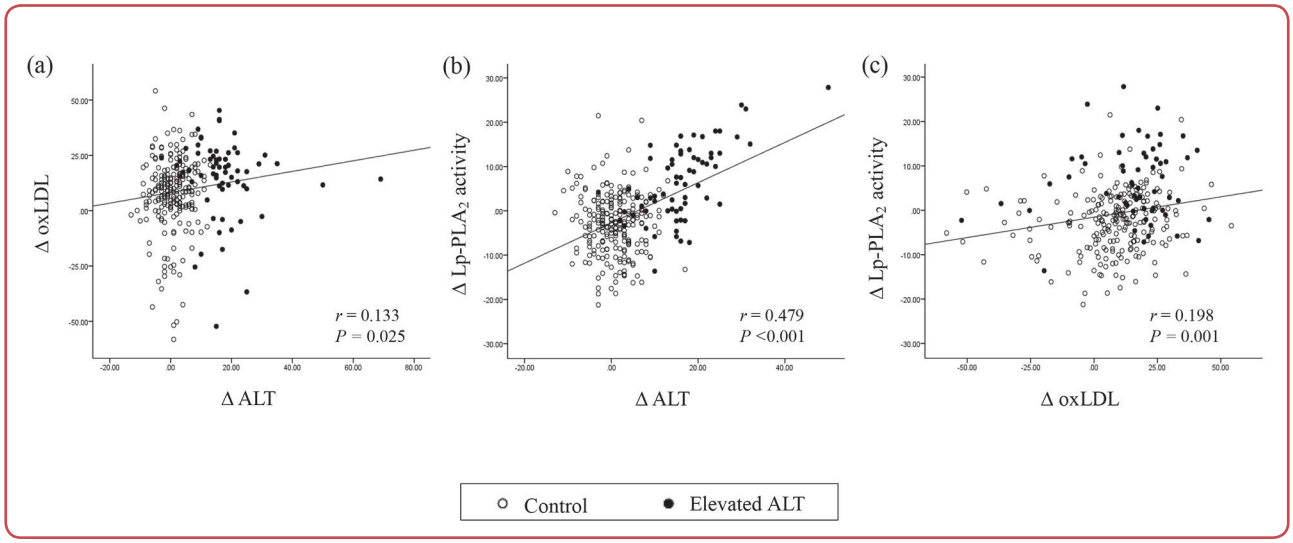

Figure 3: Correlations between changes (Δ) in ALT and ox-LDL, ALT and Lp-PLA$_2$ activity and ox-LDL and Lp-PLA$_2$ activity across all study participants.

(a) Correlation between Δ ALT and Δ ox-LDL. (b) Correlation between Δ ALT and Δ Lp-PLA$_2$ activity. (c) Correlation between Δ ox-LDL and Δ Lp-PLA$_2$ activity.

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Discussion

In this prospective study, aim was to find anthropometric/ biochemical indicators significantly associated with the increase in ALT for three years in healthy populations. As a result, the link between ALT rise and changes in Lp-PLA_2 activity was verified.

Over the course of the 3-year study, the group with increased ALT levels displayed a noteworthy 1.9-fold rise in serum ALT levels. Generally, ALT concentrations decrease with aging.[11] Therefore, the increase in ALT over three years in presented study could result from abnormal metabolism, including liver damage.[11] Mild increases of ALT and AST (less than five times the upper limit of normal) are typical in primary care in the USA, it is estimated that subjects with elevated transaminase levels account for about 10 % of the population.[12] Indeed, confirmed changes in ALT were directly and strongly linked to changes in circulating Lp-PLA_2 activity and other liver enzyme activities such as AST and γ-GT. Additionally, alteration of ALT levels was positively linked to inflammation, oxidative stress and arterial stiffness indicators. Presented results suggest a significant association between elevation in Lp-PLA_2 activity, along with other CVD risk factors and elevated ALT levels.

This research discovered alteration in circulating Lp-PLA_2 activity and other liver enzyme activities (AST and γ-GT) were directly correlated with ALT changes, which positively correlated with indicators of oxidative stress, inflammation and vascular stiffness. The findings suggested that the inflammatory response in NAFLD contributes to systemic inflammation prompted by the interaction between elevated liver enzymes[6] and increased Lp-PLA_2 activity. Several studies are lined with presented research. A recent study demonstrated positive relationships between serum Lp-PLA_2 activity and ALT levels.[13] Another study carried out by Colak et al[14] revealed that serum Lp-PLA_2 levels were considerably elevated in NAFLD patients compared to normal subjects. Besides, the changed value of ALT and AST levels were substantially more extensive in individuals with increased ALT compared to those with normal ALT in the present study. This finding implies that liver enzymes, including ALT, could be rapidly abnormal in individuals with ALT levels at the upper portion of the reference range. Therefore, both serum ALT levels and Lp-PLA_2 activity could serve as valuable non-invasive markers for detecting the advanced status of NAFLD.

Lp-PLA_2 is produced primarily by various cell types, including mast cells, monocytes, liver cells, macrophages and T lymphocytes.[15, 16] Hepatic macrophages, which generate various inflammatory mediators, including Lp-PLA_2, regulate the phenotype of neighbouring cells.[17] Just as lipoprotein particles infiltrate the vascular wall, the buildup of fat in the liver triggers the release of hepatic cytokine, aggravating increased CRP levels. Indeed, Lp-PLA_2 activity and hs-CRP concentration were positively correlated with alteration in ALT in the present study. Similarly, Kerner et al[18] observed a correlation between elevated liver enzymes and CRP concentration.

The interplay between ox-LDL and Lp-PLA_2 within the vascular wall generates oxidised fatty acids and lysophosphatidylcholines, the strongest inflammatory and atherogenic factors.[19] At first, it was considered that circulating Lp-PLA_2 strongly preferred phospholipids with short fatty acid chains at the sn-2 position; no significant activity was detected in chains longer than nine carbons.[20, 21] However, oxidatively modified phospholipids such as 8-epi-$PGF_{2\alpha}$[22] and phospholipid hydroperoxides[23] have been newly identified as plasma Lp-PLA_2 substrates. Urinary 8-epi-$PGF_{2\alpha}$ is considered the most reliable biomarker for assessing non-enzymatic lipid peroxidation and oxidative stress.[24] Throughout the study, substantial increases in Lp-PLA_2 activity, ox-LDL, as well as urinary levels of 8-epi-$PGF_{2\alpha}$ were observed in the subjects with elevated ALT. Additionally, meaningful correlations were found among these variables, with the highest degree of association found between Lp-PLA_2 activity and ALT. This result points to a potential hepatic role of systemic inflammation in modest ALT elevation status.

Furthermore, elevated Lp-PLA_2 levels are linked with higher PWV values.[8] Lp-PLA_2 activity has been considered a primary trigger of the pro-inflammatory process that causes elastin loss and increased collagen deposition, resulting in faster arterial stiffness.[8] The ba-PWV is a straightforward index used to assess the severity of arterial atherosclerosis and stiffness, reflecting the stiffness of both peripheral and central muscular arteries.[25-27] Changes in ba-PWV were closely linked to variations in liver enzymes, Lp-

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PLA_2 and hs-CRP in this study. Individuals with elevated ALT levels had a markedly greater increase in ba-PWV than controls. Consequently, the current study proposed that a mild increase in ALT levels could contribute to arterial stiffening with an impact on inflammatory markers.

It should be mentioned that the number of subjects who participated in this study was small. In addition, correlation analysis was performed only between the change value of indicators. Despite these limitations, an increase in Lp-PLA_2 activity and other CVD risk factors linked to elevation of ALT levels was confirmed. However, further analysis is needed to verify presented results.

Conclusion

It was revealed that elevated ALT levels were linked to rises in Lp-PLA_2 activity and additional risk factors for CVD in healthy populations. Therefore, the modest ALT increase could be associated with chronic low-grade inflammation. If this correlation is substantiated through further research, the combination of ALT and Lp-PLA_2 may be utilised as an integrated tool for the management of chronic disease risk.

Acknowledgement

None.

Conflict of interest

None.

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ORIGINAL ARTICLE

Sex Differences in the Hepatotropic Effects of Antiulcer Drugs and Placenta Cryoextract in an Experimental Rat Liver Injury Model

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Abstract

Background/Aim: Sex-related variances in drug metabolism provide a foundation for refining treatment protocols for prevalent conditions based on the patient's sex. Tailoring treatment strategies based on sex is particularly noteworthy among patients with comorbid illnesses due to the potential for drug interactions and the impact of concurrent diseases on clinical outcomes. Aim of this study was to assess the hepatotropic effects of antiulcer drugs (esomeprazole, clarithromycin and metronidazole – E/C/M) and placenta cryoextract (CEP) within a simulated model of tetrachloromethane (CCl_4)-induced hepatitis combined with underlying ethanol-induced liver cirrhosis (EILC), with a focus on the role of subjects' sex.

Methods: Using 112 male and female rats, the research explored the effects of different sex hormone levels. Chronic EILC was induced by administering a 50.0 % CCl_4 oil solution (8 mL/kg) twice a week, combined with a 5.0 % ethanol solution, over 45 days. Total protein (TP) levels and alkaline phosphatase (AP) activity were measured spectrophotometrically.

Results: The research findings indicate that the onset of EILC and the administration of E/C/M resulted in a significantly greater 10.8 % ($p = 0.03$) reduction in TP levels among females compared to males, without altering hormonal status. Introducing CEP led to a noteworthy ($p < 0.001$) rise in TP levels, by 30.8 % in males and 33.9 % in females, in the context of EILC and E/C/M administration, while maintaining hormonal status.

Among male rats, the most elevated AP activity was observed with excess testosterone propionate administration (5.0 [5.0; 5.9] µmol/L), while the lowest level was recorded in rats after testectomy, measuring 3.8 [2.5; 4.7] µmol/L, exhibiting a significant 20.8 % decrease ($p < 0.05$) compared to male rats without hormonal status changes. In female rats, the study revealed that against the backdrop of EILC and E/C/M administration, the highest AP level was seen in ovariectomised females, reaching 5.8 [5.1; 6.2] µmol/L, reflecting a substantial 9.4 % increase compared to rats without hormonal status changes.

Conclusions: The administration of CEP under similar experimental conditions led to the recovery of the liver's protein-synthesising function in both male and female rats. When female sex hormones were introduced to sham-operated female rats, a significant 20.8 % greater reduction in AP levels was observed. Additionally, gonadectomy led to a more pronounced decrease in this enzyme's levels in male rats compared to female rats, indicating the cytoprotective properties of female sex hormones.

Key words: Cryopreserved placenta extract; Peptic ulcer; Hepatitis; Liver cirrhosis; Sexual dimorphism; Comorbidity.

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Introduction

Considering sex-based disparities in the pharmacodynamics and pharmacokinetics of medicinal products (MPs) is a critical factor in achieving effective pharmacological treatment. Both endogenous and exogenous sex hormones can directly or indirectly impact the metabolism of MPs. Additionally, certain drugs possess the potential to induce changes in hormonal signalling pathways.[1] The oxidative biotransformation of medicinal products in the liver, mediated by a series of cytochrome P450 (CYP) isoenzymes, plays a crucial role in the therapeutic effectiveness of these products.[1, 2] Individual variations in the expression of key enzymes involved in drug metabolism, including CYP P450, sulfotransferases, glutathione transferases and glucuronosyltransferases are linked to significant individual differences in the bioavailability and clearance of drugs and other xenobiotics. Given the central role of hepatic enzymes in regulating the pharmacological and biological activity of drugs, as well as steroids and other endobiotics, it is imperative to comprehend the regulatory characteristics that contribute to individual disparities in their expression.[3] It is noteworthy that the most crucial isoform of CYP in drug metabolism, CYP 3A4, exhibits higher expression levels in the liver of women as compared with men.[4, 5]

Table 1: Biologically active substances present in placental cryoextract[10, 11]

Biologically active substances	Properties	Range values
α-fetoprotein	Growth regulator of embryonic, transformed, activated immune competent cells	429 ± 75 mIU/mL
Chorionic gonadotropin	Immune system activator, promotes the production of steroid hormones (testosterone and oestradiol)	26.8 ± 8 mIU/mL
Estradiol	Reproductive function, cardioprotective action	755 ± 48 pmoL/mL
Progesterone	Reproductive function, cardioprotective action	226 ± 110 nmoL/mL
Prolactin	Impact on the development of secondary sexual characteristics, erythropoietic action, regulation of lipid metabolism	705 ± 129 mIU/mL
Fertility α-microglobulin	Readiness for pregnancy, the conception process and the normal development of the foetoplacental unit	1470 ± 173 ng/mL
Lactoferrin	Stimulation of lactation	1270 ± 223 ng/mL
Somatotropin	Growth hormone, anabolic action	5.64 ng/mL
Luteinising hormone	Pituitary hormone, secretion of oestrogens, progesterone, testosterone	7.8 ± 1.9 IU/L
Follicle-stimulating hormone	Pituitary hormone, promotes follicle maturation in ovaries and spermatogenesis	7.1 ± 2.3 mIU/L
Testosterone	Differentiation and functioning of the reproductive system, anabolic action	3.68 ± 1.06 nmoL/mL
Thyroid-stimulating hormone	Stimulation of thyroid function, immunomodulatory action	291 ± 13 mIU/L
Triiodothyronine	Stimulation of metabolism, growth and tissue differentiation, reproduction, haematopoiesis	2.1 ± 0.6 pmoL/L
Thyroxine	Stimulation of metabolism, growth and tissue differentiation, reproduction, haematopoiesis	5.6 ± 0.99 pmoL/L
Cortisol	Metabolism of proteins, carbohydrates, fats and nucleic acids	1392 ± 515 nmoL/mL
Colony-stimulating factor	Proliferation of bone marrow cells	9.87 ng/mL
Tumour necrosis factor-α	Inhibitor of cancer cell proliferation	84.5 pg/mL
Interleukin 1β	Regulation of pluripotent stem cell differentiation and the immunendocrine system	201.7 pg/mL
Interleukin 4	Regulation of pluripotent stem cell differentiation and the immunendocrine system	21.7 pg/mL
Interleukin 6	Regulation of pluripotent stem cell differentiation and the immunendocrine system	114.9 pg/mL
Total protein	Plastic function	76.5 ± 14.0 mg/L g of tissue
Proteins with a molecular mass of 20–100 kDa	Plastic function	70–80 %
Proteins with a molecular mass below 20 kDa	Plastic function	20–30 %

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As a potential agent capable of mitigating the hepatotoxic effects of medicinal products while exhibiting its own anti-ulcer activity, attention of authors was drawn to placental cryoextract (CEP). This cryoextract was developed and introduced into practice by scientists from the Institute of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine (Institute), who have also devised a methodology for its long-term storage in a low-temperature environment.[6, 7] The placenta serves as a natural depot and producer of a wide range of biologically active substances (Table 1), which facilitate the growth and development of the foetus during intrauterine development. It supports processes such as trophic interactions and protein synthesis, gas exchange, hormone secretion, blood pressure regulation, blood clotting, detoxification, metabolite excretion, deposition of biologically active substances, immune regulation, regulation of lipid peroxidation and more.[8-10]

The aim of the study was to characterise the sex-specific features of the hepatotropic effects of anti-ulcer agents and placental cryoextract in the context of experimental chronic liver injury.

Methods

The experimental research was conducted on 112 male and female rats weighing 200–220 g, divided into four groups of 28 animals each: Group I (males) and Group III (females) – rats with simulated tetrachloromethane (CCl_4)-induced hepatitis alongside ethanol-induced liver cirrhosis. These rats were subjected to daily intragastric administration for 7 days of esomeprazole (50 mg/kg), clarithromycin (91 mg/kg) and metronidazole (91 mg/kg) (E/C/M);[12, 13] Group II (males) and Group IV (females) consisted of rats with simulated CCl_4-induced hepatitis alongside ethanol-induced liver cirrhosis. These rats were subjected to daily intragastric administration of E/C/M for 7 days following the same scheme as described earlier. Additionally, from the 3rd to the 7th day of administration of anti-ulcer agents (5 administrations), placental cryoextract (CEP) was introduced (0.16 mg/kg, intramuscularly). Each group comprised 4 subgroups with varying hormonal statuses, each containing 7 animals:

Subgroup a) – Rats of both sexes that underwent sham surgery and received replacement hormone therapy (excess).
Subgroup b) – Rats of both sexes that underwent sham surgery without a change in hormonal status (comparison group).
Subgroup c) – Rats of both sexes that underwent testectomy or ovariectomy.
Subgroup d) – Rats of both sexes that, after gonadectomy, received replacement hormone therapy.

The animals were removed from the experiment 24 hours after the last administration of CEP using cervical dislocation under inhalation anaesthesia.

Modelling experimental pathology

Chronic CCl_4-induced hepatitis with concurrent ethanol-induced liver cirrhosis (EILC) was replicated through intragastric administration of a 50.0 % oil solution of CCl_4 at a dose of 8 mL/kg animal body weight, twice a week, combined with a 5.0 % ethanol solution for drinking over a period of 45 days.[13, 16]

Modulation of sex hormone levels was achieved through surgical ovariectomy or testectomy in female and male rats, respectively, using established methods.[13-15] The investigations were carried out 21 days after gonadectomy. Untreated animals in the control groups underwent an incision of the anterior abdominal wall followed by wound closure (sham-operated animals). Substitution and excessive hormone therapy were conducted for 14 days in males by subcutaneous administration of testosterone propionate (*PAT Pharmak*, Ukraine) at a dose of 1 mg/kg once a day and in females by intragastric administration of oestradiol hemihydrate (*Abbott Biologicals* BV, Netherlands) at a dose of 150 mg/kg.[17–22] CEP was obtained from the Interdepartmental Scientific Centre of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, National Academy of Medical Sciences and Ministry of Health of Ukraine, in the form of an ampoule product named *Cryocell – placenta cryoextract*.

Biochemical research methods

The study material consisted of serum from whole peripheral blood. Samples of mixed blood were collected in centrifuge tubes after decapita-

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tion of the animals. Serum was separated by centrifugation for 15 min at 3000 rpm.

The content of total protein (TP) was determined using a spectrophotometric method based on the biuret reaction. In this method, under alkaline conditions, divalent copper ions ($CuSO_4$) react with proteins to form a violet-coloured complex. The protein concentration was measured spectrophotometrically by light absorption at a wavelength of λ = 546 nm and expressed in g/L.[23]

The activity of alkaline phosphatase (AP) was determined using a spectrophotometric method based on the property of AP to hydrolyse the ether bond in β-glycerophosphate, releasing phosphoric acid. The generated phosphate content was determined using a reaction with a molybdenum reagent in the presence of ascorbic acid. The intensity of the resulting molybdenum blue coloration is proportional to the amount of phosphate.[24]

Bioethical aspects of the study

All experimental research on laboratory animals followed Good Laboratory Practice standards, as outlined in "Medicinal Products. Good Laboratory Practice," approved by the Ministry of Health of Ukraine. The research also adhered to the Council of Europe Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, Directive 2010/63/EU and relevant Ukrainian laws. The comprehensive research program was approved by the Bioethics Committee at the Institute (Protocol No 2, 3 January 2022; Protocol No 5, 22 November 2022).

Statistical analysis

The distribution of variables within each group was assessed using the Shapiro-Wilk test. Variance homogeneity was examined through Levene's test. For normally distributed independent variables, pairwise group differences were analysed using Student's t-test and ANOVA with Fisher's parametric F-test. Non-normally distributed data comparisons utilised the non-parametric Mann-Whitney rank test and Kruskal-Wallis rank-based analysis. Normally distributed data was presented as "M ± m" (M ± SE), where M represents the mean and m (SE) corresponds to the standard error of the mean, along with a 95 % confidence interval (95 % CI). Non-normally distributed data were denoted as Me [LQ; UQ], where Me indicates the median and [LQ; UQ] signifies the upper and lower quartile bounds.[25]

Results

The study revealed that the progression of CCl_4-induced hepatitis and the administration of E/C/M resulted in a 10.8 % greater ($p = 0.03$) decrease in TP levels in females compared to males without a change in hormonal status (Table 2). The administration of testosterone propionate led to a statistically significant ($p < 0.01$) 12.2 % greater decrease in TP levels in male rats with CCl_4-induced hepatitis in the presence of E/C/M. In contrast, in castrated males, TP levels were 11.0 % higher than those of rats without a change in hormonal status (Table 3). Notably, ovariectomy in females with CCl_4-induced hepatitis in the context of E/C/M administration resulted in a 11.6 % decrease ($p = 0.01$) in TP levels in peripheral blood compared to females without a change in hormonal status.

Administration of CEP resulted in a statistically significant ($p < 0.001$) increase in TP levels by 30.8 % in males and 33.9 % in females without a change in hormonal status, in the context of CCl_4-induced hepatitis and E/C/M administration (Table 3). Notably, the smallest increase in TP levels following CEP administration was observed in females receiving excess oestradiol hemihydrate, which was in line with the highest levels of the investigated parameter in female rats not receiving CEP, at 54.1 ± 1.30 g/L and 70.4 ± 1.32 g/L, respectively. These findings suggest a protective role of female sex hormones in liver toxic injuries. Conversely, in male rats with CCl_4-induced hepatitis, E/C/M administration, excess testosterone propionate and CEP, the most significant increase in TP levels was observed ($p < 0.001$). Meanwhile, male rats not receiving CEP showed the lowest TP levels, at 47.9 ± 1.56 g/L (Table 2). The obtained data indicate the ability of CEP to restore liver protein synthesis function in both male and female rats with CCl_4-induced hepatitis, in the context of E/C/M administration.

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Table 2: Effect of CEP and E/C/M on the serum total protein content in chronic ethanol-tetrachloromethane-induced liver injury in male and female rats, g/L (M ± m (95 % CI), n = 112)

The study parametre	Group	Males		Females	
		Group I	Group II	Group III	Group IV
		EILT + E/C/M	EILT + CEP + E/C/M	EILT + E/C/M	EILT + CEP + E/C/M
		7*	7*	7*	7*
Without alterations to the hormonal status	a	54.60 ± 1.72 (95 % CI: 51.20–57.90)	71.40 ± 1.86 (95 % CI: 67.80–75.10) $p_{1-2} < 0.001$	49.30 ± 1.38 (95 % CI: 46.60–52.00) $p_{1-3} = 0.030$ $p_{2-3} < 0.001$	66.00 ± 1.91 (95 % CI: 62.20–69.80) $p_{1-4} < 0.010$ $p_{2-4} = 0.600$ $p_{3-4} < 0.001$
Hormone therapy	b	47.90 ± 1.56 (95 % CI: 44.80–50.90) $p_{a-b} = 0.190$	65.90 ± 2.60 (95 % CI: 60.80–71.00) $p_{a-b} = 0.110$ $p_{1-2} < 0.001$	54.10 ± 1.30 (95 % CI: 51.60–56.70) $p_{a-b} = 0.020$ $p_{1-3} < 0.010$ $p_{2-3} < 0.010$	70.40 ± 1.32 (95 % CI: 57.80–73.00) $p_{a-b} = 0.080$ $p_{1-4} < 0.001$ $p_{2-4} = 0.140$ $p_{3-4} < 0.001$
Gonadectomy with hormonal replacement therapy	c	58.30 ± 2.01 (95 % CI: 54.30–62.20) $p_{a-c} = 0.190$ $p_{b-c} < 0.010$	73.70 ± 3.03 (95 % CI: 67.80–79.70) $p_{a-c} = 0.530$ $p_{1-2} < 0.010$ $p_{b-c} = 0.070$	45.60 ± 1.60 (95 % CI: 42.40–48.70) $p_{a-c} = 0.100$ $p_{1-3} < 0.001$ $p_{b-c} < 0.010$ $p_{2-3} < 0.001$	62.40 ± 1.19 (95 % CI: 60.10–64.80) $p_{a-c} = 0.140$ $p_{1-4} = 0.100$ $p_{b-c} < 0.001$ $p_{2-4} < 0.010$ $p_{3-4} < 0.001$
Gonadectomy	d	60.60 ± 3.84 (95 % CI: 53.10–68.10) $p_{a-d} = 0.180$ $p_{b-d} < 0.010$ $p_{c-d} = 0.610$	74.60 ± 6.19 (95 % CI:62.40–86.70) $p_{a-d} = 0.640$ $p_{1-2} = 0.080$ $p_{b-d} = 0,220$ $p_{c-d} = 0.900$	43.60 ± 1.32 (95 % CI: 41.00–46.20) $p_{a-c} = 0.100$ $p_{1-3} < 0.001$ $p_{b-c} < 0.010$ $p_{2-3} < 0.001$	58.70 ± 2.86 (95 % CI: 53.10–64.60) $p_{a-d} = 0.160$ $p_{1-4} = 0.700$ $p_{b-d} < 0.010$ $p_{2-4} = 0.040$ $p_{c-d} = 0.250$ $p_{3-4} < 0.001$

*Notes. Indices $_{1, 2, 3, 4}$ indicate the group number depending on the investigated drugs, between the indicators of which a comparison has been conducted; Indices a, b, c, d indicate the group number depending on the hormonal status, between the indicators of which a comparison has been performed; p_{2-1} – the level of statistical significance of the difference between the groups; CEP, cryoextract of placenta; CI, confidence interval; E/C/M, esomeprazole, clarithromycin and metronidazole; EILT, ethanol-induced liver cirrhosis; HRT, hormonal replacement therapy; *: number of animals in group;*

Table 3: Effect of E/C/M and HRT on alkaline phosphatase activity in blood serum in chronic ethanol-tetrachloromethane-induced liver injury in male and female rats, g/L (M ± m (95 % CI) or Median [interquartile range], n = 112)

The study parametre	Group	Males		Females	
		Group I	Group II	Group I	Group II
		EILT + E/C/M	EILT + CEP + E/C/M	EILT + E/C/M	EILT + CEP + E/C/M
		7*	7*	7*	7*
Without alterations to the hormonal status	a	4.80 ± 0.10 (95 % CI: 4.60–5.00)	2.60 ± 0.09 (95 % CI: 2.40–2.70) $p_{1-2} < 0.001$	5.30 ± 0.27 (95 % CI: 4.80–5.80) $p_{1-3} = 0.100$ $p_{2-3} < 0.001$	2.70 ± 0.12 (95 % CI: 2.40–2.90) $p_{1-4} < 0.010$ $p_{2-4} = 0.510$ $p_{3-4} < 0.001$
Hormone therapy	b	5.0 [5.0; 5.9] $p_{a-b} = 0.03$	3.0 [2.5; 3.4] $p_{a-b} = 0.080$ $p_{1-2} < 0.001$	5.1 [4.5; 5.3] $p_{a-b} = 0.260$ $p_{1-3} = 0.140$ $p_{2-3} < 0.010$	2.0 [1.8; 2.7] $p_{a-b} = 0.060$ $p_{1-4} < 0.001$ $p_{2-4} = 0.020$ $p_{3-4} < 0.001$
Gonadectomy with hormonal replacement therapy	c	4.10 ± 0.23 (95 % CI: 3.70–4.60) $p_{a-c} = 0.020$ $p_{b-c} < 0.010$	2.20 ± 0.14 (95 % CI: 1.90–2.50) $p_{a-c} = 0.030$ $p_{1-2} < 0.001$ $p_{b-c} < 0.010$	5.20 ± 0.15 (95 % CI: 4.90–5.50) $p_{a-c} = 0.100$ $p_{1-3} < 0.001$ $p_{b-c} < 0.010$ $p_{2-3} < 0.001$	3.10 ± 0.11 (95 % CI: 2.90–3.30) $p_{a-c} = 0.010$ $p_{1-4} < 0.010$ $p_{b-c} < 0.010$ $p_{2-4} < 0.001$ $p_{3-4} < 0.001$
Gonadectomy	d	3.8 [2.5; 4.7] $p_{a-d} < 0.050$ $p_{b-d} < 0.010$ $p_{c-d} = 0.330$	2.0 [1.7; 2.5] $p_{a-d} = 0.090$ $p_{1-2} < 0.010$ $p_{b-d} = 0.020$ $p_{c-d} = 0.420$	5.8 [5.1; 6.2] $p_{a-d} = 0.240$ $p_{1-3} = 0.010$ $p_{b-d} = 0.030$ $p_{2-3} < 0.001$ $p_{c-d} = 0.080$	3.5 [3.5; 3.7] $p_{a-d} < 0.001$ $p_{1-4} = 0.400$ $p_{b-d} < 0.001$ $p_{2-4} < 0.001$ $p_{c-d} = 0.010$ $p_{3-4} < 0.001$

*Notes. Indices $_{1, 2, 3, 4}$ indicate the group number depending on the investigated drugs, between the indicators of which a comparison has been conducted; Indices a, b, c, d indicate the group number depending on the hormonal status, between the indicators of which a comparison has been performed; p_{2-1} – the level of statistical significance of the difference between the groups; CEP, cryoextract of placenta; CI, confidence interval; E/C/M, esomeprazole, clarithromycin and metronidazole; EILT, ethanol-induced liver cirrhosis; HRT, hormonal replacement therapy; *: number of animals in group;*

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In the context of E/C/M administration and the development of CCl_4-induced hepatitis, there was a parallel increase in AP levels in peripheral blood in both male and female rats, reaching 4.8 µmol/L and 5.3 µmol/L, respectively (Table 3). Male rats exhibited their highest AP activity levels in response to excessive testosterone propionate administration (5.0 [5.0; 5.9] µmol/L), while the lowest levels were observed in rats after testectomy, registering at 3.8 [2.5; 4.7] µmol/L. This difference was statistically significant ($p < 0.05$), with a reduction of 20.8 % compared to male rats without hormonal alterations (Table 3). These findings underscore the potential of male sex hormones to amplify destructive processes, especially within the liver. The study revealed that female rats, in the context of CCl_4-induced hepatitis progression and E/C/M administration, displayed the highest AP activity level among rats that had undergone ovariectomy, measuring 5.8 [5.1; 6.2] µmol/L. This level outpaced that of rats without hormonal changes by 9.4 %.

The introduction of CEP led to a significant reduction in AP activity levels in both male and female rats. Female rats with CCl_4-induced hepatitis who received E/C/M, oestradiol hemihydrate and CEP exhibited the most substantial decrease in AP activity. This parameter showed a marked statistical reduction ($p < 0.001$), dropping by 60.8 % compared to female rats not administered CEP (Table 3). Among female rats with CEP administration, the least reduction in AP activity was observed in rats after ovariectomy, with a decrease of 39.7 % compared to the group without CEP administration ($p < 0.001$). Among male rats, during CCl_4-induced hepatitis and E/C/M administration, as well as CEP treatment, the AP activity level decreased by an average of 44.9 % (ranging from 40.0 % during excessive hormonal therapy to 47.4 % during gonadectomy), compared to rats without CEP administration (Table 3).

Discussion

The development of CCl_4-induced hepatitis is known to be associated with a reduction in the TP level in peripheral blood, which is due to impaired liver protein synthesis function.[35] The study revealed that the progression of CCl_4-induced hepatitis and the administration of E/C/M resulted in a 10.8 % greater ($p = 0.03$) decrease in TP levels in females compared to males without a change in hormonal status.

To assess destructive processes in liver tissues, the study examined the AP activity in peripheral blood, as this enzyme is present, among other places, in the walls of liver bile ducts and reflects their integrity. It was observed that in the context of E/C/M administration and the development of CCl_4-induced hepatitis, there was a parallel increase in AP levels in peripheral blood in both male and female rats, reaching 4.8 µmol/L and 5.3 µmol/L, respectively. This elevated AP activity suggests the development of cholestasis, which is consistent with existing literature findings.[36]

Freire et al[2] have comprehensively examined gender-related differences in gastrointestinal tract (GIT) functionality. Their study reveals that stomach pH tends to be higher in women than in men, whereas the transit time of chyme through the stomach and intestines is comparatively shorter. Notably, certain disparities are associated with progesterone and oestrogen levels, which are influenced by menstrual cycle phases or pregnancy. The interplay of pH in the lumen and GIT motility significantly affects drug bioavailability, impacting both drug degradation rates and transit times. This dynamic, for instance, can result in an extended postprandial waiting period for drug intake.

Considering gender differences in drug metabolism and the physiological characteristics of the GIT between men and women, the optimisation of treatment regimens for the most prevalent digestive system disorders, taking into account sex hormone levels, becomes a pivotal goal in contemporary gastroenterology and hepatology.[3,4] Of particular interest is the gender-based differentiation of treatment approaches in patients with comorbid conditions, due to the risk of interactions between drugs and the influence of concomitant diseases on clinical outcomes. In patients with liver diseases, the most common comorbid conditions among others include arterial hypertension, oesophagitis, dyslipidaemia, diabetes mellitus and peptic ulcer disease (PUD).[26] According to Kim et al,[27] the prevalence of PUD among cirrhosis patients is reported to be 24.3 %, while the prevalence of *Helicobacter pylori* infection among patients with virus-induced liver cirrhosis (42.5 %) is significantly higher than among alcohol-induced liver cirrhosis patients (22.0 %, $p < 0.001$). In their examination of 619 outpatient patients with liver cirrhosis and 142 healthy control subjects, Saboo et al[28] in a study conducted in 2021, found that a greater proportion of liver cirrhosis patients were taking proton pump inhibitors

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(PPIs) as compared with the control group ($p < 0.0001$) and more men than women were using PPIs. However, it's noteworthy that PPIs, while reducing stomach acid levels, can decrease the bioavailability of medications that require intra-gastric acidity to maximise their absorption and bioavailability.[29]

The need to use medications from different pharmacological groups in patients with chronic liver diseases accompanied by PUD heightens the risks of pharmacodynamic interactions and the development of undesirable side effects, particularly hepatotoxicity associated with medications used to treat peptic ulcers.[30–34]

Presented study underscores the significant influence of sex hormones on the development of CCl_4-induced hepatitis and the response to this condition. In general, CCl_4-induced hepatitis is accompanied by a decrease in TP levels in the blood, indicating impaired liver protein synthesis function. The results reveal gender-specific differences in the response to hepatitis and hormone administration. Females experience a 10.8 % greater decrease in TP levels compared to males when E/C/M is administered, even in the absence of hormonal changes. Conversely, the administration of testosterone propionate to male rats with CCl_4-induced hepatitis in the presence of E/C/M results in a substantial 12.2 % greater decrease in TP levels, highlighting the potential negative impact of excessive male sex hormones. Castrated males, however, show an 11.0 % increase in TP levels compared to male rats without hormonal changes, indicating a potentially protective effect of removing male sex hormones. Ovariectomy in females with CCl_4-induced hepatitis and E/C/M administration leads to an 11.6 % decrease in TP levels compared to females without hormonal changes. As a result, research showed that sex hormones, particularly testosterone and oestradiol, have a significant impact on the progression of CCl_4-induced hepatitis and the therapeutic response to CEP. The results suggest a potential protective role of female sex hormones and a detrimental effect of excessive testosterone. Moreover, CEP appears to have a positive influence on TP levels and AP activity, potentially ameliorating the liver damage caused by hepatitis. Further research and a deeper understanding of these interactions are essential for the development of effective treatments for liver diseases.

Conclusion

1. The combined use of anti-ulcer drugs in the context of CCl_4-induced hepatitis displayed gender-determined differences. Specifically, female rats without hormonal alterations exhibited lower TP levels. Administering CEP under analogous experimental conditions led to the restoration of liver protein synthesis function in rats of both genders.

2. Among female rats subjected to sham surgery, a 20.8 % greater reduction in AP levels was observed when exposed to female sex hormones, whereas gonadectomy resulted in a more pronounced decrease in this enzyme's levels in male rats compared to female rats. This suggests the cytoprotective properties of female sex hormones.

Acknowledgement

None.

Conflict of interest

None.

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ORIGINAL ARTICLE

Public Perception and Willingness Towards Bystander Cardiopulmonary Resuscitation (CPR) Training and Performance in Pakistan

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Abstract

Background/Aim: Bystander cardiopulmonary resuscitation (CPR) during out-of-hospital cardiac arrest increases both survival rates and neurological recovery, but in Pakistan, an alarmingly low 2.3 % of these individuals receive bystander CPR. This study was designed to identify the reasons that affect the perception and willingness of the public toward bystander CPR training and performance in Lahore, Pakistan.

Methods: A CPR master trainer from the USA visited various organisations from 1 December 2022 to 31 January 2023, to conduct training sessions. Before and after the training, a questionnaire was distributed among respondents to fill in. The subjects were asked to answer questions about their perception and willingness to perform bystander CPR.

Results: Out of 401 participants, 240 completed the survey, with a response rate of 59.85 %. The majority of them were males [146 (60.8 %)], 215 (89.6 %) were below the age of 40, 107 (44.6 %) were graduated, 182 (75.8 %) never participated in any CPR training, mainly due to their ignorance towards the importance of bystander CPR (52.8 %) and 152 (63.3 %) were eager to participate in the CPR training course. Furthermore, the leading problem in providing bystander CPR was lack of technique or fear of possible harm that can be proved fatal (48.8 %), followed by concerns related to involvement in any legal procedure (10.0 %).

Conclusions: Bystander CPR is still uncommon in Pakistan. Participants were reluctant to perform bystander CPR because of various concerns and fears. Lack of proper skill and causing additional harm were the main reasons associated with this. Hence, while improving CPR training and public education, these findings must be considered.

Key words: Cardiopulmonary Resuscitation (CPR); Pakistan; Survey; Questionnaire.

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Introduction

Sudden cardiac arrest remains one of the most significant health issues and continues to be the leading cause of death despite all the advancements in the medical field.[1, 2] Every year, more than 3 million people experience cardiac fatalities worldwide, with the survival rate as low as 8 %.[3] For some, it is a natural death while for others, it represents an unforeseen episode that occurs before the time. Cardiac arrest can occur both in and out of the hospital. In cases of out-of-hospital cardiac arrest (OHCA), public engagement and immediate intervention from a fellow being,

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known as a bystander plays a pivotal role, making rapid resuscitation an urgent imperative. The chance of survival increases two to four times through effective and timely cardiopulmonary resuscitation (CPR).[4] Although OHCA ensures a survival rate of up to 20-70 %, the death rate is high because of insufficient practice of bystander CPR around the world.[5]

The developing countries show the gloomy side of the picture where there is insufficient knowledge related to bystander CPR. The survival rate in those regions, varied from country to country, from as low as 0 % in Mexico to 2 % in Islamabad, Pakistan.[6] It must be noted that all age groups from infants to adults experience OHCA.[7,8] As CPR is considered to be a substantial factor in survival, it is highly recommended to teach and practice this life-saving skill throughout the world because it improves the survival rate when properly managed by an individual before the arrival of medical staff.[9] Globally, many efforts are being made to improve the quality of CPR and develop the interest of people to get trained in resuscitation at the same time.[4] However, the results are quite the opposite and disappointing because insufficiently trained personnel exist.[10]

The data collected from Pakistan also suggests that the public has a poor understanding related to CPR.[11-13] This lack of knowledge can bring serious consequences and give birth to medico-legal complications. On the contrary, unacceptable techniques and insignificant knowledge can become counter-productive as they may cause CPR-related injuries. Through this study, public perception and willingness towards bystander CPR training and performance will be evaluated. Moreover, those factors will be highlighted that caused individuals hesitant to perform CPR or attend CPR training courses.

Methods

Study setting and participants

This cross-sectional study was conducted from 1 December 2022 to 31 January 2023 in Lahore, Pakistan. An American Heart Association (AHA)-certified CPR master trainer from the USA visited various organisations in Lahore, Pakistan to conduct CPR training sessions. A total of 401 participants were recruited and selected from the Falah Foundation, Hunar Foundation, Punjab Red Crescent Society and a random selection of individuals from the general population. The majority were selected from societies because of their nature of work. Their profession demands activation, more specifically, in times of emergencies or crises, they act as first responders. Hence, this training enables them to fit into the real world by equipping them with practical skills and knowledge. Informed consent was obtained from each participant. The inclusion criteria were that individuals had to be between the ages of 15 and 80 years old.

Sample size

The World Health Organization (WHO) sample size software was used to determine sample size, with a 95 % confidence level, assuming an expected population proportion of 50 % and a margin of error of 5 %. The sample size was 401 but after the exclusion of 161 participants, there were 240 respondents. These subjects were excluded due to the contradictory answers and an incompletely filled questionnaire.

Data collection tool

A detailed and well-structured questionnaire was designed as per the recent AHA guidelines and distributed to participants. It was available as both a Google form and a hard form. Most of the subjects who were below the age of 30 and at least went to college preferred to fill out online questionnaires with the help of smartphones because they found it easy. There were three sections. Part one included demographic information like age, gender and education; part two was about willingness towards bystander CPR before training and the third section consisted of knowledge and attitude of participants after training.

Statistical analysis

Data collected from the questionnaire was transferred into using IBM Statistical Package for Social Sciences (SPSS) 28.0.1 version for analysis. Descriptive statistics was used to review, tabulate and statistical analysis of demographic information and presented as frequencies and percentages. For the comparison of different variables, a Chi-square test was employed. The p-value < 0.05 was considered statistically significant. The validity and reliability of the questionnaire were assessed by Cronbach's alpha. The reliability is directly proportional to the number of items. If there were more than ten items, a Cronbach alpha of ≥ 0.7 was considered acceptable while > 0.5 was acceptable for scales consisting of less than ten items.[14]

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Ethical approval

The study was conducted after attaining authorisation from the ethical committee of respective organisations. The participants were informed and written consent was obtained from them. The anonymity was ensured by assigning unique numbers to respondents.

Results

Out of 401 participants, only 240 responses were included and analysed, with a response rate of 59.85 %. The remaining 161 respondents were excluded due to contradictory answers and an incomplete filled questionnaire. The demographic attributes of participants are exhibited in Table 1.

Table 1: Demographic attributes of participants

Demographic characteristics	n	%
Gender		
Male	146	60.8
Female	94	39.2
Age		
< 20	55	22.9
20-29	145	60.4
30-39	15	6.3
40-49	16	6.7
50-59	7	2.9
≥ 60	2	0.8
Educational background		
Primary	9	3.8
Secondary	36	15.0
Intermediate/ A-levels	72	30.0
Graduation	107	44.6
Post-graduation	16	6.7
Association with organisation		
Falah Foundation	66	27.5
Hunar Foundation	74	30.8
Punjab Red Crescent Society	53	22.1
General population	47	19.6
Have you ever participated in CPR training?		
Yes	58	24.2
No	182	75.8
What type of courses have you attended?		
Chest compression and mouth-to-mouth	56	23.3
Never attended	184	76.7
Willing to participate in a free CPR training course		
Definitely yes	152	63.3
Maybe	72	30.0
Refused to answer	16	6.7

CPR, Cardiopulmonary resuscitation;

Among the respondents, there were 146 (60.8 %) males, 94 (39.2 %) were females and 215 (89.6 %) were below the age of 40. Most of the participants 107 (44.6 %) were graduated. The majority of the subjects 193 (80.4 %) were associated with organisations or foundations, mainly from the Falah Foundation 66 (27.5 %), 74 (30.8 %) from the Hunar Foundation and 53 (22.1 %) from the Punjab Red Crescent Society. Furthermore, the test validity was found to be 0.750 (Table 2).

Table 2: Statistics on questionnaire reliability (Cronbach's alpha: α, 0.750; n, 240)

Number of items	Internal consistency	Variance	Mean score
5	0.750	0.404	2.121

For CPR background, 182 (75.8 %) never participated in any CPR training, with 56 (23.3 %) had attended both chest compression and mouth-to-mouth courses. There were some reasons for not participating in CPR training (Table 3), largely because of the unawareness of the significance of bystander CPR (n = 127; 52.8 %). However, participants 152 (63.3 %) stated that they were prepared to participate in the CPR training course.

Table 3: Reasons for not participating in CPR training courses

Reasons	n	%
There is a lack of awareness of the importance of bystander CPR	127	52.8
I have participated in a CPR training course	56	23.3
I feel I do not need to learn this	54	22.4
It should be specific for professionals only	36	14.9
It is too difficult for me	21	8.7
I am not sure when to attend the course	17	7.0
I do not have enough time	5	2.0

CPR, Cardiopulmonary resuscitation;

Before training, when participants were asked about their level of knowledge about CPR training, 68 participants (28.3 %) reported being able to perform CPR. However, a significant number of participants (87 participants; 36.2 %) stated that they did not know anything about CPR training, while 64 participants (36.78 %) reported only knowing the name of CPR. Regarding the perceived permissible delay in initiating CPR for its effectiveness, 80 participants (33.3 %) believed that a delay of 1 minute or less is acceptable. Other responses included a delay of 5 minutes (22 participants; 9.2 %), 10 minutes (29 participants; 12.1 %), 30 minutes (30 participants; 12.5 %) and having no idea (79 participants; 32.9 %). After the training, they not only became confident in performing CPR smoothly but their knowledge was also improved (Table 4).

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Table 4: Knowledge of the participant pre and post-CPR masterclass

Questions	Answers	Pre-training n (%)	Post-training n (%)
How much do you know about performing CPR?	Call for people or telephone	85 (35.4)	0.0
	Can smoothly perform CPR	68 (28.3)	240 (100.0)
	Do not know at all	87 (36.2)	0.0
What do you think "heart massage" mean?	Have no idea	52 (21.7)	0.0
	To compress the chest strongly	144 (60)	240 (100.0)
	To rub chest	44 (18.3)	0.0
Which one of the following is the most important step during CPR?	Artificial breathing	15 (6.3)	0.0
	Heart massage	63 (26.2)	0.0
	Both heart massage and artificial breathing	106 (44.2)	240 (100.0)
	Have no idea	56 (23.3)	0.0
How much time delay in CPR is permissible in CPR for its maximum effectiveness?	1 min	80 (33.3)	240 (100.0)
	5 min	22 (9.2)	0.0
	10 min	29 (12.1)	0.0
	30 min	30 (12.5)	0.0
	Have no idea	79 (32.9)	0.0

CPR, Cardiopulmonary resuscitation;

Table 5: Causes to avoid to perform CPR, even if trained

Reasons	n	%
Fear of performing immature techniques or possible harm that can be caused as a result of improper CPR	117	48.8
Agreed to perform CPR	97	40.4
No difference between performing CPR immediately and waiting for the emergency personnel to arrive	33	13.8
Avoid legal problems	24	10.0
Fear of performing mouth-to-mouth resuscitation	12	5.0
Afraid of getting an infectious disease	10	4.1
Social/ religious issues	3	1.2

CPR, Cardiopulmonary resuscitation;

Table 6: Public perception and willingness to perform bystander CPR in Pakistan

Parameter	Have you ever participated in CPR training?	Are you willing to provide simple assistance such as checking consciousness, breathing and dialling 1122?	Should the public learn CPR to help their family members or someone else when necessary?	Do you think your family members/ friends would approve of you performing CPR for strangers?	Would you prefer to perform only chest compressions, without using mouth-to-mouth resuscitation, to a stranger who needs first aid or whose breathing or heartbeat has stopped accidentally?
Yes	58 (24.2)	223 (92.9)	131 (54.6)	169 (70.4)	137 (57.1)
Maybe	0 (0.0)	17 (7.1)	70 (29.2)	52 (21.7)	18 (7.5)
No/refused to answer	182 (75.8)	0 (0.0)	39 (16.3)	19 (7.9)	29 (12.1)
Gender	0.692	0.001	< 0.001	0.002	< 0.001
Age group	< 0.001	0.835	< 0.001	< 0.001	< 0.001
Education	< 0.001	0.401	< 0.001	< 0.001	< 0.001
Association with any organisation	< 0.001	0.110	< 0.001	< 0.001	< 0.001

CPR, Cardiopulmonary resuscitation;

CONTENT

Table 7: Stratification of advanced knowledge of CPR after training

Question	Answer	General population n = 47 (%)	Studying in Falah Foundation n = 66 (%)	Vocational trainee at Hunar Foundation n = 74 (%)	Volunteer working in Red Crescent n = 53 (%)	p-value
How much do you know about the CPR training courses?	Can smoothly perform CPR	0 (0.00)	0 (0.00)	4 (8.51)	53 (100.00)	< 0.001
	Do not know at all	7 (14.89)	27 (79.41)	15 (31.91)	0 (0.00)	
	Know only the name	40 (85.11)	7 (20.59)	28 (59.57)	0 (0.00)	
How much time do you think the delay of CPR can be permissible for its effectiveness?	1 min	1 (2.13)	0 (0.00)	5 (6.76)	49 (92.45)	< 0.001
	10 min	11 (23.40)	1 (2.94)	16 (21.62)	0 (0.00)	
	30 min	23 (48.94)	7 (10.61)	8 (10.80)	0 (0.00)	
	5 min	7 (14.89)	0 (0.00)	10 (13.51)	4 (7.55)	
	Have no idea	5 (10.64)	58 (87.88)	35 (47.30)	0 (0.00)	
How much do you think CPR provided by laypeople is effective?	Have no idea	5 (10.64)	62 (93.94)	27 (36.49)	0 (0.00)	< 0.001
	Little effective	32 (68.09)	0 (0.00)	30 (40.54)	2 (3.77)	
	Moderately effective	2 (4.26)	0 (0.00)	6 (8.11)	5 (9.43)	
	Not effective	8 (17.02)	4 (6.06)	5 (6.76)	0 (0.00)	
	Very effective	0 (0.00)	0 (0.00)	6 (8.11)	46 (86.79)	
How prevalent do you think CPR by lay people is in Pakistan?	Have no idea	1 (2.13)	60 (90.90)	27 (36.49)	1 (1.89)	< 0.001
	Little prevalent	43 (91.49)	0 (0.00)	30 (40.54)	29 (54.72)	
	Moderately prevalent	0 (0.00)	0 (0.00)	3 (4.05)	2 (3.77)	
	Not prevalent	3 (6.38)	6 (9.09)	14 (18.92)	21 (39.62)	
	Highly prevalent	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	
Where did you just attend the course?	Falah Foundation	0 (0.00)	66 (100.00)	0 (0.00)	0 (0.00)	< 0.001
	Hunar Foundation	0 (0.00)	0 (0.00)	36 (76.60)	0 (0.00)	
	Red Crescent	47 (100.00)	0 (0.00)	11 (23.40)	53 (100.00)	
If you witness the collapse of your families or friends hereafter, what actions will you take?	Attempt CPR	35 (74.47)	63 (97.06)	47 (100.00)	53 (100.00)	0.041
	Call for an ambulance	12 (25.53)	1 (2.94)	0 (0.00)	0 (0.00)	
If you witness the collapse of strangers hereafter, what actions will you take?	Attempt CPR	9 (19.15)	27 (79.41)	37 (78.72)	53 (100.00)	0.02
	Call for an ambulance	36 (76.60)	7 (20.59)	9 (19.15)	0 (0.00)	
	Call people or telephone	2 (4.26)	0 (0.00)	1 (2.13)	0 (0.00)	

CPR, Cardiopulmonary resuscitation;

Participants reported many hurdles while performing CPR. Among them, performing the immature technique or possible harm that can be caused while performing improper CPR (48.8 %), afraid of being involved in legal issues (10.0 %), getting any infectious disease (4.1 %), afraid of performing mouth-to-mouth resuscitation (5.0 %) and other social, cultural or religious issues (1.2 %) were some of the reasons of the participants, showing their unwillingness to not perform CPR during medical emergencies, even if they were trained (Table 5). The summarised comparison of individual questions related to their perception and willingness to perform bystander CPR, along with p values is shown in the Table 6. On stratification, it was found that gender (male), education (higher education), age group (20-29 years) and association with any organisation or society play a significant role in performing CPR except the willingness to assist in consciousness, breathing or dialling 1122 (Table 7).

On detailed analysis, it was concluded that participants' beliefs about the effectiveness of CPR provided by laypeople varied. Ninety-four respondents (39.17 %) stated that they had no idea about the effectiveness of CPR performed by laypeople. Similarly, training played a significant role in changing the perception of professionals including medical staff, social workers, or basic life savers as they showed positive attitudes and willingness towards performing CPR after the masterclass as compared to the general population who was only interested in performing CPR on their family members or friends, even if trained.

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Discussion

Cardiovascular emergencies are quite common and life-threatening occurrences that must be identified and treated without delay to increase the survival rate of patients.[15] For this, bystander CPR is considered an essential and significant factor for OHCA survival. Despite widespread recognition as a life-saving ability, the rate of bystander CPR is less than 50 % on average,[16, 17] with its restricted training and performance implementation in low and middle-income countries. Pakistan is not an exception.[6] This study was devised to highlight the low rate of bystander CPR performance and undercover the obstacles in improving the rate and ability to perform CPR by a layman.

In study, the main reason for not learning and taking part in CPR training was insufficient behaviour and knowledge of the importance of bystander CPR. They did not know that they could also play their part in saving victims with the help of proper training. Chen et al[3] also demonstrated in their research that bystander CPR provided by the common man is based on the behaviour of kindness and goodwill towards strangers. Thus, people are willing to perform CPR out of goodwill towards strangers. But several factors stop them from doing so. First and the foremost reason is their fright of the consequences when they cause harm unintentionally, followed by their involvement in legal actions. Cheskes et al[18] also identified the barriers and driving forces in performing bystander CPR and concluded that compassionate and humane beliefs urge participants to perform CPR. Similarly, Daud et al,[19] Johnston et al,[20] Mao et al[21] and Dobbie et al[22] pointed out common barriers including fear of transmission of disease, blood, perceived danger, legal actions and become a reason of injury unintentionally. Attitude towards culture or religion is another neglectful, yet important barrier in performing CPR. The study concluded that culture is an unrecognised barrier but doctors in Pakistan did not consider it as a barrier while performing CPR.[23]

On stratification of results for variables, it was found that gender, age, education and belonging to any society or foundation plays a significant role in willingness towards bystander CPR training and performance on strangers. In a retrospective study, it was observed that the majority of individuals who participated in training were between the age of 20-29 and this age group was also less hesitant to perform CPR on strangers. Similarly, as compared to females, males showed their willingness. Those who were reluctant to perform CPR, even after training had primary or secondary education. A similar study was conducted by Huang et al[5] in which gender (more men as compared to women) were more inclined to perform bystander CPR. This was in contradiction with the observations made by Ahmed et al[24] where a high number of females performed bystander CPR.

Studies also revealed that many times, cardiac arrests occur at home or out of the hospital and demand instant treatment. Therefore, bystander CPR plays a significant role. But it must not be performed without proper training. In the present study, 63.3 % of participants were eager to participate in a CPR training course. From table 6, it can be clearly assumed that professionals are more inclined to perform CPR but the general population is reluctant to perform CPR, specifically on strangers. Iqbal et al[23] also stated that CPR is one of the most influential components in the cycle of survival and 82.3 % of doctors believed that without any training, bystanders should not perform CPR. According to Park et al[26] and Khan et al[6] the performance of bystander CPR is essential along with the pre-hospital emergency care system. They also reinforced that through bystander CPR, OHCA outcomes can be improved.

In summary, this study suggests that the government along with organisations need to make a strategic plan so that bystander CPR performance can be improved. Firstly, the public should be educated in this regard so that the situations can be controlled and improved for those who need CPR outside the hospital.[27] Secondly, legislation must be introduced to protect bystanders who came forward to rescue and put aside all their fears. Thirdly, the authoritative bodies need to create an encouraging atmosphere socially and culturally for those who give a helping hand to others in need. Finally, targeting a large number of the population irrespective of their race, language, gender, etc to get CPR training through many national programs and make it compulsory at the school and college level.[26]

Limitations

This study has several limitations. Firstly, it involved a very limited number of individuals from the general population which cannot be considered the representation of Pakistan. Secondly, research was conducted through a questionnaire and participants' behavioural intentions were analysed hypothetically. As the behaviours in the real world vary from situation to situation, thus, the responses of positive behaviour do not mean to occur in real scenarios. It is recommended to investigate actual CPR performance for future studies.

Conclusion

In conclusion, the number of people realising the importance of bystander CPR is increasing around the globe. But, in Pakistan, CPR training and bystander CPR are quite uncommon to date. The population has started to show interest and willingness towards CPR training and bystander CPR irrespective of gender, age and education. The obstacles that cause reluctance in performing this life-saving action are fear of disease transmission, involvement in legal procedure and low confidence in performing CPR ie fear they may adopt the wrong technique and eventually harm the injured person. However, it is highly suggested that governments, organisations and responsible personnel join hands together to create awareness among common citizens, provide effective and high-quality training and sound legislation, include CPR in the curriculum and encourage culture to offer assistance to those needed to increase the willingness to perform bystander CPR without any fears.

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Conflict of interest

None.

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ORIGINAL ARTICLE

The Influence of Socioeconomic Status and General Health on the Fracture Incidence

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Abstract

Background/Aim: There is an association between patient general health, socioeconomic status and fracture incidence. Aim of this study was to demonstrate the relation between the socioeconomic and health status and the occurrence of fractures in Al-Kindy Hospital residents.
Methods: A prospective cross-sectional study was performed by using data collected from fractured patients in Al-Kindy Teaching Hospital / Baghdad Orthopaedics Outpatient Clinic, between 12 December 2021 and 8 March 2022. A hundred patients aged 20 to 75 years were included in this study. The data were collected using a researcher-administered questionnaire, which included items to assess patient's socioeconomic status (accommodations, occupation, income status, level of education, owing motor vehicle) and assessed patient's health status (height, weight, presence of chronic disease(s), smoking status, alcohol consumption, water source quality, sun exposure, nutrition status).
Results: A 100 patients from Al-Kindy Teaching Hospital between the age 20-75 participated in the study. The incidence of fractures was higher in younger and more active males (68 %) than females (32 %). Highest percent appeared in lower group of socioeconomic status (58 %) and in lower group of health status (44 %). There was a significant relation between health status and body mass index (BMI). Obese and overweight patient were the more vulnerable to fractures.
Conclusion: Fractures are more likely to happen to people with low socioeconomic status, low general health status and overweight people.

Key words: Socioeconomic status; Fractures; Body mass index; General health.

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Introduction

A fracture refers to the occurrence of a discontinuity or disruption, often seen in the structure of a bone. An open or complex fracture is the term used when a shattered bone penetrates the skin. Fractures often occur as a result of vehicular collisions, accidental falls, or sports-related traumas. Additional factors contributing to bone deterioration include inadequate bone density and the presence of osteoporosis.[1]

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Socioeconomic status refers to the societal position or socioeconomic class occupied by a person or a collective entity. Socioeconomic status may be determined by many factors, including education level, occupation, house ownership (weighted by a factor of 0.5), car ownership (weighted by a factor of 0.1) and an adjustment for age relative to 20 (divided by 100). Additionally, those who are retired, jobless, or died are also taken into account when assessing socioeconomic status.[2]

The construct is often quantified as a composite of educational attainment, financial earnings and professional vocation. Analyses of socioeconomic position often uncover disparities in resource accessibility, as well as concerns pertaining to privilege, power and authority. Individuals with less educational attainment may have a higher likelihood of encountering risk variables that make them susceptible to experiencing a fracture, thereby elevating their potential mortality risk associated with such fractures. There is an observed correlation between lower educational attainment and increased death rates associated with fractures. This implies that efforts aimed at preventing and treating fractures should prioritise those with limited educational backgrounds. Moreover, there exists a correlation between lower income and an elevated likelihood of experiencing fractures. Nonetheless, there exists a dearth of knowledge on the influence of one's career, specifically in terms of its degree of physical activity and the socioeconomic status it signifies, on the susceptibility to hip fractures in the latter stages of life.

The elucidation of the connections between socioeconomic status and health often revolves around three primary causal hypotheses: (i) socioeconomic status serves as a determinant of health outcomes, (ii) health outcomes operate as determinants of socioeconomic status, or (iii) both socioeconomic status and health outcomes are influenced by a same underlying element. The existence of significant disparities in health status is a well-documented phenomenon, both among nations categorised as developed or developing, as well as within individual countries across various demographic groupings.[3]

Aim of this study was to demonstrate the relation between the socioeconomic status and the occurrence of fractures in Al-Kindy Hospital residents and to demonstrate the impact of level of education and type of occupation on occurrence of fractures, as well as to determine whether there was association between general health and fracture incidence and measuring the body mass index (BMI) of each patient and determine its effect on occurrence of fractures.

Methods

This was a prospective cross-sectional study. Data were collected from fractured patients in Al-Kindy Teaching Hospital / Baghdad Orthopaedics Outpatient Clinic, between 12 December 2021 and 8 March 2022.

A detailed baseline survey was performed from young and middle-aged adults including socioeconomic characteristics (accommodation, occupation, educational level, number of family members, income status, owning of motor vehicle). The socioeconomic index (SEI) was calculated based on careful assessment performed on a particular reference to:
SEI = education + occupation + house ownership x 0.5 + car ownership x 0.1 + (age—20)/100— retired/unemployed/deceased.[2]

Physical activity, past medical and drug history, sun exposure, nutritional status and water source quality, lifestyle risk factors (smoking, alcohol consuming) were obtained. Smoking behaviour recorded as the number of cigarettes smoked per day as (heavy, moderate, light). Physical activity and alcohol consumption were self-reported by patients. Anthropometric measures included height and weight and BMI was calculated (weight (kg) / (height (m) x height (m)). BMI was categorised as: underweight, normal weight, overweight, obese.[4]

A 100 patients participated in this survey. Inclusion criteria were patients with fracture, aged between 20-75 years. Non-inclusion criteria were patients below 20 years of age and where all data could not be collected. Informed consent was obtained from all participants verbally and in written form. This study was approved but the Scientific and Ethical Committee of Al-Kindy College of Medicine with the registration number EA-5842.

Statistical Analysis

Data were presented in tables and graphs. SPSS version 25 was used to calculate the associations of social and economic factors and various as-

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pects of public health with different age groups of the members participating in the study. Data were compared by using Chi-square test. P-value of < 0.05 was considered statistically significant.

Results

Of the 100 fractured participants 68 % were male, 32 % were female. Most patients were among young age group (49 %) and middle age group (40 %), while 11 % were elderly patients (Table 1).

Table 1: Demographic data of the studied sample and their socioeconomic index (SEI)

Variable	Categories	N	%
Gender	Male	68	68.00
	Female	32	32.00
Age categories	Young age	49	49.00
	Middle age	40	40.00
	Elderly	11	11.00
SEI	Upper	8	8.00
	Middle	34	34.00
	Lower	58	58.00
Income status	Poor	10	10.00
	Intermediate	52	52.00
	Good	31	31.00
	Very good	7	7.00
Owning motor vehicle	No	52	52.00
	Yes	48	48.00
Health status	Upper	16	16.00
	Middle	40	40.00
	Lower	44	44.00
Physical activity	No	86	86.00
	Yes	14	14.00
Chronic disease	Yes	28	28.00
	No	72	72.00
Smoking	Non smoker	47	47.00
	Moderate smoker	24	24.00
	Heavy smoker	29	29.00
Sun exposure	Poor	14	14.00
	Intermediate	22	22.00
	Good	39	39.00
	Very good	25	25.00
BMI	Underweight	1	1.00
	Normal weight	27	27.00
	Overweight	38	38.00
	Obese	34	34.00
Site of fracture	Upper extremity	31	31.00
	Lower extremity	64	64.00
	Others	5	5.00

BMI: body mass index;

Fractures increased with reduced physical activity (86.0 % reported lack of exercise) and increased BMI (38.0 % were overweight and 34.0 % were obese). Majority of the patients reported with lower extremity fracture - 64.0 % (leg 35 %, foot 17 %, thigh 7 %, knee 4 %, pelvis 3 %, ankle 1 %). Fifty-three percent were smokers (moderate smoker 24.0 % and heavy smoker 29.0 %), 28.0 % of patients had chronic disease, 14 % reported poor sun exposure and 22 % intermediate exposure.

Fractures increased with increased socioeconomic deprivation. This category of people with low socioeconomic status 58.0 % included low level of education (illiterate 22.4 %, primary 34.5 %, intermediate 17.2 %, secondary 24.1 % and only 1.7 % who got bachelor or higher degree) comparing with the category of people with higher socioeconomic status where 100.0 % were bachelor and higher (Table 2). In crowded houses in 50.0 % of cases were 5 to 10 members in the house and in 24.1 % more than 10 members.

Table 2: The number of family members according to socioeconomic status

Socioeconomic status	Number of family members	N	%
Upper	None	0	0.00
	3 to 5	3	37.50
	5 to 10	5	62.50
	More than 10	0	0.00
Middle	None	0	0.00
	3 to 5	19	55.90
	5 to 10	14	41.20
	More than 10	1	2.90
Lower	None	1	1.70
	3 to 5	14	24.10
	5 to 10	29	50.00
	More than 10	14	24.10

Majority low socioeconomic status patients (51.7 %) inhabit North Rusafa (Madinah, ALShaab, Jamilah, Binouk ,Saba Abkar, Hay Sumer, AL-Thaealiba, Al-Hussainiah and others) (Table 3).

Upper class people tended to have higher number of bachelor and other higher studies qualifications as compared with other classes (Table 4). People of low socioeconomic class tended to have higher number of unemployed people as compared to other classes (Table 5).

No significant difference was found according to Chi-squared test between BMI and different location of fractures (upper extremities, lower extremities, other sites) (Table 6).

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Table 3: The residence according to socioeconomic status

Socioeconomic status	Residence	N	%
Upper	North Rusafa	5	62.50
	Karkh	1	12.50
	South Rusafa	0	0.00
	West Rusafa	0	0.00
	Central Rusafa	2	25.00
	Outside Baghdad	0	0.00
Middle	North Rusafa	16	47.10
	Karkh	7	20.60
	South Rusafa	6	17.60
	West Rusafa	0	0.00
	Central Rusafa	5	14.70
	Outside Baghdad	0	0.00
Lower	North Rusafa	30	51.70
	Karkh	7	12.10
	South Rusafa	10	17.20
	West Rusafa	3	5.20
	Central Rusafa	7	12.10
	Outside Baghdad	1	1.70

Table 4: The distribution of educational level according to socioeconomic status

Socioeconomic status	Level of education	N	%
Upper	Illiterate	0	0.00 %
	Primary	0	0.00 %
	Intermediate	0	0.00 %
	Secondary	0	0.00 %
	Bachelor and higher	8	100.00 %
Middle	Illiterate	0	0.00 %
	Primary	1	2.90 %
	Intermediate	3	8.80 %
	Secondary	11	32.40 %
	Bachelor and higher	19	55.90 %
Lower	Illiterate	13	22.40 %
	Primary	20	34.50 %
	Intermediate	10	17.20 %
	Secondary	14	24.10 %
	Bachelor and higher	1	1.70 %

Table 5: The distribution of occupational status according to socioeconomic status

Socioeconomic status	Occupational status	N	%
Upper	Employee	8	100.00 %
	Freelancer	0	0.00 %
	Unemployed	0	0.00 %
Middle	Employee	18	52.90 %
	Freelancer	5	14.70 %
	Unemployed	11	32.40 %
Lower	Employee	2	3.40 %
	Freelancer	32	55.20 %
	Unemployed	24	41.40 %

Table 6: The body mass index (BMI) in patients with fractures

BMI	Site of fracture (N; %)								p-value
	Upper extremity		Lower extremity		Other site		Total		
Underweight	0	0.00	1	1.56	0	0.00	1	1.00	0.693
Normal weight	11	35.48	15	23.44	1	20.00	27	27.00	
Overweight	12	38.71	23	35.94	3	60.00	38	38.00	
Obese	8	25.81	25	39.06	1	20.00	34	34.00	
Total	**31**	**100.00**	**64**	**100.00**	**5**	**100.00**	**100**	**100.00**	

p-value was calculated by Chi-squared test;

Table 7: The socioeconomic index (SEI) in patients with fractures

BMI	Site of fracture (N; %)								p-value
	Upper extremity		Lower extremity		Other site		Total		
Upper	3	9.68	4	6.25	1	20.00	8	8.00	0.434
Middle	9	29.03	22	34.38	3	60.00	34	34.00	
Lower	19	61.29	38	59.38	1	20.00	58	58.00	
Total	**31**	**100.00**	**64**	**100.00**	**5**	**100.00**	**100**	**100.00**	

p-value was calculated by Chi-squared test;

The prevalence of the fracture was higher among participants who were with lower socioeconomic status (58 %) and lower level of health status (44 %), but no significant difference was found between groups (Table 7).

Discussion

The association between SEI and fracture and other musculoskeletal disorder is little understood, despite there being an inverse relationship between SEI and most causes of morbidity.

In this study these two variables were chosen (socioeconomic status, general health status) due to the importance of them to determine whether they have a significant relation to the possibility of fractures incidence or not. Hypothesis stated that people with low SEI and low general health status would have a higher incidence rate than the others with higher SEI and general health levels.

There were 68 males with an average age of 35 and 32 females with an average age of 50.4 years which is acceptable since the incidence of fractures are higher in younger and more active males than females despite the increasing potentiality of fracture occurring in older females. This finding goes with results of study by Khalid[5] and is in contrast with finding of Kiebzak et al.[6]

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Moreover, 75 % of females were housewives and the rest were working in a relatively safe jobs like an employee compared to 52.9 % male workers, 16.2 % unemployed and 30.9 % employees. It is needed to be mentioned that even the safe occupations (eg, employee) have some kind of risk including transportation accidents and so on. Researchers noticed a pattern, so their opinion was that the occupation does have an effect on the probability of fracture incidence which was observed and found to be significant in this study. The more dangerous the occupation a higher probability of fracture occur. More male patients in this randomly taken sample were observed and most of them were prone to more danger than the females because of their occupational status. It also should be mentioned that most of these patients with relatively risky occupation belong to the low SEI class (when only males were considered).

In this study most patients were distributed geographically among the regions of Russafa / Baghdad (84 %) with the majority of their accommodations located in north Russafa (51 %) mainly Madinah (Madinah-t-Al-Sadder) and AL-Shaab and about 19 % were distributed among south and east Russafa mainly Baghdad AL-Jadeda, AL-Amin, AL-Mashtal and AL-Obaidi and a smaller percentage of 14 % and 15 % for central Russafa (eg Ziyouna, Karada, AL-Kiffah, Al-Baladiat) and Karkh (eg Jihad, AL-Mansur, AL- Salhia, AL-Baiaa, AL-Sakan), respectively. It was observed that the majority of the patients were from regions known for their active lifestyle considering the relationships between their residents and environmental interactions of them, this might cause a higher probability of getting involved in different accidents that will possibly lead to a higher rate of bone fracture incidences among the people living in this kind of regions, which goes with findings of Li et al.[7] It was also noticed that regions with more disadvantages would have a higher incidence rate of bone fractures, similar with findings of Lo et al.[8]

The effect of diet (nutritional status) on the probability of fracture incidence was also analyses and the BMI was chosen as the indicator of this variable due to a relatively good indication of body fatness.[9, 10] Of the 100 patients it was found that 34 % were obese, 38 % overweight, 27 % normal weight and 1 % underweight and a significant relation between the health status and the BMI index was found. Therefore, the obese participants made about 60 % of the lower health status class in this sample which indicates more health problems for this group. Some studies suggest that being a bit overweight may not be so bad for the individuals.[11-13]

Moreover, obese patients constitute 39 % of the lower extremity fractures and about 25 % of the upper extremity fractures which does not go with Saverio et al[14] as they found that higher BMI levels is associated with higher incidence of upper extremity fractures (specifically humerus bone) but it does go with Ong et al[15, 16] who found that it is more likely for the obese people to break their lower extremity (specifically, ankle) as well as the upper extremity.

The 100 patients were arranged in three groups depending on the SEI - upper 8 %, middle 34 % and lower 58 %. In this study it was notice a higher incidence of fracture among the lower class of socioeconomic status and that goes with Valentin et al.[17, 18] Also, most of the fractures of the lower class were in the lower extremity which goes against Carlen et al who found that the wealthier population is at 30 % higher risk of lower extremity fractures incidence (specifically hip bone fractures).[19]

As for the general health status, patients were also arranged in three groups upper (16 %), middle (40 %) and lower (with slightly more individuals than the middle group with 44 %). Most of the patients were not exercising on regular bases (86 %) which does not indicate a good health status and that goes with Han et al[20] who found that higher levels of physical activity are associated with fracture prevention.

Limitations

The main limiting factor were the participants, most of them were suspicious and almost scared, even the individuals who agreed to cooperate were giving misleading answers to the questions due to cultural or personal reasons such as feeling ashamed of the answer. Eg there were only three patients who admitted that they have been consuming alcohol which is in some way suspicious and 47 % individuals responded that they had a good diet with a protein source and an amount of vegetables but actually 72 % of them were overweight and obese so it can be assumed that they were ashamed and it necessary to considered these results to be potentially misleading.

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Conclusion

The incidence of fracture is more likely to happen to people with low socioeconomic status and low general health status. The incidence of fracture was higher among men which may be due to their more active lifestyle. Obese and overweight patients were the most vulnerable to fractures, with the lower extremity to be the most common site of fracture in both of them. It was also noticed that the regions with a faster and more active lifestyle and regions with less advantages were associated with a higher incidence of bone fracture among their residents.

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Conflict of interest

None.

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ORIGINAL ARTICLE

The Correlation Between Nurses' Knowledge of Triage and the Accuracy of Triage Level Interpretation in the Emergency Department

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Abstract

Background/Aim: Knowledge about triage has been identified as one of the factors influencing patient outcomes in the emergency room. Nurses' knowledge regarding triage is necessary in order to work effectively in managing patients, as swift and accurate actions depend on the nurses' acquired knowledge. The aim of this research was to determine the correlation between nurses' knowledge of triage and the accuracy of triage-level interpretation.
Methods: The data from 145 nurses were collected by using a questionnaire sheet for the triage knowledge variable and an observation sheet for the variable of accuracy in triage level interpretation. This research was conducted in May-June 2023 at Tugurejo Regional General Hospital, KRMT Wongsonegoro Regional General Hospital and Dr Kariadi General Hospital. The independent variable in this study was nurses' knowledge and the dependent variable was the accuracy of triage level interpretation. The statistical analysis included descriptive statistics and bivariate analysis using the Chi-square test for comparison between groups.
Results: Based on the results of the bivariate analysis, nurses' knowledge has a correlation with the accuracy of triage-level interpretation with a p-value of < 0.001.
Conclusion: There is a correlation between nurses' knowledge of triage and the accuracy of triage-level interpretation.

Key words: Emergency department; Triage knowledge; Accuracy of triage level interpretation.

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Introduction

The emergency department (ED) is the initial department when a patient enters a hospital to receive medical care for emergency conditions. Emergency condition refers to a situation in which a patients require immediate assistance to save their life. Upon arrival at ED, patients are sorted and prioritised to receive care based on the issues they are facing during the triage process.[1, 2]

According to the data, it was reported that patients visits to ED have been consistently increasing each year, with an approximate 30 % rise compared to all emergency units worldwide.[3] Based on the data, patients visits to EDs in Indonesia reached 4.402.205 in 2017. The data further indicates that patients visits to EDs in Central Java amounted to 1.990.104 in 2018.[4] Knowl-

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edge of triage has been identified as one of the factors influencing patients outcomes in the ED. ED nurses require in-depth knowledge and clinical expertise to effectively manage patients and handle various situations, such as overcrowded, proficiently.[5]

Nurses' knowledge regarding triage is essential for managing as many patients as possible within a short timeframe in ED, as swift and accurate actions depend on the nurses' acquired knowledge. Adequate triage knowledge equips nurses to effectively apply their expertise in their professional practice, thereby impacting the quality of patients' lives and satisfying the families' needs.[6,7]

The aim of this research was to determine the correlation between nurses' knowledge of triage and the accuracy of triage-level interpretation.

Methods

The research site was at Tugurejo Hospital, KRMT Wongsonegoro Hospital and Dr Kariadi Hospital. The study as performed from May to June 2023.

The sample covered by this study included 145 nurses in the ED at Government Hospitals in Semarang. Nurses included in the criteria had at least one year of experience in the emergency room and have received previous triage training.

The data taken included: age, sex, length of work (in years), education, nurses' knowledge of triage and the accuracy of triage level interpretation. The research was conducted on the correlation between nurses' knowledge of triage and the accuracy of triage level interpretation.

The statistical analysis included descriptive statistics, bivariate analysis using the Chi-square test for comparison between groups. Data obtained were analysed using the IBM SPSS Version 24 program. The level of statistical significance was set at $p < 0.05$.

Ethical consideration

This study was approved by Health Research Ethics Committee RSUP Dr Kariadi Semarang (No 1451/EC/KEPK-RSDK/2023).

Results

A descriptive analysis was conducted to obtain an overview of the independent and dependent variables. The respondents were mostly (53.1 %) female, the average age of nurses was 34.3 ± 5.6, the youngest being 21 years old and the oldest being 53 years old, the majority of nurses (61.4 %) had completed a diploma degree in nursing and mostly (44.1 %) had a work experience of 1-5 years.

Table 1: Descriptive analysis data of nurses' knowledge of triage and the accuracy of triage level interpretation

Triage categories [n (%)]	Nurses' knowledge of triage		
	Good	Sufficient	Inadequate
Under triage	13 (9.0)	5 (3.4)	21 (14.5)
Accurate triage	90 (62.1)	7 (4.8)	2 (1.4)
Over triage	1 (0.7)	3 (2.1)	3 (2.1)
Total	**104 (71.7)**	**15 (2.2)**	**26 (17.9)**

Table 2: The relationship between nurses' knowledge and accuracy of triage level interpretation

Triage categories	Nurses' knowledge of triage								p-value
	Good		Sufficient		Inadequate		Total		
	n	%	n	%	n	%	n	%	
Under triage	13	9.0	15	3.4	21	14.5	39	26.9	< 0.001
Accurate triage	90	62.1	7	4.8	2	1.4	99	68.3	
Over triage	1	0.7	3	2.1	3	2.1	7	4.8	
Total	**104**	**71.7**	**15**	**10.3**	**26**	**17.9**	**145**	**100.0**	

There was correlation between nurses' knowledge of triage and the accuracy of triage level interpretation (Chi-squared test, $p < 0.001$) (Table 2).

Discussion

Based on the results of this research, the majority of nurses possessed a good level of knowledge, accounting for 71.8 %, indicating that there were still nurses with sufficient and insufficient knowledge, comprising 28.2 %. Knowledge is influenced by various factors, including age. Age impacts an individual's comprehension and cognitive abilities. As age increases, an individual's understanding and thought processes develop, leading to an enhancement in the acquired knowledge.[8,9] In this research, nurses with a good level of knowledge were most prevalent in the early adulthood age group, specifically between 21 and 35 years, comprising 44.8 % of the total. As an individual's age increases, their manner

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of thinking and working matures, consequently exerting a greater influence on the accumulation of knowledge regarding triage and experience in effectively and accurately managing patients.[9]

Based on this research, the obtained average tenure of the respondents' work was 7.7 years, with a minimum work period of 1 year and a maximum of 30 years. An individual's length of service significantly influences their knowledge.[10] The more experience an individual accumulates, the greater their understanding of the matter will become.[11] This indicates that the longer the tenure, the higher the level of cognitive maturity an individual possesses, thereby leading to an increase in the acquired knowledge.[12–14] Based on this research, the majority of nurses in interpreting triage levels fall within the appropriate triage category, accounting for 68.3 %. The results of this research also indicate that there are still nurses with triage level application falling within the under triage and over triage categories, amounting to 31.7 %. This is influenced by the knowledge and the often-occurring patient congestion in the ED, which results in inadequate triage determinations.

A strong knowledge base yields proficient action. Adequate nurse knowledge about triage enhances their skills in implementing triage, subsequently influencing the effectiveness of care delivery.[15, 16] Knowledge also serves as the foundation in the process of implementing triage.[17] Knowledge is a dominant factor that supports decision-making in establishing patient triage priorities.[18, 19] Based on this research, it is evident that there is a correlation between nurses' level of knowledge about triage and the accuracy of triage level interpretation in the Emergency Department of the Government Hospitals in Semarang. Nurses with a good level of knowledge fall within the appropriate triage category, accounting for 62.1 %. This indicates that the better the nurses' knowledge level, the more accurate their triage level interpretations tend to be.

The results of this research are in line with previous research that has proposed that knowledge has a significant influence on the implementation of triage.[19] The knowledge of ED nurses about triage greatly aids them in identifying emergency cases, which not only has a positive impact on the quality of care but also can prevent an increase in mortality and disability.[20,21] Other research results also indicate that a significant relationship exists between the level of knowledge and the application of triage in ED. The results reveal that as one's knowledge level improves, their ability to carry out triage also improves. Conversely, a lower level of knowledge corresponds to a reduced ability in implementing triage.[22] Possessing knowledge about triage is crucial for a triage nurse as it provides an understanding of a patient's condition.[23, 24]

Nurses' knowledge and experience regarding the accuracy of triage in the ED constitute specialised skills that ED nurses must possess.[25–27] Adequate nursing knowledge holds a significant correlation with the implementation of triage. Knowledge within the nursing realm is of paramount importance, particularly for the nurses themselves. With sufficient knowledge about triage, it is expected that nurses will be able to conduct triage accurately and precisely in the future.[26]

Based on this research, the majority of nurses exhibit a satisfactory level of knowledge, although there are still nurses with moderate and insufficient knowledge. Another result of this research also indicates that there are still ED nurses categorised under both under triage and over triage. The consequences of inadequate knowledge and inaccuracies in triage determination by an ED nurse can indeed pose serious and perilous implications for patient safety. Moreover, it can also have an impact on the quality of care provided. It is crucial for ED nurses to continually enhance their triage knowledge and skills through advanced education, training and on-the-job experiences, ultimately yielding favourable outcomes for both patients and the care rendered.

Conclusion

A high level of nurses' knowledge is correlated with the accuracy of interpreting triage levels in the ED. The results of this research are expected to serve as a reference for enhancing knowledge about triage and improving abilities related to the implementation of triage.

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CONTENT

Conflict of interest

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REVIEW ARTICLE

The Impact of Antioxidant Diets, Nutraceuticals and Physical Activity Interventions in the Prevention of Cardiometabolic Diseases: An Overview

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Abstract

Hippocrates – Father of Medicine (ca 460-370 BC) – endorsed the cuative effects of foods; he said: "Leave your drugs in the chemist's pot if you can heal the patient with food". This review focuses on the management of cardio-metabolic diseases (CMDs) with nutraceuticals and antioxidant diets such as *Allium sativum*, turmeric, soybean, peptides, phytosterols, resveratrol, polyphenolic substances etc. CMDs are a cluster of conditions linked to altered fat and carbohydrate metabolism as well as macro- and micro-vascular problems. CMDs cause severe pathophysiological and metabolic alterations in the body, resulting in the occurrence of chronic diseases like atherosclerosis, coronary heart disease and stroke, neurodegenerative ailments, fatty liver, kidney malfunction, hypercholesterolaemia, hyperlipidaemia, insulin resi-tance and some cancers, consequently imposing a very high economic burden on the healthcare costs. Currently used pharmacotherapies are not only expensive but also are associated with undesirable adverse events. Thus, there is an urgent need for affordable, cost-effective and alternative safe therapies for the prevention and management of CMDs. Holistic approaches targeted for health promotion and prevention of CMDs include the intake of antioxidant-rich diets, anti-inflammation wholesome foods and moderate physical activity (about 30 min/day). Such strategies will not only prevent obesity-related CMDs, type 2 diabetes mellitus (T2DM), coronary heart disease and stroke, but also will improve the quality of patient's life and consequently reduce healthcare burdens. Nutraceuticals and probiotics exhibit anti-inflammation, anti-aging, anti-obesity and anti-diabetic effects, thereby reducing the adverse health risks associated with CMDs. Antioxidants protect cell membranes and DNA from excessive free radicals, which contribute to CMD related diseases. Physical exercise along with dietary interventions helps to mitigate oxidative stress, improve blood triglyceride levels, increase HDL-cholesterol and reduce LDL-cholesterol and reverse the biological markers associated with CMDs. Many studies have provided robust scientific evidence and demonstrated links between dietary interventions, nutraceuticals, probiotics, wholesome foods and physical activity for the prevention of CMDs. The major limitations in promoting nonpharmacological therapies for health and well-being benefits are a lack of public awareness and a paucity of clinical nutrition instruction for medical students on the merits of complementary methods for the prevention and management of CMDs. The goals of this review are to provide up-to-date knowledge about selected nutraceuticals, wholesome foods and physical activity in the prevention of CMDs and the underlying mechanisms associated with each intervention, which will ultimately improve patient's quality of life and assist in reducing healthcare costs globally.

Key words: Cardiometabolic diseases; Cardiovascular diseases; Coronary heart disease; Hypertension; Antioxidant and anti-inflammation diets; Nutraceuticals; Physical activity.

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Introduction

The cardiometabolic diseases (CMDs) consist of a wide array of disorders characterised by abnormalities in glucose and fatty acid metabolism, which can lead to both systemic and localised vascular dysfunctions. CMD-induced pathophysiological changes cause hypertension, hyperlipidaemia, atherosclerosis, greater likelihood of developing coronary heart disease and stroke, non-alcoholic fatty liver, hyperglycaemia, insulin resistance, kidney dysfunction and some cancers.[1-4] The occurrence of non-communicable diseases (NCDs) like obesity, type 2 diabetes mellitus (T2DM), CMDs, neurological ailments, osteoarthritis and cancer are escalating all over the world. NCDs inflict a very high financial toll on the healthcare systems because prolonged hospital time is required for recovery, accompanied by high drug and physician expenses. It has been estimated that one in every three individuals suffers from CMDs worldwide[5] and CMDs enhance the risk of cardiovascular diseases and neurological disorders such as dementia and cognition problems.[6]

Currently, CMDs are treated with pharmaceutical agents such as antihypertensives, antidiabetics and lipid-lowering drugs. However, these therapies are not only expensive but are also associated with undesirable adverse effects.[7] Therefore, there exists an urgent need for cost-effective and alternative safe therapies for the management of CMD-related disorders. Some alternate strategies include dietary interventions (eg, lesser consumption of carbohydrates and saturated fat diet), weight reduction in obese patients through physical activity and lesser use of antihypertensive, antidiabetic and lipid-lowering drugs.[7] Pharmaceutical therapies often target enzymatic pathways in patients. However, some drug therapies may produce undesirable side effects, thereby compelling the patients and the healthcare practitioners to make a risk-benefit analysis.[8-12] For example, switching over to angiotensin-converting enzyme (ACE) inhibitors and dose reduction of lipid-lowering statins for treating hypertension and reducing hypercholesterolaemia, respectively. The prolonged usage of ACE inhibitors is sometimes associated with adverse iatrogenic effects, including decreased kidney function, elevated potassium levels, hypotension, chronic cough, skin rashes, angioedema and foetal abnormalities due to oligohydramnios.[13] The most common side effects of cholesterol-lowering statins are drowsiness, insomnia, headache, muscle weakness muscle aches (myalgia) and flushing of the skin. Due to the occurrence of unwanted side effects of drugs, the usage of alternative remedies, eg, nutraceuticals and phytotherapies, antioxidant and anti-inflammation dietary interventions, functional foods, probiotics and physical activity have gained momentum for the management of CMDs.[14, 15]

It's now well recognised that wholesome foods, antioxidant and anti-inflammation diets can have a critical influence in the prevention of CMDs associated with T2DM and obesity. Lifestyle modifications such as smoking cessation, less alcohol consumption and moderate exercise (30 min/day) are significant contributors to the prevention of metabolic syndrome (MS), hypertension and some cancers. Obesity, T2DM and unhealthy dietary habits are considered the primary cause of CMDs and cardiovascular diseases (CVDs).[16] The wide variety of risk factors associated with CMDs and CVDs are illustrated in Figure 1. The industrial revolution followed by the green revolution, agriculture mechanisation, urbanisation and technological advancements have profoundly altered the way we live and work, consume unhealthy processed foods and travel by car. Gadgets such as television, cell phones and tablets are frequently used for social and leisure activities. Less physical activity, night-time shift work, less sleep and less exposure to sunlight, intake Western-style diet and sugar-loaded drinks, saturated fat and fast-salty foods and unhealthy dietary habits contribute greatly to causing NCDs.[17-19] Non-genetic factors involved in promoting obesity consist of overconsumption of carbohydrate-rich diets and lesser consumption of fibrous foods, as well as lesser expenditure of energy in the biochemical processes and the basal metabolic functions.[20] There is overwhelming evidence that wholesome foods and Mediterranean-type diet rich in fibber, nuts and seeds, omega-3 fatty acids, less red meat, low-fat dairy products and probiotics play a significant role in promoting healthy microbiota in the gastrointestinal tract and assist in the prevention of CMDs. Furthermore, nutraceuticals and antioxidant diets, consumption of fruits and vegetables also decrease insulin resistance and MS and consequently promote cardiovascular health.[20]

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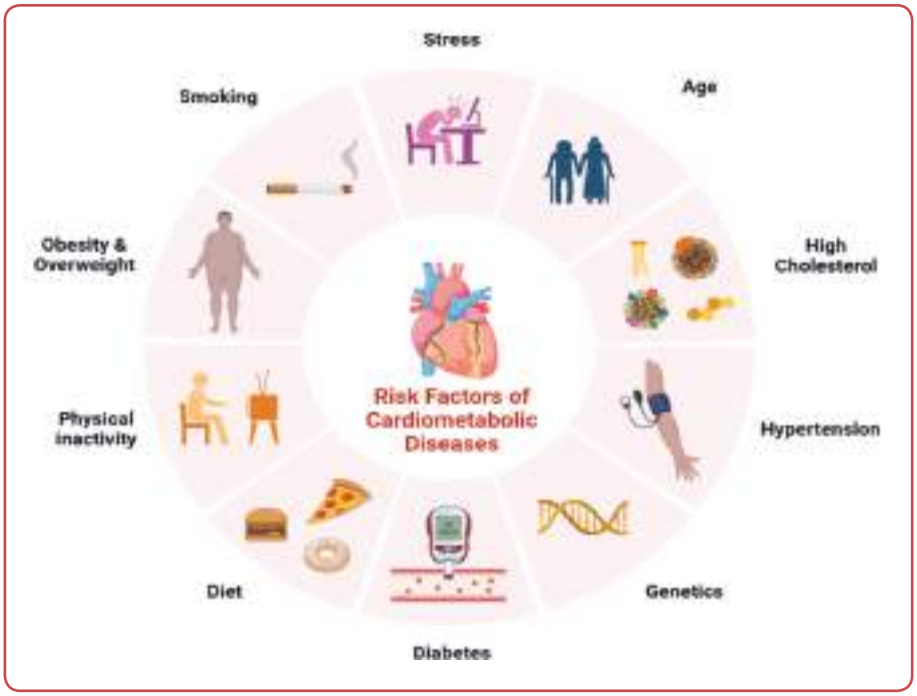

Figure 1: Risk factors associated with cardiometabolic and cardiovascular diseases

Many researches have shown that healthy eating habits and healthy dietary behaviour, as well as lifestyle modifications (eg, smoking cessation, low alcohol consumption, exercise) play a crucial role in the management and prevention of many nongenetic chronic illnesses. A number of studies have demonstrated a direct connection between the high incidence of CMDs and dietary habits, including high intake of ultra-processed foods, Western-style diets, foods and drinks spiked with sugar and high fat content and reduced consumption of fresh fruits and vegetables.[21-23] Antioxidant bioactive compounds present in wholesome foods have depicted a myriad of advantages, including anti-inflammation activity and protection against excessively produced free radicals which cause cell membrane injury and DNA damage.

The term "nutraceutical" refers to nutrient plus pharmaceutical that provides both therapeutic and health promotion advantages besides acting as food for energy.[24] Nutraceuticals are natural substances which are comprised of prebiotics, herbal remedies and plant-based substances with antioxidant and anti-inflammation properties, polyunsaturated fatty acids (PUFA) and culinary spices,[25-27] thus offering an alternative means to confer therapeutic and health promotion activities and well-being objectives.[28] As a result, people are becoming more conscious about the consumption of high-quality wholesome foods, as well as doing physical exercise and smoking cessation, which can collectively be beneficial in their quality of life, healthy aging and consequently prevention of non-communicable chronic diseases.[29-31]

Nutraceuticals

As alluded to earlier, the term nutraceutical was coined by combining pharmaceutical and nutrient words. Nutraceuticals are referred to as substances/ingredients of natural origin that are found in foods or isolated from plants, chemically purified and concentrated for use in health and disease conditions, as well as for the prevention of chronic ailments.[32] Therefore, the nutraceuticals exhibit both health protection and health promotion properties and can be used in the prevention and management of CMDs. They are generally recommended as part of the preventive strategy or treatment regimen for conditions such as hypertension, angina, arrhythmias, congestive heart failure (CHF) and hyperlipidaemias.[33] The subsequent sections of this review will discuss the mechanisms of action, efficacy and safety of delectated nutraceuticals that are efficacious in the prevention and management of CMDs. Table 1 summarises the results of clinical trials that have demonstrated the health advantages of nutraceuticals against the occurrence of CMDs. Additionally, Table 2 summarises the biological actions

Table 1: Questionnaire about knowledge and opinions about artificial intelligence

N	Nutraceutical molecule	Description	Result	Reference
1	Omega-3 fatty acid	11,324 participants were included in an open-label experiment and monitored for 3.5 years.	Fish oil consumption was connected to a 45 % decrease in sudden deaths and a 30 % and 20 % reduction in coronary and total mortality respectively.	[40]
2	Garlic	13 placebo-controlled studies were conducted including 781 participants to understand the garlic supplementation effect on cholesterol levels.	A daily dose of standardised extract (600-900 mg) was shown to lower blood cholesterol levels by 0.41 mmol/L.	[41]
3	Garlic	Double-blinded, placebo-controlled study involving 51 subjects suffering from coronary heart disease.	150 mg tablet twice a day for a year reduced CVD risk by 1.5 times in males and up to 1.3 times in females. The mode of action involved the reduction of LDL cholesterol levels.	[42]
4	Garlic	30-month long; double-blinded, controlled, random study involving 90 subjects.	Supplementation of garlic leads to improvement in the cardiometabolic indices, intestine transit time and lipid accumulation product.	[43]
5	Garlic	Human pilot scale study involving 9 subjects.	Raw garlic juice was consumed by subjects for 7 days who were high trimethylamine N-oxide (TMAO) producers. The study showed a reduction in the formation of TMAO and an improvement in gut microbiota.	[44]
6	Dietary fibre	Ten prospective cohort studies including 245,186 women and 91,058 men followed up for 6 to 10 years were performed.	Every 10 g increment in overall dietary fibre content daily was correlated with a 27 % and 14 % decrement in the rate of coronary death and the risk of occurrence of all cardiac issues.	[45]
7	Soybean	A randomised controlled trial was conducted on 22 normolipidemic volunteers (5 males and 17 women).	HDL cholesterol and Apolipoprotein A-1 levels are increased by soy protein supplementation (56 mg).	[46]
8	Soybean	A study was conducted with 41 postmenopausal women and hyperlipidaemic men.	Soy foods minimize the chances of developing coronary artery disease (CAD) by reducing oxidised LDL cholesterol, blood lipids and blood pressure.	[47]
9	Probiotic and synbiotic supplementation	Controlled randomised trial involving 120 adults.	Both probiotic and symbiotic groups saw a decrease in hyperglycaemia. Further, hypertension and low HDL cholesterol were decreased in probiotic groups.	[48]

CVD: cardiovascular disease; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; MS: metabolic syndrome; NO: nitric oxide;

and underlying mechanisms of different nutraceutical molecules.

a) Polyunsaturated fatty acids (PUFA)

PUFAs are present in eggs, fish meat and fish oil, which include eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).[34] Multiple studies have been conducted to evaluate the preventive actions of PUFAs in CMDs. Omega-3 fatty acids have shown protection against cardiac arrhythmias by improving the electrical consistency of myocardial cells and lengthening their relative refractory time.[35] They also have a protective potential against CMDs. In fact, scientific research has provided evidence that omega-3 fatty acids have a similar or better effect than statins in the prevention and treatment of hypercholesterolaemia.[36, 37] Statins are known to substantially reduce the risk of stroke, myocardial infarction, coronary heart disease and overall cardiovascular risk, according to the results of a meta-analysis of 63 randomised controlled trials comparing

CONTENT

Table 2: Summary of biological actions and underlying mechanisms of nutraceuticals

N	Nutraceutical	Action/Pathway	Mechanism	Reference
1	Omega-3 fatty acids (eg EPA, DHA)	Conversion to bioactive lipid mediators (eg, resolvins, protectins) via enzymatic pathways	Modulate inflammation, reduce oxidative stress, improve lipid profiles, lower blood pressure and reduce thrombosis, thereby preventing CVDs and MS.	[77–79]
2	Garlic	Cholesterol biosynthesis	Garlic compounds inhibit HMG-CoA reductase, a key enzyme in cholesterol biosynthesis, leading to reduced cholesterol synthesis and lower LDL cholesterol levels.	[80]
		NO production	Allicin and S-allyl cysteine present in garlic enhances the production of NO in endothelial cells, which promotes vasodilation and helps maintain vascular health.	[81]
		Blood pressure regulation	By increasing NO production, lowering oxidative stress and blocking the activity of ACE, a crucial blood pressure regulator, allicin and S-allyl cysteine, two compounds found in garlic, lower blood pressure.	[82]
		Antioxidant enzymes	Superoxide dismutase (SOD) and catalase, two antioxidant enzymes that guard against oxidative stress-related cardiovascular damage,+ are upregulated by allicin and allyl sulphides.	
3	Dietary fibres	Anti-inflammatory effects	Fibers can have anti-inflammatory effects by modulating the production of inflammatory markers and cytokines, such as interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α), which are implicated in CVDs.	[83]
		Modulation of gut microbiota	Fibers can modulate the composition and activity of gut microbiota, producing short-chain fatty acids (SCFAs), which have been associated with improved cardiovascular health through various mechanisms, including reduced inflammation and improved lipid metabolism.	[84]
		Blood glucose	Fiber-rich diets, particularly those high in insoluble fibres, can slow down the digestion and absorption of carbohydrates, improving glycaemic control and reducing the risk of developing T2DM, a major risk factor for CVDs.	[85]
4	Soybean	Reduction of LDL-cholesterol	It has been demonstrated that soy protein, especially isoflavone-rich soy protein, lowers total and LDL cholesterol levels in both human and animal models. Soy decreases bile acid and cholesterol absorption from the gastrointestinal tract, raises bile acid excretion and improves liver LDL receptor activity and cholesterol production, leading to an increase in the elimination of cholesterol from the blood via the LDL receptor.	[86]
		Antioxidant action	Since soy isoflavones function as antioxidants, they lessen the likelihood that LDL cholesterol may oxidise. This is crucial in lowering the risk of CVDs and atherosclerosis. As measured by indicators of lipid oxidation such as 8-epi prostaglandin F2 and thiobarbituric acid reactive compounds, it enhances the LDL's resistance to oxidation. Additionally, it increases the overall antioxidant capacity and, specifically, the alpha-tocopherol level in serum and liver, supporting the body's natural antioxidant defence.	[86]
		Faecal excretion of bile acids	Soy protein has been shown to increase faecal excretion of bile acids, which may contribute to the cholesterol-lowering effects of soy. Higher faecal steroid excretion has been observed in isoflavone-rich soy protein compared to isoflavone-depleted soy protein.	[86]
5	Peptides	Competitive inhibitor of ACE enzyme	Inhibit ACE, a major target for developing antihypertensive agents, leading to decreased blood pressure. Up-regulate expression of ACE2, suppress inflammation, increase NO-mediated vasodilation and improve endothelial function, contributing to antihypertensive activity.	[87]
		Regulation of blood pressure	Increase plasma levels of SOD and catalase, decrease total plasma level of peroxide and reduce blood pressure.	[87]

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6	Prebiotics	Reduction of serum cholesterol	Prebiotics have been demonstrated in hypercholesterole animal models and human trials to lower total serum cholesterol levels. This is done through up-regulating the genes that make bile and produce cholesterol, as well as by increasing caeca digest, which may improve the body's ability to eliminate cholesterol.	[88]
		Reduction of liver triacylglycerol accumulation	It has been shown to reduce triacylglycerol accumulation in the liver, which is often associated with obesity, a risk factor for CVDs. This suggests that prebiotics may have anti-obesogenic effects, potentially reducing the risk of CVDs in obese individuals.	[88]
		Reduction of liver triacylglycerol accumulation	Prebiotics' potential for preventing and treating hypertension, a major CVD risk factor, is still being researched. However, prebiotics have been suggested to have potential blood pressure-lowering effects, which may contribute to the prevention of CVDs.	[88]
7	Prebiotics	Reduction of cholesterol levels	Probiotics, particularly *Bifidobacterium* species, reduce cholesterol levels *via* various processes. This includes bile acid amide bond hydrolysis, which results in the release of primary bile acids that are easily ejected from the gastrointestinal system, as well as cholesterol assimilation or precipitation in the gut, which reduces its absorption into the blood. In hypercholesterolemic adults, probiotic supplementation has been demonstrated to considerably decrease total and LDL cholesterol levels.	[88]
		Improvement of vascular endothelial function	Some probiotics, such as *Lactobacillus plantarum* 299v, have been shown to improve vascular endothelial function, which is important for maintaining healthy blood vessels. This is performed by modulating the genes involved in intestinal cholesterol transport and liver cholesterol homeostasis.	[88]
		Oxidative stress and inflammation reduction	Probiotics, such as *Lactobacillus* and *Bifidobacterium* species, have been found to suppress lipid peroxidation and the formation of reactive oxidative species (ROS), which may assist in alleviating oxidative stress and inflammation, both being prominent in CVDs.	[88]

CVD: cardiovascular disease; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; MS: metabolic syndrome; NO: nitric oxide;

the action of statins and omega-3 supplements on cardiovascular events. On the other hand, as compared to the control group, only omega-3 supplementation significantly decreased the risk of myocardial infarction and coronary heart disease (CHD). Furthermore, when compared to omega-3 supplements, pravastatin and atorvastatin were found to be more effective in reducing the risk of CHD, myocardial infarction and overall CVDs. Therefore, omega-3 dietary supplements may not be as effective in preventing CVDs as pravastatin and atorvastatin.[38] There are three omega-3 fatty acid supplements currently marketed: namely, ethyl ester of omega-3 fatty acids, primarily EPA and DHA; ethyl eicosapentaenoic acid (IPE), the ethyl ester of EPA; and omega-3 carboxylic acids, a combination of long-chain omega-3 fatty acids in the form of free fatty acids: EPA and DHA make up the bulk of this mixture.[39]

b) *Allium sativum*

Numerous biological properties like anti-hyperlipidaemic, anti-diabetic, anti-hypertensive and immunomodulatory actions have been attributed to *Allium* species and highlighted by research studies.[49] To investigate the anti-hypertensive activity of garlic, a clinical trial was done in 56 CAD patients given garlic powder pills for three months. The placebo group experienced a significant increase in both systolic and diastolic blood pressure. On the other hand, the garlic supplementation group showed a significant decline in systolic blood pressure, especially in hypertensive patients. In view of these findings, garlic-based therapy can successfully reduce blood pressure in CAD patients, making it a safe adjunct therapeutic option for the high-blood pressure population.[50] Another randomised single-blinded and placebo-controlled trial demonstrated the anti-hyperlipidaemic action of garlic in 70 recruited patients with T2DM and dyslipidaemia. Garlic supplementation (300 mg tablets, twice a day) markedly improved the lipid profile. The experimental group subjects experienced considerable reductions in total and LDL cholesterol, as well as slightly high HDL cholesterol.[51] A similar outcome

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was found in a clinical trial involving 150 hyperlipidaemic patients. Results of the six-week trial pointed to a considerable drop in total and LDL cholesterol as well as an increase in HDL cholesterol.[52] Both studies revealed marked beneficial impact of garlic products intervention in treating hypercholesterolemia. A very recent study also reported the beneficial actions of allicin in a preclinical model of metabolic syndrome. Weight increase, blood pressure, glucose intolerance and kidney damage markers were significantly reduced after allicin treatment. It was noted that the kidneys' inflammation and oxidative stress were also decreased. The authors concluded that these positive effects of allicin were mediated *via* the Nrf2 pathway.[53] Garlic produces antihyperlipidemic action by increasing the elimination of end products of cholesterol in the faces and decreasing endogenous cholesterol production. These actions markedly improve HDL : LDL ratio.[54] The phytochemical makeup of garlic could be a highly promising therapy for treating various cardiovascular conditions. Organosulfur-containing components are the main bioactive compounds present in allicin and alliin.[55] Thus, garlic's cholesterol-lowering ability has been linked to different organosulfur compounds as well as to a range of steroidal saponins. When the bioactive components of garlic: namely allicin and alliin are preserved from the biodegradation action of stomach acid, they can invoke additional beneficial effects to lower cholesterol levels.[56] Further research is needed to validate these findings and explore the pharmacological activities of *Allium* species bioactive compounds for the prevention or mitigation of CMDs and CVDs as well as determination of optimal therapeutic doses, efficacy, mechanisms and long-term safety.

c) Dietary fibres

Insoluble dietary fibres lower the risk of colon cancer and inflammatory pathology of the colon called diverticulitis, whereas soluble fibres considerably reduce serum cholesterol concentration, thereby lowering the risk of CVDs.[57, 58] It has been reported that the insoluble flaxseed dietary fibre contains 6 % mucilage, which helps to reduce CHD risk.[59] Dietary fibres not only protect against CHD by reducing cholesterol levels, but also lower plasma triglyceride levels and high blood pressure, as well as help to stabilise postprandial blood glucose levels.[60] A number of studies have shown that dietary fibres adsorb bile acids and inhibit fat absorption from the gut and also promote healthy microflora in the gastrointestinal tract.[61, 62]

d) Soybean

Soybeans have a wide variety of nutrients, while the two that have received the most focus include soy isoflavones and soy proteins.[33] Soybean isoflavones decrease LDL-cholesterol oxidation, prevent atherosclerosis and enhance vascular responsiveness.[63] According to Hermansen et al, high concentration of soy phospholipids, fibres and isoflavones not only lower LDL-cholesterol, improve LDL- : HDL-cholesterol ratio, triglycerides and total cholesterol levels by 15 %, 20 %, 6 % and 10 %, respectively; but also increase HDL-cholesterol by 5 %.[64]

e) Peptides

Casein and whey proteins present in milk are rich in ACE inhibitor peptides and their intake is linked with the improvement of coronary heart disease.[65] Arginine is found in high concentrations in vegetable proteins. L-arginine serves as a precursor for nitric oxide synthase enzyme, enhances the production of nitric oxide (NO), which acts as a vasodilator and also contributes to the improvement of coronary vessels sympathetic responses. According to Palloshi et al, hypertensive and angina patients may benefit by taking L-arginine orally.[66]

f) Prebiotics and probiotics

The consumption of prebiotics improves the composition of the intestinal microflora, leading to the predominance of certain gut bacteria that are beneficial for health and well-being. The beneficial bacteria include *Lactobacilli* and *Bifidobacterium* species. The prebiotics supplements enhance good gut microbes in the host, improve immune function and assist to prevent serious illnesses like cancer and CVDs.[67] Prebiotics containing nondigestible oligosaccharides promote healthy intestinal microbiota.[68, 69] Probiotics when consumed in adequate amounts, have an advantageous influence on the host's health.[70] Probiotics from fermented dairy products cause decrease in blood cholesterol levels, whereas prebiotics with non-digestible fermentable carbohydrates can lower triacylglycerol levels. It has been reported that *Lactobacillus acidophilus*, *Lactobacillus bulgaricus* and *Bifidobacterium bifidum* significantly decrease cholesterol in the body.[71, 72] Some investigators have suggested that the consumption of *Lactobacillus* and *Bifidobacterium* containing probiotics can help to reduce the incidence of CHD, stroke, hypertension and improve cholesterol and triglyceride levels.[33]

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g) Phytosterols

Since phytosterols have closely resembling chemical structures with cholesterol, phytosterols compete for the absorption of cholesterol in the small intestine and consequently retard the systemic bioavailability of cholesterol and its liver uptake, resulting in the lower levels of cholesterol in the bloodstream.[73] Consumption of plant sterols were found to reduce LDL-cholesterol by 8-15 %.[74] Some studies found that dietary intake of 2-3 mg of plant sterols or stanols/per day can markedly decrease LDL-cholesterol levels by 9-20 %.[75] A number of investigators have suggested that phytosterol-ester from margarines could be used as statin replacement therapy and fibrate-based hypercholesterolemia.[75, 76]

Antioxidant diets

Among other factors, oxidative stress and inflammation have been reported to be involved in the initiation and development of different chronic diseases, including cancer, atherosclerosis, diabetes, cardiovascular and neurodegenerative diseases. Antioxidants are polyphenolic substances that include catechins, flavonoid glycosides, isoflavones and anthocyanins. Polyphenols are bioactive chemicals that have anti-inflammatory, anti-aging, anti-diabetes and anti-cancer properties.[89] The influence of flavonoids on arachidonic acid metabolism has been linked to their ability

Table 3: Clinical trials done with different antioxidant compounds

N	Compound	Description	Result	Reference
1	Red wine (Resveratrol)	15 CAD-suffering individuals were involved.	Purple grape juice or red wine consumption provided greater protection against oxidation of LDL-cholesterol as well as better endothelial function.	[106]
2	Mixture of turmeric (*Curcuma longa*) and *Spirulina maxima*	In a study involving patients with abdominal obesity was administered with 156.6/266 mg of Turmeric (*Curcuma longa*). *Spirulina maxima* for 12 weeks.	Patients who took Spirulina supplements had spiked serum levels of antioxidants than those who took a placebo.	[107]
3	α-Carotene and β-Carotene	A population-based trial including 392 tobacco users was conducted to assess the link between blood levels of antioxidants and atherosclerotic risk.	An inverse relationship was identified between α and β-carotene and atherosclerosis.	[108]
4	Ascorbic acid	A "Coronary Artery Risk Development in Young Adults" (CARDIA) study involving 2637 individuals.	Ascorbic acid levels in the blood were discovered to be inversely correlated with calcium in the blood arteries, a key sign of atherosclerosis.	[109]
5	Lycopene	A randomised trial involving 20 males and 4 females.	Supplementing lycopene decreased levels of total and LDL cholesterol.	[110]
6	Cocoa	A meta-analysis of data from 12 trials that explored the positive benefits of cocoa on cardiometabolic biomarkers in T2DM.	Cocoa-rich products lead to a reduction in LDL cholesterol, c-reactive protein, triglycerides and blood glucose in the long term.	[111]
7	*Camellia sinensis* capsules	The randomised, placebo-controlled and double-blind study which included 111 individuals.	*Camellia sinensis* capsules lowered blood pressure, LDL cholesterol, oxidative stress and a marker of chronic inflammation which are risk factors for CVD.	[112]
8	Coenzyme Q10 (CoQ10)	The meta-analysis study comprised 12 studies involving a total of 650 individuals suffering from diabetes complications and MS.	It has been demonstrated that CoQ10 reduces total and LDL cholesterol levels, which are significant CVD risk factors. Additionally, it has been hypothesised that CoQ10 may enhance vascular tone and endothelial function.	[113]

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9	Amino acid L-arginine	The randomised, controlled, single-blind study involved 90 individuals who were obese.	L-arginine supplementation led to significant reductions in anthropometric variables (such as BMI, WC, TS and SS), blood pressure levels (SBP, DBP), FBS, HbA1c, LDL and MDA, as well as significant increases in HDL. Additionally, the intervention group's TG and TC levels were substantially reduced.	[114]
10	Quercetin	An evaluation of the impact of quercetin supplementation on lipid profiles and inflammatory markers in patients with MS and associated illnesses using data from 16 randomised clinical trials.	Total and LDL cholesterol and C-reactive protein were considerably lowered by quercetin administration.	[114]

CVD: cardiovascular disease; CAD: coronary artery disease; BMI: body mass index, WC: waist circumflex; SBP: systolic blood pressure; DBP: diastolic blood pressure; FBS: fasting blood sugar; MDA: malondialdehyde; TG: triglyceride; TC: total cholesterol; T2DM: type 2 diabetes mellitus;

to reduce the risk of CVDs.[90] Table 3 summarises the results of clinical trials and the beneficial effects of antioxidants against the occurrence of CMDs.

The free radicals play a vital part in the life of all organisms through their involvement in cell-cell communication and cell signal mechanisms. However, the unabated excessive production of reactive oxidative species (ROS) can lead to the oxidation of biomolecules (eg, nucleic acids, lipids and proteins) or the activation of proinflammatory signalling pathways, resulting in the activation of several transcription factors or the dysregulation of gene and protein expression followed by cell injury or cell death. Excessive ROS can be generated in the human body by mitochondrial damage, oxidative stress, dysregulation of basic metabolic functions, diabetes mellitus, obesity, as well as external factors such as cigarette smoking, X-rays, air pollution, ozone and industrial toxins.[91] Increased production of ROS has been linked to the development of several chronic illnesses, including cancer, cardio-respiratory disorders, dementia and Alzheimer's disease, osteoarthritis and inflammatory bowel disease (IBD). Under normal physiological conditions, ROS are scavenged by endogenous antioxidants (eg, glutathione, dismutase enzyme, vitamins, minerals). Antioxidant deficiency combined with starvation makes people more susceptible to oxidative stress, thereby increasing the risks of CMDs, CVDs and some cancer types. The regulation of metabolic processes essential to the proper functioning of body organs is dependent on the presence of dietary antioxidants, amino acids, vitamins (A, C, D), trace metals (Zn, Cu, Se) and synthesis of glutathione. The multifaced advantages of antioxidant diets in health and disease have been reported by many clinical investigations because the dietary antioxidants mitigate the oxidative stress and prevent the cellular damage caused by the highly reactive free radicals.[92]

Antioxidant flavonoids are abundantly found in green and black teas, fresh fruits and vegetables and red wine.[54, 93] Ignarro et al observed that lycopene present in tomatoes can decrease the oxidation of LDL-cholesterol in the blood stream.[94] Furthermore, lycopene consumption was found to be inversely related with the occurrence of CVDs.[95, 96] Another study by Vita revealed that higher intake of polyphenols was associated with the decreased risk of CVDs.[97] Polyphenols were also reported to exert anti-tumour activities against prostate, larynx, lung, colon, tongue, gastric and breast cancers.[98, 99] Foods rich in antioxidants, such as spirulina and turmeric play an important preventive role in the management of CMDs by curtailing the metabolic processes that trigger their onset.[100]

To determine the effects of polyphenol supplementation in combination with calorie restriction and physical activity on body weight and fat deposition, body mass index (BMI) and waist circumference in overweight and obese persons, Llaha et al examined the findings of 15 randomised clinical studies. The results of these studies showed that consumption of isoflavone supplements helped non-Asian postmenopausal women to lose weight and more fat while exercising, thereby suggesting that intake of polyphenol supplements may have a positive health effect.[101] An extensively researched flavonoid/flavonol for its anti-inflammatory properties is juglanin found in *Polygonum aviculare.* It was found that juglanin exerts its anti-inflammatory activity by blocking the TLR4/MAPK/NF-B pathway, which decreased the production of TNF-, IL-1 and IL-6.[102, 103] Juglanin also reported to decrease the necrosis and excessive permeability of the blood-

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brain barrier in the ischaemic condition of the brain by interfering with the vascular endothelial growth factor (VEGF) and VEGFR2 (VEGF-receptor 2) pathway.[104] Sun et al investigated the amelioratory effects of juglanin in myocardial injury and apoptosis of cardiomyocytes and found that it suppressed the inflammatory response after myocardial infarction by inactivating the MAPK pathway.[105]

Physical activity

Physical activity refers to the general movement of the body in different situations and settings. Physical exercise provides multiple health advantages, including lower the risk of CVD related morbidity and mortality, prevent obesity and T2DM, improve cholesterol and lipid levels, reduce hypertension and coronary heart disease, decrease the risks of prostate, colon and breast cancer, reduce stress and enhance brain functions.[116, 117] Beneficial neurological/psychological health outcomes have also been reported in several epidemiological and clinical investigations, that physical exercise can reduce stress and depression, enhance cognition, lower the risk of dementia and promote bone health.[118] Physical exercise reduces the incidence of cardiometabolic illness.[119] Regular physical exercise can lower diastolic and systolic blood pressure, with data supporting the advantages of both moderate-intensity (such as walking) and strenuous activity.[120] According to Carnethon et al, physical activity and aerobic exercise are inversely related to the development of hypertension and coronary heart disease risk. They found nearly 34 % reduction of hypertension in 4,618 men and women involved in the physical activity experiment.[121] Excessive amount of systemic cholesterol as well as pathophysiology of atherosclerosis can also be reduced by physical activity.[122] Intense physical exercise has been linked to the improvement of cholesterol levels by boosting the concentration of HDL-cholesterol while preserving and balancing elevations in triglycerides and lowering LDL-cholesterol.[123, 124] Regular physical activity can reduce body weight and waist circumference in overweight and obese people and significantly reduce the incidence of cardiometabolic syndrome.[125] A comparative study involving 70 younger and 43 older (> 65 years) individuals was performed where the intricate relationship between physical activity and cardiometabolic disease-causing risk factors was determined.[126] Another cohort study involved 24,960 respondents of at least 35 years suffering from diabetes, hypertension and/or heart disease. Out of these, 52 % of adults were victims of cardiometabolic diseases and were not physically active in their daily life and 34 % were residents of least activity-friendly areas, thus proving the importance of physical activity.[127] Similarly, another cohort study involving 341,331 participants suggested the importance of physical activity amongst other factors for the survival of both men and women suffering from cardiometabolic disorders.[128]

Concluding remarks and future strategies

In this comprehensive review, efforts have been made to emphasise the significant roles of nutraceuticals, phytosterols, soybeans and polyphenolic diets containing high amounts of antioxidant and anti-inflammatory compounds as well as omega-3 PUFAs, which can help in preventing obesity, T2DM, CHD, CMD, CVD and other chronic non-communicable illnesses.

There is an upward trend of sedentary lifestyles among all age groups and genders, especially in the urban areas of India and other developing countries, along with the growing consumption of sodium chloride salt and sugar-loaded drinks, fatty food and unhealthy dietary habits.[129] The sedentary lifestyles have contributed to the high occurrence of obesity, T2DM and greater prevalence of CMDs, CVDs and NCDs. This review emphasises the impact of nutraceuticals, wholesome foods, probiotics and physical activity as cost-effective measures to counter the increased occurrence of cardiometabolic problems and associated disorders. Nutraceuticals are useful in risk management and prevention of CMDs like hypertension, CHF, hyperlipidaemias and coronary ailments. Antioxidant diets help to counter the oxidative stress and ROS-induced cellular damage by mopping ROS, as well as by interfering with various metabolic pathways and arresting the oxidative stress cycle, thus exhibiting properties like anti-inflammatory, anti-cancer, anti-obesity and anti-aging effects.[130] Physical exercise and wholesome diet interventions have an additive or synergistic actions in controlling conditions

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like obesity, insulin resistance, stimulation of immune function, reduction of hyperglycaemia and hypercholesterolemia and consequently reducing the risk of CMDs.[131]

Evidence is now emerging that perturbation of gut microbiota can lead to an increased permeability of gut epithelium and leakage of microbial toxins into the systemic circulation, thus becoming a trigger for chronic NCDs ranging from IBD, CMD, CVD, T2DM, obesity and neurological illnesses. The consumption of fermented dairy products, including prebiotics and probiotics help to restore healthy microbiota in the host's gut and consequently reduce the risk of metabolic conditions, atherosclerosis and NCDs.

Nevertheless, the most important factor is to increase public and healthcare providers awareness regarding the pivotal role of nutraceuticals, dietary supplements, antioxidant-rich diets and physical activity in the prevention of CMDs, CVDs and NCDs. National policy guidelines can be developed which should include public health policies to promote educational settings to incorporate physical activity along with healthy dietary habits. In addition, national programs should be developed to enhance awareness among the physicians and general public regarding the important roles of physical activity and lifestyle modifications in the prevention of CMDs and CVDs at the primary healthcare levels. There is a large gap between scientific proof regarding these factors, the public health needs and implementation. The important task is to figure out how to translate the laboratory research findings into the successful public health and education programs. The public awareness and education strategies developed should comprise holistic approaches tailored towards the country's vegetarian and non-vegetarian populations. Hence, physical activity, dietary interventions and smoking cessation are needed to be prioritised as part of the CMD/CVD prevention agenda.[132] In addition, medical students should be taught about the role of clinical nutrition and the merits of complementary methods for the prevention and management of NCDs, CMDs, CVDs etc.

Numerous epidemiological studies, meta-analysis, clinical trials and experimental findings have unequivocally shown the beneficial effects of dietary interventions, quitting cigarette smoking and low alcohol consumption, healthy dietary habits and lifestyle modifications can improve overall health and well-being and reduce the risk of CMDs, CVDs, and NCDs. Also, lesser intake of sugar loaded drinks, carbohydrate containing deserts and processed foods, less red meat, low fat dairy products, and heart healthy diets can reduce the risk of CVDs by about 75 % to 80 %. Before some of the significant underlying causes of CVDs substantially affect an individual or a population at large, preventive interventions against CVDs must be directed at the primary health promotion level. Such preventive measures would not only assist in lowering hospital and medicine expenditures that burden the healthcare sectors of developed and developing nations, but will also reduce workforce absenteeism.[133]

Recently Lee et al did meta-analysis of a large study sample comprised of 1,561 healthy men of middle ages (mean age 53-years) and reported that men with anxiety, intense worry and mood disorders are more prone to high incidence of cardiometabolic diseases such as CHD, stroke, hypertension and diabetes at earlier age and remain on a stable trajectory of heightened risk into older age. The authors suggested that assessment of cardiometabolic and psychological risk factors earlier in life would be helpful to reduce early morbidity and mortality caused by CHD and CVD.[134] The authors of this review propose that physical activity or exercise therapy and intake of wholesome foods, probiotics and omega-3-PUFA and avoidance from substances of abuse, would be impactful in treating neurotic and worrisome individuals with depression and anxiety disorders. Such approach will not only be cost-effective in treating mental disorders (depression, anxiety, mood swings), but also reduce the adverse reactions caused by antipsychotic and antidepressant drugs.

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Conflict of interest

None.

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CURRENT TOPIC

A Literature Review of the Relation Between Iron Deficiency Anaemia, Physical Activity and Cognitive Function in Adolescent Girls

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Abstract

Women, especially those young and/or pregnant, are at high risk of experiencing an iron deficiency. Low iron stores in the body can affect cognitive function and physical activity in adolescents, but the results of epidemiological studies about the effect of iron deficiency on cognitive function decline and physical activity in adolescents are not consistent. Therefore, it is necessary to review the literature on the relationship between iron deficiency, cognitive function and physical activity in women and girls. This study is an article review using sources from Google Scholar, PubMed and ProQuest database searches from 2014 to 2021. The keywords used were iron deficiency OR anaemia AND adolescent girls OR young women AND activity AND cognitive. By using review criteria, sources were limited to those in English that used a sample of adolescents or young women. In the initial search stage, 63 reviews were obtained and based on the predetermined criteria, 12 reviews were selected to be analysed. Eleven studies examined the relationship between iron deficiency and cognitive decline, there was one study that showed a non-significant relationship between the two; ten studies showed a significant effect of iron deficiency on cognitive decline in the domains of attention tasks, memory tasks and executive function. Three studies showed their effect on decline in adolescents by using different measuring tools. Lack of iron in the body caused a decrease in cognitive function, especially in the domain attention tasks, memory task domains and executive functions domains. In addition, iron deficiency can cause a decrease in physical activity in adolescents due to the low supply of oxygen to the blood and tissues. Therefore, it is necessary to research nutrition intervention programs to improve iron anaemia status in adolescent girls and prevent a decline in cognitive function and physical activity as a result of the impact of iron deficiency.

Key words: Iron deficiency; Adolescence; Activity; Cognitive.

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Introduction

Anaemia due to iron deficiency develops when body stores of iron drop too low to support normal red blood cell (RBC) production, indicated by low levels of haemoglobin. Iron deficiency (ID) is defined as a decrease in the total iron content in the body or having iron stores below normal for physiological status. There are many types of anaemia but anaemia due to iron deficiency is the most common type of anaemia.[1] Anaemia affects 1.62 billion people in the world, especially in developing and developed countries. Iron deficiency is the cause of 50 % of cases of anaemia worldwide and represents a global health problem.[2] Teenage girls or women of childbearing age often experience iron deficiency, which is at risk of anaemia; due to adolescents experience

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rapid growth, requiring high iron intake during puberty,[3-5] as well as loss of iron during menstruation.[6]

Adolescence is marked by physical growth and cognitive and socio-emotional development, which are the characteristics seen in adolescence and this period is strongly influenced by the social, cultural and economic environment.[7] Adequate nutrition in adolescence affects the future growth and health in adulthood. It will also have an effect on offspring, so adequate nutrition in adolescence has influence on the next generation.[8]

Anaemia due to iron deficiency can cause delays in individual psychological and physical development, cognitive dysfunction, low immune status, decreased physical capacity and performance and increased foetal morbidity and mortality. In addition, iron deficiency also causes reduced attention, decreased memory and school attendance, which in turn affects school achievement in adolescents.[9, 10]

Several preliminary studies have investigated the importance of iron in cognitive function and found a link between iron deficiency, diet and changes in psychological development and cognitive function.[11-13] Experimental studies in both animals and humans suggest that iron deficiency has the potential for cognitive impairment, with damage to brain mitochondria as the basis for these changes. Cognitive impairment, including those associated with impaired attention, intelligence and perception sensory, emotional and behavioural and generally, is associated with iron deficiency anaemia.[12]

Furthermore, there are very few studies on iron deficiency associated with decreased physical activity. Decreased physical activity is caused by a decrease in iron stores and haemoglobin concentration, which causes a reduction in the availability of oxygen to the tissues and work of the heart.[18] Although various studies on the effects of iron deficiency on decreased cognitive function and activity have been investigated, the results are still inconsistent, considering the relevance of iron deficiency problems to the development of cognitive function, relative changes that can last into adulthood as a result of iron deficiency in the body.[12]

This paper aimed to review the results of research from several existing observational studies and randomised controlled trials (RCTs) to determine the effect of iron deficiency on decreased cognitive function and physical activity in adolescent girls.

Methods

The article search process was based on three relevant databases (Google Scholar, ProQuest and PubMed). The search started from the year 2014 and ended in 2021. The studies included in this review met the following criteria: 1) focus on impaired cognitive function and decreased physical activity, 2) observational studies and RCTs, 3) articles in English, 4) sample of adolescents or young women. Exclusion criteria were 1) review articles, unpublished work and study protocols, 2) populations with nonspecific iron deficiency-related problems. Key terms used were: iron deficiency OR anaemia AND young women OR adolescent girls AND activity AND cognitive.

Initial search results obtained 63 references. These references were included in the Mendeley library and filtered by title and abstract. Furthermore, an analysis was carried out based on the full text of the selected references. Finally, based on the exclusion criteria, 12 articles were analysed.

Results

Twelve published journal articles were found and the source of each article was checked (Table 1). The first study was conducted in Peshawar, Pakistan.[14] The second research was conducted in the city of Rwanda, France with the subjects being university students.[15] The third study was conducted at Idaho University, Moscow with female students who were not anaemic.[16] The fourth study was conducted in urban areas on non-anaemic women of reproductive age,[6] the fifth study was conducted in metropolitan (Sydney) and regional/rural (Bathurst) areas in the state of New South Wales in Australia.[17] The sixth study was conducted at the Pennsylvania State University with the subjects being female students.[18] The 7th and 8th study were conducted in India[19] and Newcastle University, Australia,[20] while the ninth study was conducted in Maharashtra, India with the subjects being female students aged 12–16 years.[21] The tenth study was conducted in El Minya province, Upper Egypt with the subjects being female teenagers.[22] Eleventh and twelfth studies were conducted in Jintan, China[23] and India,[24] respectively. There were three journals that discuss research output on physical activity.[6, 14, 19]

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Table 1: The relationship between iron deficiency and decreased cognitive function and physical activity in adolescents

N	Author (year)	Study participant	Study design	Outcome Measure/Tools	Findings
1	Abbas Khan et al (2019)	Teenage girl, 10-14 years old; N = 100	Cross-sectional. Study duration: 6 weeks	Physical work capacity up and down stairs (Modified Harvard's Steep)	Anaemic girls had significantly lower physical work capacity than non-anaemic girls.
				Coloured progressive cognitive functions (Raven Matrix)	Anaemic girls had significantly lower cognitive function compared to non-anaemic girls.[14]
2	Laura et al (2017)	Female aged 10-27 years; N = 150	RCT. Study duration: 18 weeks	Cognitive function (computerised MJW and DMDX program) with 5 domains: ATN, SRT, GNG, CRT, SMS.	Iron biofortified nuts consumed for 18 weeks can improve iron status and cognitive function, especially ANT and SMS in young adult women.[15]
3	Blanton C (2014)	Non-anaemic women aged 18-30 years; N = 42	RCT. Study duration: 16 weeks	Cognitive function (CANTAB) with 5 areas: 1. Motor screening test; 2. Verbal recognition memory; 3. SWM; 4. One touch stocking; 5. Rapid visual information processing.	Iron in the body was significantly associated with cognitive function of SWM and One touch stockings of Cambridge (OTS) speed.[16]
4	Dziembowska et al (2019)	Healthy women aged 20 – 32 years; N = 23	Cross-sectional	Physical activity energy expenditure units per week (IPAQ);	Low levels of iron and ferritin caused decreased activity and perceived endurance.
				Cognitive function intelligence test - APM Raven.	Raven's APM cognitive scores in iron-deficient women were not significantly different from iron-enough women, but iron-deficient women needed more time to complete a given task compared to iron-enough women.[6]
5	Rebecca et al (2017)	Healthy young woman aged 18-35 years; N = 300	Cross-sectional	Cognitive function (computerised Integneuro, brain resource) with 5 cognitive domains: 1. GNG; 2. Attention; 3. The switching of attention test; 4. The memory recognition; 5. EF.	Women with IDA obtained significantly lower cognitive scores in the attention domain than women with ID, but cognitive function in the other four domains was not significant.[17]
6	Samuel et al (2015)	Female 18-35 years old; N = 127	Cross-sectional	Cognitive functions (PEBL platform computing, through 5 tasks): 1. GNG; 2. ANT; 3. SMS task; 4. EF; 5. Card sorting.	Adequate iron in the body can give a better performance on ANT cognitive function and executive function planning ability.[18]
7	Laura et al (2021)	12–16-year-old school children; N = 130	RCT	Light, moderate and strong physical activity (Accelerometer)	Children who consumed iron biofortified pearl millet had 22.3 minutes more LPA than children who did not consume pearl millet.[19]

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8	Alecia et al (2014)	Healthy women aged 18-35 years; N = 84	RCT	Cognitive Function (Battery of Integneuro Cognitive Test) includes 7 domains: 1. Memory; 2. Response speed; 3. GNG; 4. Attention; 5. Information processing; 6. EF; 7. Emotion identification.	Women who were given iron supplements had higher changes in cognitive scores for the impulsivity (GNG) and attention domains than women who were not given iron supplements.[20]
9	Samuel et al (2018)	Children aged 12-16 years; N = 140	RCT	Cognitive Functions (DMDX Program MJW Software Computing) includes 5 cognitive tasks: 1. SRT; 2. GNG; 3. ANT; 4. CRT; 5. CFE.	Iron biofortified pearl millet consumed by children can improve cognitive function on attention tasks (STR, GNG and ANT and memory tasks (CFE and CRT).[21]
10	Suzan et al (2016)	Teenage girls 12-15 years old; N = 151	Cross-sectional	Cognitive functions mathematical score (Stanford Binet intelligence scale V edition)	Adolescents with iron deficiency anaemia had significantly lower math scores than iron-deficient adolescents and also lower than adolescents who were not anaemic.[22]
11	Xiaopeng et al (2017)	Teenagers aged 11-14 years; N = 428	Longitudinal study	Neuro cognitive function (CNB), with 4 domains: 1. EC; 2. EM; 3. CC; 4. SS.	Iron deficiency leaded to decreased spatial processing ability, reflecting CC function and decreased abstraction reflecting EC function in adolescents.[23]
12	Shaik et al (2019)	18–22-year-old students; N = 100	Cross-sectional	Mental health cognitive function, MMSE and MoCA	There were significant differences between students with anaemia and non-anaemia in the values of Hb, MCV, MCH and MCHC, and MMSE and MoCA cognitive scores.[24]

RCT: Randomised controlled trial; ANT: Attentional network task; SRT: Simple reaction; GNG: Go no go (GNG); CRT: Cued recognition task; SMS: Sternberg memory search task; SWM: Spatial working memory; OTS: One touch stockings of Cambridge; IPAQ: Physical activity energy expenditure units per week; APM: advanced progressive matrix; EF: Executive functions; IDA: iron deficiency anaemia; ID: iron deficiency; LPA: light activity per day; CFE: Composite face effect; CNB: computerised neurocognitive battery; EC: executive control; EM: Episodic memory; CC: Complex cognition; SS: Sensorimotor speed; MMSE: Mini mental state examination; MoCA: Montreal cognitive assessment; Hb: haemoglobin, MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration;

Subject characteristics and study designs of the twelve studies found were labelled according to when they were conducted and published (Table 1). In general, the samples in the study were school age young women and female students of childbearing age up to 35 years of age. There were three types of initial sample conditions, (1) Anaemic adolescent girls or women (studies number 2, 7 and 9), (2) healthy women (study numbers 3-6, 8) and (3) a combination of anaemic and healthy women (study numbers 1, 11 and 12). There were six studies with a cross-sectional study design and six studies conducted with a randomised, double-blind, controlled design.

The 11 studies used different cognitive function instruments with different domains to give different results as a result of iron deficiency on cognitive decline. As reported in the study above, some of the individual tests used were: Modified Harvard's Steep,[14] CANTAB,[15] computerised neurocognitive battery (CNB),[20, 23] Stanford-Binet Scale,[22, 23] Raven Matrix,[6, 18] MJW and DMDX programs,[15, 21] IntegNeuro, Brain Resources[17] and Mini mental state examination (MMSE) Questionnaire, Montreal cognitive assessment (MoCA).[24]

Three studies described the relationship between iron deficiency and physical activity in adolescents and young women, using different measuring instruments. The three studies showed a significant relationship between iron deficiency and physical activity, including decreased work activity in children,[14] decreased activity, endurance and performance in completing tasks[6] and light activities (LPA) with less time per day compared to children who had enough iron in their bodies.[19]

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Discussion

Relationship of iron deficiency with decreased cognitive function

An important component of heme, which is required for the synthesis of haemoglobin, is iron; iron deficiency can lead to low haemoglobin levels. Iron that comes from food in the form of ferric ions is reduced to ferrous ions before being absorbed. This ferrous form is then absorbed by the mucosal cells of the small intestine and undergoes oxidation to form ferric ions again. A small portion of ferric ions form ferritin and most of them are reduced to ferrous ions which are released into the bloodstream and ferrous ions are re-oxidised to form ferric ions which then bind to transferrin and are stored as reserves in the liver, spleen and bone marrow in the form of ferritin.[25, 26]

Iron is an essential component for brain development and is needed for cell differentiation, protein synthesis, haemoglobin synthesis, neurotransmitter production and energy metabolism.[27] Iron deficiency can cause abnormalities in three brain domains, namely a decrease in dendritic structure and an increase in glutamate in the hippocampus, nerve hypomyelination and changes in the metabolism of the neurotransmitter dopamine.[28, 29] Changes in dopamine metabolism can reduce the ability of the striatum and amygdala to regulate motivation. This decrease in motivation can reduce learning abilities and can reduce children's cognitive function.[30]

Diversity cognitive function tests are grouped into different domains, multiple construct measurement tests and a variety of tests to evaluate specific cognitive domains. There is currently no consensus on the classification of cognitive tests and domains,[31] which may explain some of the differences between studies. The classification of the executive function domain includes attention and impulsive action; the working memory domain, which measures multiple constructs, includes information processing, memory and executive function planning.[31, 32] Continuous performance tests that measure sustained attention and capacity to inhibit impulsive responses over time, were used to assess the attention domain.[32]

Of the 11 studies that examined the relationship between iron deficiency and decreased cognitive function, there was one study that showed a non-significant relationship between iron deficiency and cognitive decline and there were ten studies that showed a significant relationship between iron deficiency and cognitive decline in children. Different domains were different. Five studies used cognitive function instruments with five domains, one study used four domains, one study used seven domains and three studies used cognitive function instruments with a total score. Several studies used the same cognitive function domain, including the impulsivity task domain (GNG), attention network task domain (ANT), memory task domain, simple reaction time task domain (SRT) and executive function domain.

Regional developmental requirements for iron differ across specific brain systems and it is likely that this underlying mechanism is still unclear across cognitive domains. The domains of cognitive function are related to different brain systems; therefore, the domains of cognitive function may vary in their sensitivity to iron deficiency or excess.[33]

The mechanism underlying changes in brain activity in relation to iron status may involve disturbances in neuroendocrine function. The enzymes that synthesize catecholamines, serotonin and thyroid hormones require an iron cofactor for their activity; changes in enzyme levels and activity are seen in iron-deficient animals and humans.[34] Iron deficiency and iron anaemia lead to lower intelligence and performance scores, disturbances in attention span and sensory perception functions, as well as functions related to emotion and behaviour, which are critical to school achievement in adolescents.[35, 37]

The effect of iron deficiency on physical activity

Iron is an important mineral that the body needs to produce one of the components of red blood cells, namely haemoglobin. Haemoglobin is a protein that functions to transport oxygen to be distributed throughout the body's tissues. When there is an iron deficiency, the body cannot produce enough haemoglobin. Lack of haemoglobin production reduces the oxygen supply in the blood so that the body does not get enough oxygen. This is what causes iron deficiency anaemia

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sufferers to become easily tired, weak, even short of breath and affect their physical activity.[38]

Anaemia due to iron deficiency can reduce the performance of young women in activities due to the low amount of oxygen supplied to tissues and cells.[14] A study reported that adolescent girls who were anaemic had significantly higher pulse rates immediately after one to two minutes of activity compared to adolescents who were not anemic,[39] this was due to the low supply of oxygen to the blood and tissues.[39, 40] Giving iron supplementation to adolescent girls can significantly reduce perceived fatigue.[41] The iron-dependent serotoninergic and dopaminergic reuptake systems function seems to be partially demonstrated by the activity.[42-47] Women who are iron deficient have decreased activity due to the changes in the brain system.[14]

Iron deficiency causes decreased energy levels and cognitive capacity due to changes in dopamine and serotonin levels that are felt in the body.[42-44] Increased sensitivity due to iron deficiency can cause difficulty in concentrating attention on tasks and cause feelings of frustration or irritability. More attention is concentrated on sensory processing. However, these results still require a more in-depth study in terms of theoretical approaches to sensitivity. Iron reduces the level of stimuli required for cognitive functioning and protects the nervous system from overstimulation .[6]

There is a relationship between haemoglobin levels and maximum oxygen volume (VO_2max), it is proven that an athlete who has high haemoglobin levels has good endurance or VO_2max. When the haemoglobin level is below normal, the oxygen level in the blood is also lower and *vice versa*. Normal haemoglobin levels make the process of transporting oxygen into the tissues more optimal.[48] A review of research has provided evidence that high-intensity exercise has a positive effect on oxygen levels. The mechanism of high intensity exercise is that the heart, lungs and muscular system are forced to work hard and one of the effects is an increase in the maximum amount of oxygen. A decrease in physical activity will affect a person's cardiorespiratory endurance in carrying out daily activities. The need for continuous oxygen supplementation can influence a 6-minute walking activity test in respondents.[49]

Conclusion

Lack of iron in the body causes a decrease in cognitive function, especially in the domain attention tasks, memory task domains and executive functions domains. In addition, iron deficiency can also cause a reduction of physical activity in adolescents due to the low amount of oxygen supplied to the blood, cells and tissues. Therefore, research is needed on nutritional intervention programs to improve iron anaemia status in adolescent girls and prevent a decline in cognitive function and physical activity due to iron deficiency.

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Conflict of interest

None.

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CURRENT TOPIC

A Scoping Review in Indian Post-Stroke Patients

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Abstract

Stroke is the second most common cause of death worldwide and the third most common cause of disability-adjusted life years (DALYs) worldwide. According to the available evidence, 85.5 % of total stroke fatalities are reported in low- and middle-income countries compared with high-income countries. In addition, the prevalence of DALYs in low-income countries is very high. The major challenge is the vastness of India and its humongous population size, which makes it nearly impossible to reach patients far away. The quality of life (QoL) of stroke survivors is an important factor in predicting the burden of the disease and determining the effectiveness of treatment. Many research studies provide an overview of the overall estimates of QoL and contribute to research on QoL after stroke in India. Owing to the bleak post-stroke rehabilitation facilities in India, stroke patients don't get the post-stroke care they ought to. The gap is not only in the patient care management system but also in the policies laid out by the government. The unmet gaps in post-stroke rehabilitation and patient care remain a major setback in patient care management, which impacts the clinical outcomes at large. These challenges are the reasons for the increasing disease burden on society and the hampering of the socio-economic status of the country at large. The government authorities should lay down the policy that will help the patient seek the correct in-time treatment for stroke and help the post-stroke patients to live a QoL.

Key words: Quality of life; Rehabilitation; Post-stroke rehabilitation; Patient care management; Patient education.

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Introduction

In India, the rate of stroke-related hospitalisations is 46 out of every 100,000 people (Figure 1 and 2). The average and median cost per stroke-related episode is approximately ₹40,360 (about 539.75 US$) and ₹17,140 (about 229.22 US$), respectively. There are a lot of out-of-pocket expenditures for post-stroke hospitalisations across different wealth quintiles. About 29 % of households looking for stroke treatment in public hospitals had to pay out of pocket, while 37 % of households had to use borrowed health financing from relatives and neighbours. On average, drugs make up 38 % and outpatient care 73 % of public sector hospitalisations. Patients who were hospitalised in a private facility and stayed there for more than 7 days were more likely to have catastrophic expenses.[1]

The unmet need for post-stroke rehabilitation services is due to the limited rehabilitation resources available in India, the development of an accessible, innovation-driven, patient-centric and culturally sensitive rehabilitation intervention has public health implications. Developing technology-based stroke rehabilitation strategies is essential for low and middle-income countries such as India to meet the increasing rehabilitation demands of stroke survivors. One

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of the main reasons for this is a lack of knowledge about stroke and how to manage stroke-related disabilities. The financial burden of stroke treatment and support increases for stroke survivors and families.

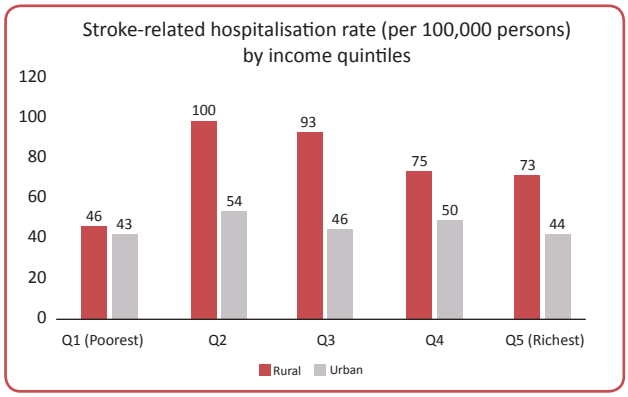

Figure 1: Stroke hospitalisation rates by income quintiles in India in 2017-2018[1]

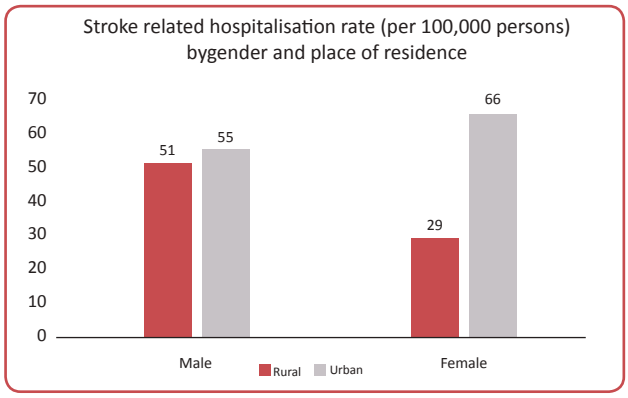

Figure 2: Stroke hospitalisation rates by gender and place of residence in India[1]

Due to a lack of rehabilitation resources in India, the development of innovative, multi-disciplinary, patient-oriented and culturally sensitive rehabilitation interventions is of paramount public health importance. In India, there is a lack of information on the rehabilitation needs of people with disabilities, particularly after stroke, where people with disabilities generally face several obstacles in accessing rehabilitation services.[2-5]

Roadblocks in post-stroke management

While the number of acute stroke units in India is growing as resources become more accessible, they serve only a small fraction of India's population and most Indians do not receive rehabilitation services either in the hospital or after discharge. Developing effective, low-cost community rehabilitation interventions for emerging chronic diseases like stroke in India has the potential to significantly impact public health. These interventions, if proven to be cost-effective and feasible, could be scaled up or generalised. The research challenge of how to develop sustainable and multifunctional rehabilitation systems for the rural population in low and middle-income countries, including providing services to the poor, was ranked as the second-most important research priority for disabled people (after equal access to healthcare) by a recently published Lancet expert panel.[6]

For stroke patients, mobility issues and the inability to do basic functional tasks like walking and feeding with the affected handled to sudden reliance on their spouse for basic care and day-to-day activities. As a result, spouses had to shoulder the burden of caregiving, while most participants had little or no access to rehabilitation or health services at a community level.[7]

The care management of stroke survivors is too poor for patients coming from humble backgrounds in India. The post-stroke care is not at all understood and well-practiced by hospitals and stroke care units. Once the patient gets discharged from the hospital the rehabilitation takes a backseat and the patient becomes dependable on his/her family for daily routines.

The most common post-stroke disability is motor impairment, which occurs either as a direct result of the loss of signal transmission from the cerebral cortex, as a gradual accumulation of cerebral injuries, or as a result of muscle atrophy caused by learned disuse. According to Divani et al,[8] the risk of fall and fall-related injury was higher among stroke elders.

Risk factors associated with an increased fall risk in stroke survivors include: gender (women are more prone to falls), poor general health, time since the first stroke, psychiatric problems, urinary incontinence, pain, previous injury from fall, psychological problems, uterine incontinence, impairment of hearing, motor impairments, multiple strokes, motor function deficits and increased fall risks.

Fall-related injuries can severely impact the patient's mobility and daily life, which limits their ability to participate in social activities and other professional activities.[8-10]

CONTENT

Lack of patient education and stroke awareness

The level of patient education and stroke awareness among common Indians is bleak and very poor. Stroke patients are not getting help once they have a stroke regarding the disease and rehabilitation which leads to poor outcomes and impacts of disability-adjusted life years (DALYs).

Patients often have very limited knowledge about the impaired mobility after a stroke which can lead to the development of pressure sores (pressure ulcers), which in turn can lead to the formation of deep vein thromboses (DVTs) and pulmonary embolisms. Pressure ulcer occurs when there is an imbalance between the external forces acting on the skin and soft tissues and the internal sensitivity of the skin and its soft tissues to injury.

The lack of knowledge of patients with pressure ulcers is more likely to die after a stroke. Both men and women aged 60 years and older are more likely to develop pressure ulcers after a stroke. Stroke patients are also more likely to develop deep DVTs and pulmonary embolisms due to their immobility and increased prothrombotic activity. The primary risk factors for poststroke DVT are advanced age, male sex, congestive cardiac failure, malignancies and fluid and electrolyte abnormalities. These lacunas often impact the clinical outcomes and patients are forced to live with disability and this leads to an increase in mortality rates.[11-14]

As the population ages, the local government has implemented several initiatives to improve access for the elderly to overcome the mobility issues that elderly stroke survivors face. These include the installation of a ramp and additional lifts at the local subway stations, the introduction of wheelchair-accessible public buses, a lift upgrading program to ensure lift access at every level of the public housing block and a heavily subsidised public housing home improvement program that includes ramp and steps at the entrance of the housing units.[15]

For elderly stroke survivors who don't have caregivers to accompany them to outpatient rehabilitation centres, authors recommend the implementation of an affordable home rehabilitation program or inexpensive tele-rehabilitation services. The ongoing local trial on telerehabilitation during the first 3 months post-stroke may provide more insight into the potential benefits and cost-effectiveness of telerehabilitation in the Singapore post-stroke population. Home based robotic therapy (HBRT) could be considered for elderly stroke survivors who had difficulty accessing outpatient rehabilitation. In addition, HBRT has been shown to reduce costs and improve access to rehabilitation for stroke survivors. To correct the common misconception that rehabilitation is the same as home exercises without the support of a rehabilitation team, it is essential to provide education on rehabilitation during acute admission. Evidence-based educational guidance has helped stroke survivors (and their families) to understand the value of rehabilitation, manage their co-morbidities and CV risk factors and reduce their recurrence risk.[16, 17]

Role of patient education in post-stroke rehabilitation

It is important for patients and the community to be informed about the signs and symptoms of stroke. Teaching the patient to return to as much self-care as possible is very important. Assistive devices should be provided as indicated in guidelines from time to time. An occupational therapist should make a home assessment and recommendations to assist the patient in becoming more independent. Coordinated care provided by multiple healthcare providers; assisted the family in planning aspects of care; advised the family that the patient may be fatigued easily, irritable and agitated by minor events and less interested in daily activities. Therapist should recommend home speech therapy; recommend family involvement, provide practical instructions to assist the family between speech therapy appointments. If necessary, talk to the physician about possible antidepressant therapy for the patient. He/she should encourage the patient to participate in community-based stroke clubs to provide a sense of belonging and companionship to others; encourage the patient to pursue hobbies, recreational and leisure interests; keep in touch with friends to avoid social isolation; encourage the family to support the patient and provide positive reinforcement, as well as remind the family that they need to attend to their health and well-being.

While stroke education during hospitalisation is necessary, its impact on secondary prevention and early detection of new events is still poorly understood. One of the reasons for this is that providing high-quality patient education

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is expensive in terms of human resources, especially when many hospitals are unwilling and unable to provide extra full-time nurses to support other aspects of care that go beyond physical needs. While hospitals typically provide written materials about stroke for patients and caregivers, it is not clear whether many utilise high-engaged stroke team (HES) members for the kind of comprehensive education authors used in a pilot study. While it was not evaluated how uneducated patients / primary caregivers would have performed on these test items, the results would likely have been similar, if not worse, compared to the pilot study cohorts. This raises the question of whether more than a written handout is necessary given such poor retention rates.[18, 19]

Requirements in post-stroke management in India

Acute ischaemic stroke is still a life-threatening condition that has a considerable impact on individuals, their loved ones and the healthcare system, despite the advances in diagnosis and treatment over the past decade.[20] As evaluations, medications and care for acute stroke have evolved over the past decade, the best practices of nursing and interdisciplinary care need to be adapted and updated to reflect the principles of best practice. Through continuous observation and assessment, nurses can identify patients at risk of clinical deterioration. Prompt and appropriate action is also taken when changes in a patient's health status are detected.[21]

Medical attendants should conduct extensive and precise actual assessments for all patients who have experienced a stroke, including checks of the primary 5 essential signs: internal temperature, pulse rate, respiration rate, chest extension, oxygen saturation and mental state/level of consciousness. Evidence-based nursing care and ongoing assessment are essential to reduce unfavourable outcomes for patients following stroke.[22-24] Better flow of stroke recovery program is necessary for better clinical outcomes (Figure 3).[26, 29]

Recent rehabilitation of stroke patients in India

Analysing the physiotherapy management, it was divided into three levels:

Level 1: easy to do at most set-ups, including District hospitals. Necessary equipment is 3-4 pillows, 2 sandbags, a shoulder sling (one can be made at home), a simple mirror and a static ankle foot orthosis.

Level 2 requires some equipment and expertise, but it's still pretty easy to get. Necessary equipment is a postural mirror, a functional electrical stimulator, an electrical neuro-muscular stimulator, a static cycle, a tre-admill and an indirect pneumatic compression unit.

Level 3 is for more advanced rehabilitation centres and necessary equipment is a surface EMG biofeedback system, body weight support

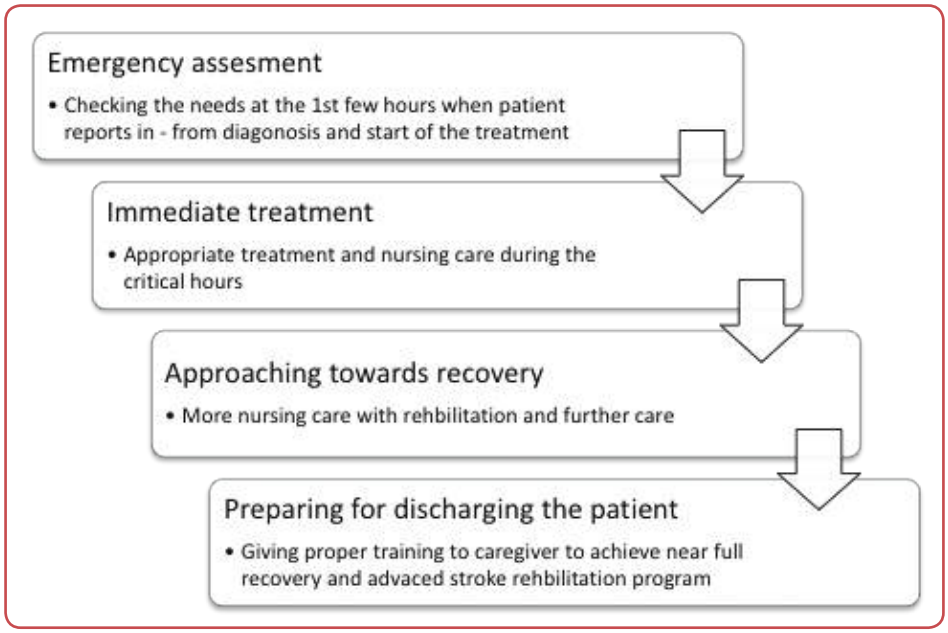

Figure 3: Flow of stroke recovery program for better clinical outcomes[26, 29]

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treadmill trainer, arm ergometry, robotics, mental imagination, visual scanning training, force platform, balance and gait analysis system.[25]

Owing to the diversity in India a multidisciplinary approach is necessary to create awareness and support groups for post-stroke patient care management. The needs are numerous and diverse for post-stroke patients and caregivers to cater to the variety of requirements for improving the DALYs. Patient education is not widely available to help patients live a quality life post-stroke when they reach home for recovery. The usual approach of post-stroke patients has not been up to the mark as compared to developed nations wherein the patients get home-based post-stroke rehabilitation and they are more likely to live an independent life. In India stroke patients specifically from the lower income groups are not getting the due rehabilitation when they reach home. The caregivers are not being educated by the nursing staff or the clinicians about post-stroke care which can be pivotal in enhancing the quality of life (QoL) of the stroke patients post-discharge from the critical care unit.[27, 28]

Rehabilitation is essential to reduce the recurrence of stroke-related complications and patients who receive ongoing professional and systematic rehabilitation after the acute phase of the stroke tend to recover quickly.[29] Currently, drug and rehabilitation therapy is practiced as a rehabilitation treatment for stroke. The various interventions that can be employed in the recovery process include bilateral training, repetition of tasks, movement therapy based on constraint, electrical stimulation, robotics and exercise.[30, 31] Exercise is of paramount importance to assist patients in returning to activities of daily living by restoring the functioning of damaged muscles and enhancing physical function. Furthermore, exercise is necessary to prevent secondary complications, as demonstrated by a study that found that post-stroke exercise and physical activity reduce the risk of recurrent cardiovascular disease and mortality.[32, 33]

The government or health ministry should create a robust plan for creating awareness about the available resources that may help post-stroke patients to a large extent in living an independent life after they get back home with limited abilities.

Conclusion

There is little or no research on the role of nurses in post-stroke management and the role of physiotherapists which is home-based. The lack of research on this topic persists despite evidence that stroke patients are better treated when treated by a multidisciplinary team. It is hoped that this review will serve as a tipping point for recommendations for the streamlined role of clinicians, the nurse and the physiotherapist in post-stroke care and rehabilitation, as well as serve as a motivation for other researchers to conduct real-world studies using larger and more representative samples for the larger good of Indian society.

Stroke rehabilitation needs are on the rise in low and middle-income countries like India, so it's essential to create technology-based stroke rehabilitation plans to meet these growing demands.

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Conflict of interest

None.

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Kolarević et al. Scr Med 2023 Dec;54(4):419-24. DOI:10.5937/scriptamed54-45969
HISTORY OF MEDICINE

Twelve Decades of Using Radium in the Treatment of Deeper Localised Cancers

Goran Kolarević,[1, 2, 3] Oliver Arsovski,[1] Branko Predojević[3]

Abstract

The end of the 19th and the beginning of the 20th century marked a period of fundamental discoveries in the physics of ionising radiation (X radiation and radioactivity). Isolating radium, a highly radioactive element, immediately opened the way to its application for medical therapeutic purposes. It turned out that the sources of ionising radiation are very effective for changes localised on the skin and at small depths under the skin but not for lesions at greater depths. Interestingly, the inventor of the modern telephone, Alexander Graham Bell, was the first to come up with the idea of placing radium sources in glass tubes and placing them directly in the pathologically changed tissues of the patients to be treated (at greater depths). That period marked the beginning of a highly successful era in radium therapy, involving the use of capsules and needles filled with radium, which eventually led to the development of modern brachytherapy. Unfortunately, for several decades people believed in the universally therapeutic properties of radium, so that (fortunately in smaller quantities) it was added to water, food, hygiene products, etc.

Key words: Radioactivity; Radium; Alexander Bell; Brachytherapy; Ionising radiation.

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Radioactivity and Radium

In February 1896, 3 months after the discovery of X-rays by Wilhelm Conrad Röntgen (1845-1923, Nobel Prize in Physics 1901), a strange phenomenon was observed by Henri Becquerel (1852-1908, Nobel Prize in Physics 1903),[1] uranium salt emits invisible and penetrating radiation similar X-rays. On March 2nd, Becquerel reported at the Academy of Sciences that this radiation arises without any stimulation by light. Names such as Becquerel rays, uranium rays and invisible fluorescence referred to the discovered phenomenon. Becquerel's discovery sparked interest in the scientific community, establishing more centres dedicated to studying this phenomenon. Without diminishing the significance of other centres, the most significant contributions in the early investigation of this phenomenon, particularly in experimental research, were made by research groups gathered around the Curies in Paris (Marie Sklodowska-Curie, Pierre Curie, Irene-Joliot Curie, Frederik Joliot), around Hahn and Meitner (Otto Hahn, Lise Meitner, Kaiser Wilhelm Institute Berlin) and Rutherford (Ernest Rutherford, McGill University in Montreal).

Several questions were formulated regarding Becquerel rays: a) which substances emit them, b) what is the nature of Becquerel rays, c) what processes lead to the emission of this radiation. These questions have determined the fundamental directions of further research. The

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most significant contribution in answering the first question in the early years of nuclear physics development was made by Marie and Pierre-Curie.

Figure 1: Marie Skłodowska-Curie and Perie Curie

Marie Sklodowska-Curie (1867-1934, Nobel Prize in Physics 1903 and Chemistry 1911), wife of physicist Pierre Curie (1859-1906, Nobel Prize in Physics 1903), was in Paris at the end of 1897 contemplating potential topics for her doctoral dissertation. The Curies (Figure 1) were very interested in radioactivity and began experiments with uranium. They concluded that radiation is a property of the atoms themselves.
In her report to the Paris Academy in April 1898, Marie Curie wrote: "Two uranium minerals: uranite (uranium oxide) and chalcolite (copper-uranium phosphate) are much more active than uranium itself. This fact is very significant and leads us to think that these minerals may contain some element that is much more active than uranium..."

Their stance was confirmed by publishing their work on the radioactivity of thorium, just days after Gerhard Schmidt.[2, 3] In the same year (1898), they managed to isolate two new elements, polonium[4] and radium,[5] the radiation of which is three million times more active than uranium.[6] Working on polonium, for the first time in the history of science, they used the word „radioactivity", a term that has remained in use to this day. Strictly speaking, the Curies did not obtain pure radium but radium chloride in their initial result. Radium as pure metal was isolated by Marie Curie and Andre Luis Debierne through the radium chloride ($RaCl_2$) electrolysis.[7]

Shortly after the discovery of radioactivity, in 1901, Pierre Curie recommended the use of radium to the French dermatologist Henri Alexandre Danlos for the treatment of skin changes. In the beginning, surface applicators were created for radium sources (for the treatment of surface-skin lesions) and we can say that surface brachytherapy was created (Figure 2).[8, 9] It was soon concluded that radium rays have the same biological properties as X-rays.

Figure 2: Treatment of skin lesions with radium, Melbourne, Australia (1905)

In June 1903, Marie Curie presented her doctoral thesis at the Sorbonne University and became *Docteur es Sciences* (PhD) and in December received the Nobel Prize. Pierre Curie was appointed as a professor and head of the Physics Department at Sorbonne University in October 1904, while Marie Curie became head of his laboratory. On April 19th, 1906 (age 47), Pierre Curie was killed by a horse-drawn carriage crossing Dauphine Street in Paris (Marie was 39 years old). Sorbonne University appointed Marie as a professor and she became the first female full professor at a French University (November 1906). The unit for measuring the activity of radioactive material (1910) was named „curie" (Ci) and defined as the activity of radium mass 1 g.[10]

Dr Alexander Graham Bell - Radium Pioneer

Alexander Graham Bell (1847-1922) patented the modern telephone as his invention. His life was made miserable by a lengthy court process due to circumstances where Elisha Gray reported the same invention to the US Patent Office just two hours after his lawyer (February 14th, 1876).

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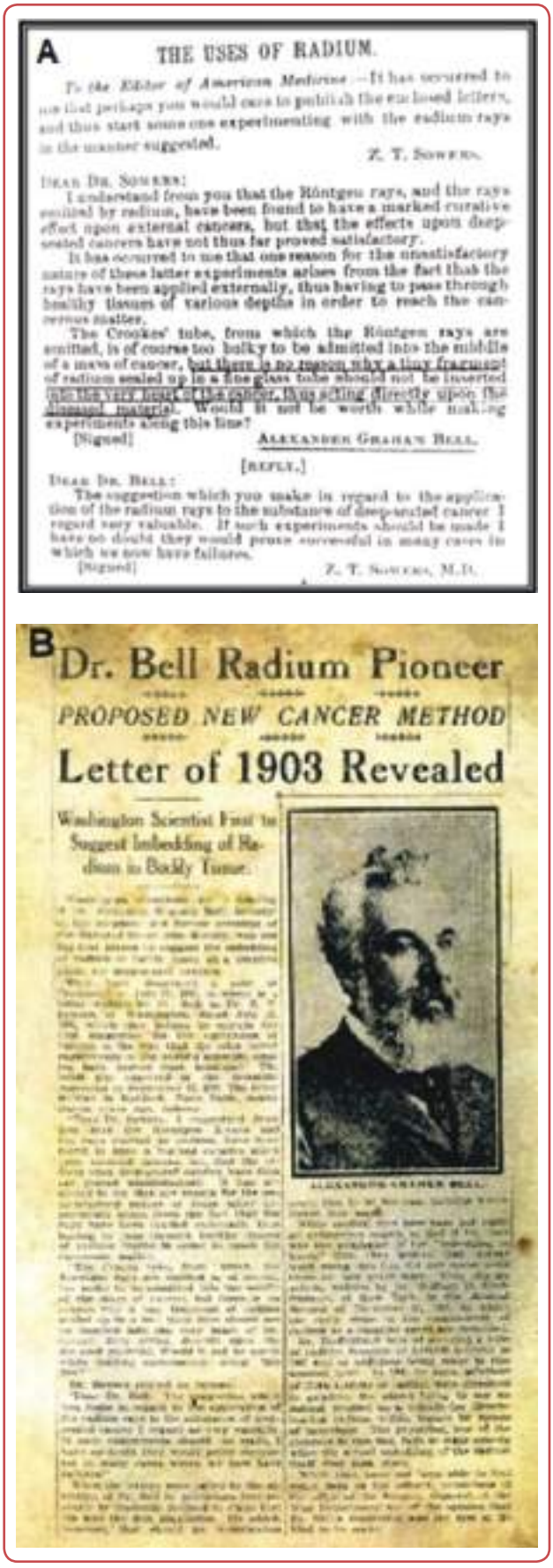

Figure 3: A) Radium and Cancer (Letter to Dr ZT Sowers). Science, July 31st, 1903, B) Medical record, New York, December 13th, 1913

Because of those two hours, Gray lost millions.[11] The letter, dated July 21st, 1903, in which Bell addresses doctor Sowers from Washington, is considered to contain the first proposal for the application of radium in the way that the latest experiments in the world's scientific centres showed to be the most useful at that time.[12] The letter was published in the Scientific American magazine on September 12th, the same year (Figure 3A) and read:

„Dear Dr. Sowers. I understand from you that the Roentgen X-rays and the rays emitted by radium have been found to have a marked curative effect on external cancers but that the effects upon deep-seated cancers have thus far proved unsatisfactory.

It has occurred to me that one reason for the unsatisfactory nature of these latter experiments arises from the fact that the rays have been applied externally, thus having to pass through healthy tissues of various depths in order to reach the cancerous matter.

The Crooks tube, from which the Roentgen rays are emitted, is of course too bulky to be admitted into the middle of the mass of cancer, but there is no reason why a tiny fragment of radium sealed up in a fine glass tube should not be inserted into the very heart of the cancer, thus acting directly upon the diseased material. Would it not be worthwhile making experiments along this line?"

Dr Sowers replied as follows:
„Dear Dr. Bell. The suggestion which you make in regard to the application of the radium rays to the substance of deep-seated cancer I regard as very valuable. If such experiments should be made I have no doubt they would prove successful in many cases where we now have failures".[13]

Today, Dr Alexander Graham Bell, inventor of the telephone and former president of the National Geographic Society, is believed to have been the first person to propose the implementation of radium into body tissue as a cure for deep-located cancers.

The article by Dr Williams Dieffenbach, in the Medical Record of December 13th, 1913 (Figure 3B), described the first steps in using radium as medicine. He talks about a solution of radium in gelatine, which was injected into a patient in 1906.

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Radium Applicators – Radium Therapy

Nineteen twenties, radium salts are inserted into tubes and thin needles with the help of jewellery experts. In Paris, platinum tubes containing 10 mg of radium and needles of various lengths were made. The platinum wall absorbed alpha, beta and gamma rays of low energy.[10]

Brachytherapy (term of Greek origin introduced in 1931) is defined as a short-distance treatment of malignant disease with radiation from encapsulated sources. The sources are placed directly (or in the immediate vicinity) into the tumour.[12] Radium (^{226}Ra, half-life 1622 years, E_γ = 0.8 MeV)[14] was an ideal choice for brachytherapy because it emitted high-energy radiation that could effectively destroy tumour tissue.

Intracavitary radium therapy is performed by insertion of the suitable applicators in the body cavities-gynaecologic cancers or rectal cancers.

Radium salts (powder) are packed in metal tubes (Figure 4A) or cylinder capsules (Figure 4B) designed for endocavitary application in the rectum or vagina/cervix (Figure 5); the thinner ones could be placed in the uterus or cervix.[15]

Cancer was treated more successfully by applying the radium by the intracavitary technique of treating the cervix. The so-called Paris system,

Figure 4: A) Cervical-uterine radium applicator (1930-1970), made from brass which is chrome plated, activities 50-100 mCi; B) Radium capsules

consisting of a uterine tube containing 33.3 mg of radium and two vaginal cylindrical plugs connected by a movable spring and containing 13.3 mg of radium each, was used for continuous irradiation for 120 hours (Figure 5).[10]

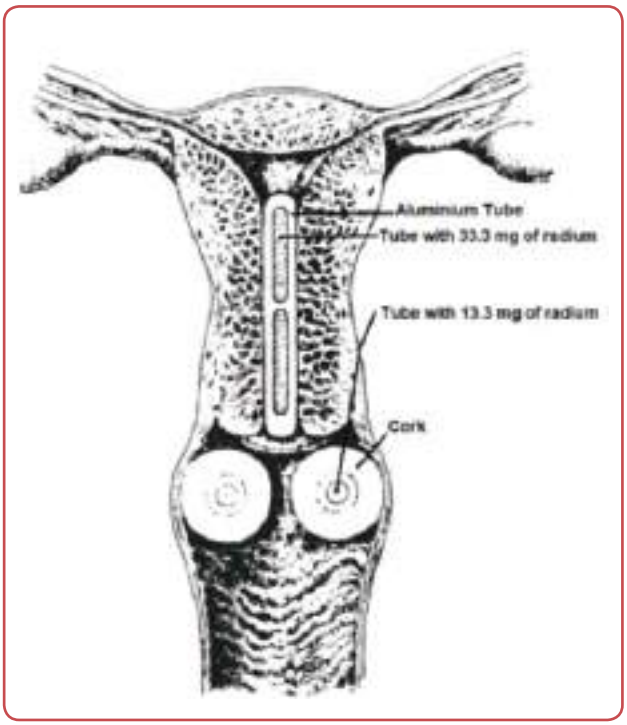

Figure 5: Intracavitary radium treatment for cancer of cervix

Interstitial radium therapy is performed by insertion of the applicators directly in the tumour tissue-head and neck cancers or prostate cancers.

One of the key advantages of using radium needles (Figure 6) in brachytherapy was the possibility of targeted tumour treatment. Radium needles were placed directly into the tumour or its surroundings, allowing focused radiation to the diseased area. This approach had an advantage over external radiotherapy (X-radiation) because it allowed a higher dose of radiation to the tumour while at the same time protecting healthy tissue from excessive radiation. Indeed these radium insertions could be considered as the first three-dimensional radiotherapy treatments.

During the 1920s, interstitial radium therapy (needles) was commonly used to treat tumours of the head and neck (Figure 7), breast, bladder, prostate and cervix (through the abdominal route).[10]

Over time, the harmful effects of radiation became better understood. Workers involved in producing radium needles and medical professionals

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Figure 6: European-type radium needles and tubes[16]

who used them were at high risk of radiation over-exposure. Thus, using radium needles in brachytherapy became controversial during the 20th century. Although this therapy was very effective in treating tumours, the radioactivity of radium caused serious consequences for the health of medical personnel who came into contact with it. Many have also developed various forms of cancer. As a result, the use of radium in medicine became less and less popular and later it was replaced by other radioactive materials (^{60}Co, ^{192}Ir, ^{125}I or ^{137}Cs).

Although the use of radium needles/capsules in brachytherapy is rare today, their importance in the history of medicine cannot be ignored. These needles represented a turning point in cancer treatment, paving the way for developing more advanced brachytherapy methods that have been successfully applied in modern oncology. The use of radium for medical purposes is definitively prohibited by law in France on October, 1976.[10]

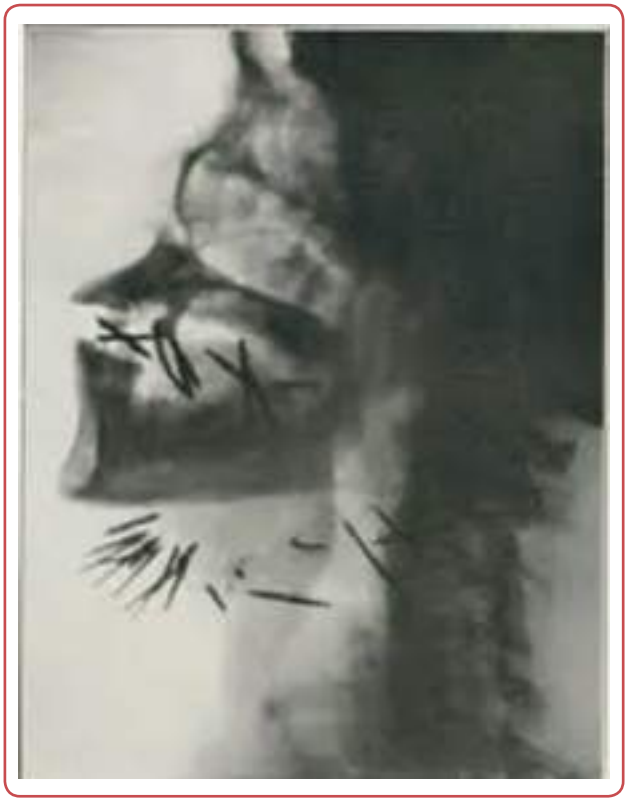

Figure 7: Loaded radium needles were inserted into malignant tumours (head and neck)

The World's Delusion

Medicine's belief in the universal therapeutic properties of radium (a cure for all diseases) lasted several decades. This had an impact on the general population and numerous consequences in medicine. The entire public was misled about the magic of radium in treating pathological changes, pain relief, regulation of physiological functions and maintenance of good health. The radioactive content of mineral waters was emphasised as an indicator of their quality as water for drinking or bathing as a prevention against all diseases. People were offered radioactive ointments and poultices to relieve pain and to treat ulcers and burns, radioactive powder for digestive problems, bathtubs made of radioactive materials, radioactive bedding, radioactive soap and cosmetic cream to prevent skin aging (Figure 8). Fortunately, all this was not too dangerous for the customers since the radioactivity of the product was minimal.[15]

Figure 8: Advertisements for „radium“ enriched products

Thomas Edison, William J Morton and Nikola Tesla were among the first to point out possible unwanted harmful effects on the human body, inappropriate exposure to radium and X-radiation.[17]

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Conclusion

The discovery of radioactivity and radium is a significant event in the history of physics. Some applications have led to progress in many areas of technique and sciences, including medicine. Brachytherapy is one such case. Radium needles/capsules were crucial in developing brachytherapy and cancer treatment. Their use ensured targeted irradiation of tumours and technological progress led to the creation of more efficient and safer methods.

Acknowledgement

None.

Conflict of interest

None.

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PROFESSIONAL ARTICLE

Resolving Discrepancies in Forward and Reverse ABO Blood Group Typing

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Abstract

Background/Aim: There is a need of investigation of high frequency of the cases of ABO discrepancy revealed by forward and reverse typing. The aim of the study was to reveal optimal methods for detection of weak subgroups of group A and B antigens on erythrocytes.
Methods: Sixteen persons with A blood group and anti-B antibody adsorbing ability (weak Bel, Bm and B_3 antigens), 5 persons with B blood group and anti-A antibody adsorbing ability (weak Ax antigen) and 2 persons with O blood group and anti-B antibody adsorbing ability (weak Bel antigen) were investigated as the cases of type II-III of ABO discrepancy and weak B antigen. Liquid chromatography, agglutination, inhibition of agglutination and adsorption reactions at 4 °C and 37 °C with prolonged incubation (12 hours) were used in the study.
Results: The persons with A blood group revealed by forward typing at 4 °C and O blood group detected by reverse typing at 37 °C showed the ability of the erythrocytes to adsorb anti-B antibody. The persons with B blood group revealed by forward typing with presence of anti-B antibodies reactive at 37 °C showed the ability of erythrocytes to adsorb anti-A antibody. Prolonged incubation at reverse typing, adsorption-elution tests, inhibition of haemagglutination and liquid chromatography were used to define blood group specificity.
Conclusion: Prolonged incubation during reverse typing at 4 °C and 37 °C, adsorption at 4 °C, heat elution and liquid chromatography might help to detect blood group type in cases of type II-III of ABO discrepancy.

Key words: Chromatography; Antigen; Adsorption; Erythrocyte; Transfusion.

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Introduction

ABO typing is the most important pretransfusion study directly related to the quality of the person's safety. The complications due to mistyping and ABO mismatched mis-transfusion are known to elevate the risk of viral infection.[1]

ABO discrepancies are known to be classified into four groups. Group I represents missing antibodies, group II is associated with unpredicted reaction due to weak antigens, group III demonstrates rouleaux formation and pseudo-aggluti-

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nation, group IV is associated with autoantibodies and polyagglutination.[2, 3] In haematological malignancies and epigenetic variations of group A and B transferases the erythrocyte antigen genes expression might be lost.[4] Tumour cells were reported to secrete serum soluble blood group substances.[5] Group II discrepancy has been observed in persons with solid organ malignancies and group III discrepancies were reported in Hodgkin lymphoma. Alterations of antigen expression have been observed in gastrointestinal cancer tissue with absence of group A, B, H or Le^b antigens in 25 % of cases.[6] The causes of ABO discrepancy due to the cold reactive autoantibodies, alloantibodies (anti-M, anti-P1, anti-N and anti-E), rouleaux formation, hypogammaglobulinaemia, anti-A1 and anti-B antibodies in A2B case and influence of autoanti-A antibodies have been described. Some cases caused by erythrocytes' problem (loss of blood antigen in leukaemia, weak group A or B antigens, acquired group B antigen, mixed field agglutination followed ABO mismatched transfusion, non-classified agglutination) have been revealed.[7]

Weak subgroups are usually detected by agglutination with antibodies, adsorption elution method, definition of secretor status.[8-13] Thirty cis-AB cases with discrepant results in automated and manual methods were reported and fifteen cis-AB cases were determined due to weak reactions in automated methods.[9, 10] Thus, the implementation of automated methods does not necessarily guarantee reducing errors in ABO typing, especially for weak ABH subgroups as cis-AB.[11, 12]

Group A, B and H antigens of erythrocytes are known to be the most important for transfusion medicine. Group A antigen is considered to have multiple structural forms. The most common is considered A1, determined in approximately 80 %. Group A2 antigen is structurally different than group A1 antigen. There are fewer copies of group A2 antigen on erythrocytes as compared to group A1 antigen.[13] Carbohydrate antigens are considered to lead to the IgM immune response with the highest reactivity at 4 °C, whereas immune anti-A and anti-B antibodies agglutinate at 37 °C. Naturally occurring IgM anti-A and anti-B antibodies were reported to cause difficulties in incompatible blood transfusion and transplantation.

The use of different temperatures while incubation of erythrocytes with sera attracted attention of many scientists due to the different reactivity of IgM and IgG antibodies at 4 °C and 37 °C.[14] Thus, antibody, IgM or IgG, may be of clinical significance if reactive at 37 °C. ABO discrepancies in persons with lymphoma and solid organ tumours have been resolved by elution and antibody screening at 37 °C and 40 °C. The search of the conditions for optimal interaction of erythrocyte antigens and antibodies continues.

The aim of the study was to reveal optimal conditions for the detection of weak group A and B antigens on erythrocytes.

Methods

Serological based ABH phenotypes were determined by agglutination testing according to standard methods and procedures described in AABB Technical Manual.[15] To obtain IgG antibodies the sera were heated for 30 minutes at 56 °C.

The polyclonal sera (*Tulip diagnostics* Goa, India) and group A, B and O erythrocytes were used for forward and reverse typing of the studied persons by tube method. The test erythrocytes and sera were taken from 25 volunteers aged 71.4 ± 1.2 years old (15 men and 10 women). The erythrocytes were washed three times with normal saline and centrifugated at 1000 g for 10 minutes.

The first results of blood typing showed 16 persons with A blood group, 5 persons with B blood group and 4 persons with O blood group.

The study of the persons' sera on the presence of anti-A and anti-B antibodies was performed after the contact with group A and B erythrocytes at 4 °C, 8 °C and 37 °C. Fifty µL of test erythrocytes were added to 100 µL of the studied serum in different dilutions. The results were recorded after microscopic investigation.[16] The strength of agglutination was graded according to the standards.[17] The studied sera showed the presence of unexpected antibodies. Thus, 11 persons with A blood group showed the presence of anti-A antibodies, active at 4 °C (9 persons) and at 37 °C (8 persons). Among them those having Bel antigen demonstrated presence of anti-B antibodies, active at 4 °C (6 persons) and at 37 °C (6 persons).

Meanwhile, persons with B blood group showed

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the presence of anti-B antibodies (3 persons), active at 4 °C (2 persons) and at 37 °C (2 persons).

An attempt to resolve the observed discrepancy led to the repeating testing using adsorption method.[18] Polyclonal antisera of human origin (O blood group) were used for adsorption. The adsorption reaction was performed after the contact of the studied erythrocytes (50 µL) with anti-A, B serum (100 µL) for 12 hours at 4 °C (1:2 volume ratio of erythrocytes and serum accordingly). Heat elution of antibodies was performed after adsorption of the studied erythrocytes with anti-A, B serum at 56 °C for 10 minutes. The technique was performed following AABB Technical Manual.[19] Thus, 13 persons with A blood group demonstrated anti-B antibody adsorbing ability, 5 persons with A blood group demonstrated anti-A antibody adsorbing ability and 2 persons with O blood group showed anti-B antibody adsorbing ability.

The analysis of the erythrocytes sample on N-acetyl-D-glucosamine presence was performed by liquid chromatography. Sample: 10 mL 10-3; column: Shodex Silica 5 NH 4D; eluent buffer (pH 7.5)/ CH_3CN=30/70. 3.5 g of KH_2PO_4 was dissolved in water in 1 L of volumetric flask, 0.25 mL of ammonium hydroxide 25 % was added, diluted with water and mixed, adjusted to pH 7.5 with H_3PO_4. Flow rate: 1.1 mL/min. Detector: UV 195 nm. Column temperature: 35 °C.

Fifteen persons with A blood group showed type II-III of blood group discrepancy (7 with weak Bel antigen, 7 with Bm antigen, 1 with weak B3), 5 persons with B blood group showed type II discrepancy (weak Ax antigen), 2 persons with O blood group showed II type of discrepancy (weak Bel antigen).

The analysis of the erythrocytes sample on N-acetyl-D-glucosamine presence was performed by liquid chromatography. Sample: 10 mL 10-3; column: Shodex Silica 5 NH 4D; eluent buffer (pH 7.5)/ CH_3CN=30/70. 3.5 g of KH_2PO_4 was dissolved in water in 1 L of volumetric flask, 0.25 mL of ammonium hydroxide 25 % was added, diluted with water and mixed, adjusted to pH 7.5 with H_3PO_4. Flow rate: 1.1 mL/min. Detector: UV 195 nm. Column temperature: 35 °C.

The study was approved by the Ethical committee of Kharkiv National Medical University (protocol 4).

Statistical analysis was performed by Statistica 10.0. The Student's and the Mann Whitney U tests were used for analysis of the mean values of agglutination. Correlation of adsorption (the degree of inhibition of haemagglutination after adsorption of the sera with studied erythrocytes) and liquid chromatography data (the concentration of N-acetyl-D-galactosamine in erythrocyte's sample) was performed using Pearson's coefficient.

Results

The cases of ABO discrepancy of the persons typed as A blood group with unexpected anti-A antibodies (II-III type of discrepancy)

1. The person of A blood group with anti-B antibody adsorbing ability. The studied erythrocytes after adsorption decreased the expression of agglutination of group B erythrocytes by anti-A, B serum in 1:32 titre (from 3+ to 1+), whereas the studied serum agglutinated group A erythrocytes after 12 hours of the contact at 4 °C (w+) and 37 °C (+). Interestingly the serum did not agglutinate group B erythrocytes at 4 °C and decreased their quantity at 37 °C (Table 1). Type II discrepancy (Bm antigen) was observed. Liquid chromatography of the erythrocytes sample determined the presence of N acetyl-D-galactosamine in 0.095 mg/mL concentration (Figure 1).

2. The person of A blood group with anti-B antibody adsorbing ability. The studied erythrocytes after adsorption led to the decrease of agglutination of group B erythrocytes by anti-A, B serum (from 1:32 to 1:16 titre). Elution reaction after adsorption of the studied erythrocytes showed the presence of anti-B antibodies.

Meanwhile, the studied plasma agglutinated group A erythrocytes after 12 hours of contact at 4 °C and at 37 °C, as well as group B erythrocytes at 4 °C and 37 °C (decreased their quantity) (type II discrepancy, Bel antigen).

3. The erythrocytes of A blood group with anti-B antibody adsorbing ability after adsorption led to the decrease of agglutination of group B erythrocytes by anti-A, B serum (from 1:32 to 1:4 titre). The studied serum agglutinated group A erythrocytes at 4 °C and 37 °C (type II discrepancy, Bm antigen). Elution after adsorption of the studied erythrocytes with anti-A, B serum showed the presence of anti-B antibodies.

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Table 1: The study of erythrocytes and serum of the persons with discrepancy in forward and reverse typing

	Erythrocytes	Adsorption with S anti-A, B at +4 °C for 12 h after contact with S from AB sample for 1 h	The studied serum or plasma with A er. at +4 °C	The studied serum or plasma with A er. at +37 °C	The studied serum with B er. at +4 °C	The studied serum with B er. at +37 °C	Elution after adsorption	Inhibition of agglutination
1	A (Bm+) (from S)	S anti-A, B: 1:32:3+; after adsorption 1:32:1+.	w+	1+	-	- (↓ n)		
2	A (Bel+) (from S)	S anti-A, B 1:32-1:16 with B er.	3+	+ aggl.	2+	1+ (↓ n)	with B er.: 1+	
3	A (Bm+) (from S)	S anti-A, B 1:32-1:4, S anti-B 1:32-1:32	+	+ strong			with B er.: 1+ with anti-B S: -	
4	A (Bel+) weak (citrated)	S anti-A, B < aggl. 1:32	1 + after 5 min - 12 h	-12 h	with unwashed: 1+	1+		↓ S anti-A ,B 1:20 in 1:2 titre
5	A (Bm+) weak	S anti-A, B 1:32 (1:16)	-	-				↓ S anti-A, B in 1:2 – 1:4 titre
6	A (Bel++) (citrated)	S anti-A, B + B 1:32-1:16	2 + 5 min 1+	+ (no ↓ n)	w+			↓ S anti-A, B (1:20) 30 in
7	A (Bel+) weak		3+	- no ↓ n	1+	+ (↓ n)		↓ S anti-A, B + A er.: ↓ in 1:2-1:16 titre, ↑ with B er.: in 1:2 titre
8	A (Bm+) weak	S anti-A, B + B er. < aggl.	2 + 5 min 1+	+ (no ↓ n)			+(aggl. of B er.)	
9	A (Bm+)	S anti-A, B + B er.: 1:32-1:16, 1:32: < aggl.	+m	-				-↓ S anti-A, B + B er.: + in 1:2-1:4 titre
10	A		-12 h	- 12 h			- with saline - with S AB	
11	A (Bm++) (citrated)		-12 h	- 12 h				
12	A (Bm+) (citrated)	S anti-A, B + B er.: 1:32-1:16	30 min + 12 h	- 30 min -12 h				↓ S anti-A + A er.: in 1:2-1:16 titre
13	A (Bel+)	S anti-A, B + B er.: 1:32-1:16	3+	+	+	-		
14	A (Bel+)	S anti-A, B+B er.: 1:32-1:8	-	-	3+	w+		
15	A (B3+) (citrated)	S anti-B + B er.: 1:32-1:16	1 h: 3+	+ + 1 h	1+	+ ↓n		

CONTENT

16	A (Bel-) (citrated)	S anti-B + B er.: no decrease	w+	+, w +	3+	-, ↓n +		
17	0 (Bel+)	S anti-B + B er.: no decrease				+	aggl. of B er. (elution with saline, AB S)	
18	0 (A-Bel++) (from S)	S anti-A, B +A er.: < aggl. in 1:32 titre; + B er.: 1:32-1:16	+	↓n	+	+ no ↓n		S anti-A, B (2:20) +A er.: -
19	0							S anti-A, B +A er.: -
20	0							S anti-A, B +A er.: - with heated: -
21	B (Ax+) (citrated) weak	S anti-A, B+ A er.: < aggl. in 1:32 titre			-	+	- (no aggl of A er.)	
22	B (Ax+) weak (from S)	S anti-A, B: < aggl. in 1:32 titre	+	+↓n	w+ 30 min	-12 h		- citrated anti-B (2:20) + B er. +8°C
23	B (Ax+) (from S)	S anti-A, B + A er.: 1:8-1:4	3+	+ no ↓ n + no ↓ n	w+	w+		↓ S anti-A, B + A er.: in 1:2-1:4 titre ↓ +B er.: in 1:2-1:4 titre ↓ S anti-B +B er.: in 1:2-1:4 titre
24	B (Ax+) (from S)	S anti-A, B +A er.: 1:32: +; after adsorption: 1:32:1+.	3+	1+	2+	w+ , ↓n		
25	B (Ax+) (from S)	S anti-A, B +A er.: 1:32: +; after adsorption: 1:16.	2+	1+	-	+ 12 h		↓ S anti-A + A er. and B er.: in 1:2 titre

Note: S - serum, h - hour, AB - AB blood group, aggl. - agglutination, er. - erythrocytes, n- quantity of erythrocytes.

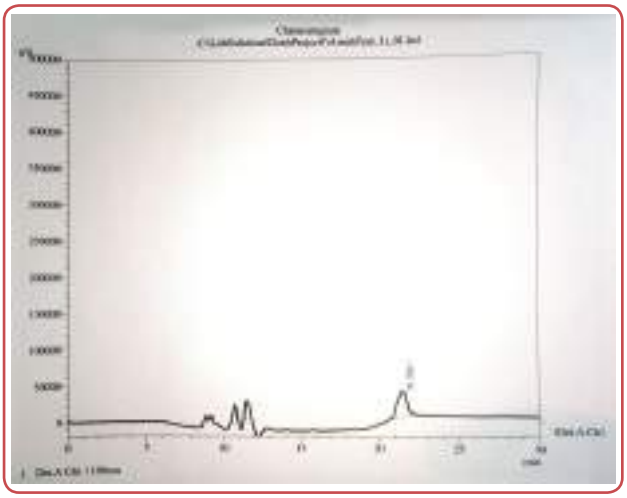

Figure 1: Chromatogram of the person with A blood group and weak group Bm antigen

4. The sample of A blood group with anti-B antibody adsorbing ability. The serum agglutinated group A erythrocytes at 4 °C after 5 minutes of contact and group B erythrocytes at 4 °C and 37 °C (type II discrepancy, Bel antigen). The serum inhibited agglutination of group A erythrocytes by anti-A, B serum in 1:2 titre. The studied serum did not agglutinate group A erythrocytes at 37 °C after 12 hours contact. The studied serum weakened the agglutinating ability of anti-A, B serum with group B erythrocytes in 1:2-1:4 titre.

5. The sample of group A erythrocytes with anti-B antibody adsorbing ability. The studied erythrocytes after adsorption led to the decrease of agglutination of group B erythrocytes by anti-A, B serum (from 1:32 to 1:16 titre).

CONTENT

The serum did not agglutinate group A erythrocytes at 4 °C and 37 °C, however inhibited agglutinating ability of anti-A, B serum in 1:2-1:4 titre (weak Bm antigen).

6. The sample of A blood group with anti-B antibody adsorbing ability. The studied erythrocytes after adsorption decreased agglutination of B erythrocytes by anti-A, B serum (from 1:32 to 1:16 titre).

The serum agglutinated citrated unwashed group A erythrocytes after 5 minutes of contact at 4 °C and 37 °C, as well as after 12 hours of contact and weakly agglutinated group B erythrocytes at 4 °C. The serum significantly inhibited agglutination of group A erythrocytes in 1:2-1:4 titre by anti-A, B serum (in 1:20 dilution) (type III discrepancy, Bel).

Anti-B serum agglutinated the studied erythrocytes after 30 minutes of incubation at 4 °C with further centrifugation. The studied serum in 1:2-1:4 titre inhibited agglutination of group B erythrocytes by serum from O blood type.

7. The person of A blood group with weak anti-B antibody adsorbing ability. The studied serum in 1:2-1:16 titre inhibited agglutination of group A erythrocytes by anti-A, B serum at 8 °C after 20 minutes of contact. The studied serum agglutinated group A erythrocytes at 4 °C, however did not agglutinate nor decrease the quantity of group A erythrocytes at 37 °C after 12 hours of contact. The serum agglutinated group B erythrocytes at 4 °C and 37 °C. Bel antigen was determined.

8. The person with A blood group and weak anti-B antibody adsorbing ability. The erythrocytes after adsorption led to the decreased agglutination of group B erythrocytes by anti-A, B serum. Elution after adsorption of anti-A, B serum with the studied erythrocytes revealed the presence of anti-B antibodies (weak Bm antigen).

9. The person with A blood group and anti-B antibody adsorbing ability. Anti-A, B serum after adsorption with the studied erythrocytes decreased agglutination of group B erythrocytes (from 1:32 to 1:16 titre). The studied serum inhibited agglutination of group B erythrocytes by anti-A, B serum in 1:2-1:4 titre (weak Bm antigen).

10. The person with A blood group. The studied serum did not agglutinate group A erythrocytes after 12 hours of contact at 37 °C and 4 °C. The elution (with saline and serum from AB blood group) after adsorption of anti-A, B serum with the studied erythrocytes did not reveal the presence of anti-B antibodies. A blood group was determined.

11. The sample of A blood group with anti-B antibody adsorbing ability. The studied citrated plasma agglutinated group A erythrocytes at 37 °C on the contrary to the reaction at 4 °C (type II discrepancy, Bm antigen).

12. The person with A blood group and anti-B antibody adsorbing ability. The studied erythrocytes after adsorption led to the weakened agglutination of group B erythrocytes by anti-A, B serum (from 1:32 to 1:16 titre). The studied plasma in 1:2-1:16 titre inhibited agglutination of group A erythrocytes by anti-A serum at 8 °C after 30 minutes of contact. The studied citrated plasma did not agglutinate group A erythrocytes at 37 °C after 30 minutes of contact, however agglutinated group A erythrocytes at 4 °C after 12 hours of contact (type II discrepancy, Bm).

13. The erythrocytes of the person with A blood group and anti-B antibody adsorbing ability after adsorption led to the weakened agglutinating ability of anti-A, B serum with group B erythrocytes (from 1:32 to 1:16 titre). The serum agglutinated group A erythrocytes at 37 °C and 4 °C and B erythrocytes at 4 °C on the contrary to 37 °C (type II discrepancy, Bel antigen).

14. The person with A blood group and anti-B antibody adsorbing ability. The studied erythrocytes after adsorption led to the decreased agglutination of group B erythrocytes by anti-A, B serum (from 1:32 to 1:16 titre). The studied serum did not agglutinate group A erythrocytes at 4 °C and 37 °C, however weak agglutination was observed with group B erythrocytes at 4 °C and 37 °C. The serum in 1:2-1:4 titre inhibited agglutination of group A erythrocytes by anti-A, B serum (weak Bel antigen).

15. The person with A blood group and anti-B antibody adsorbing ability. The studied erythrocytes after adsorption led to the decreased agglutination of group B erythrocytes by anti-B serum (from 1:32 to 1:16 titre). Citrated anti-B plasma agglutinated the studied erythrocytes at 37 °C after 1 hour of contact, however did not agglutinate at 4 °C. The studied citrated plasma

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agglutinated group A erythrocytes at 4 °C and 37 °C (with decrease of their quantity) and group B erythrocytes at 4 °C and 37 °C (with decrease of their quantity) (type III discrepancy, antigen B_3).

16. The person with A blood group. The studied erythrocytes after adsorption did not lead to decrease of agglutination of group B erythrocytes by anti-B serum. Anti-B serum did not agglutinate the studied erythrocytes at 37 °C after 1 hour contact. The studied citrated plasma agglutinated group A and B erythrocytes at 37 °C and 4 °C (with decrease of their quantity) (Bel antigen, type II discrepancy).

Thus, persons of A blood group and presence of anti-A antibodies reactive at 37 °C or 4 °C (II type of discrepancy, presence of B_3, Bm and Bel antigens) showed the ability of the erythrocytes to adsorb anti-B antibody. The persons with weak B antigen (type II discrepancy) demonstrated ability of the sera to inhibit haemagglutination of specific sera. The persons with type III discrepancy demonstrated haemolysing activity of the serum towards A and B erythrocyte antigen.

The persons with O blood group type (weak Bel antigen)

17. The elution with saline and serum from AB group after adsorption of O blood group erythrocytes with anti-B antibody adsorbing ability showed the presence of anti-B antibodies (presence of weak Bel antigen, II type of discrepancy).

18. The sample of O blood group with anti-B antibody adsorbing ability. The studied erythrocytes after adsorption led to the decreased agglutination of group B erythrocytes by anti-A, B serum (from 1:32 to 1:16 titre) (presence of weak Bel antigen, II type of discrepancy).

The studied serum after 30 minutes of contact did not inhibit agglutination of group A erythrocytes by anti-A, B serum (in 2:20 dilution) at 4 °C. Anti-A serum led to the weak agglutination of the studied erythrocytes at 4 °C after 30 minutes of contact with further centrifugation. The studied serum agglutinated group A erythrocytes at 4 °C and 37 °C (with decrease of their quantity) and group B erythrocytes at 4 °C and 37 °C (without decrease of their quantity).

19. The serum from O blood group did not inhibit the agglutination of group A erythrocytes by anti-A, B serum. O blood group was determined.

20. The serum of O blood group (as well as the heated serum) did not inhibit agglutination of group A erythrocytes by anti-A, B serum. O blood group was determined.

The described cases of O blood group with anti-B antibody adsorbing ability of erythrocytes (weak Bel antigen, II type of discrepancy) demonstrated the ability of the studied serum to inhibit haemagglutination of erythrocytes by specific antisera and presence of anti-B non-haemolysing antibodies.

The cases of B blood group persons with unexpected anti-B antibodies reactive at 4 °C or 37 °C (type II discrepancy, Ax antigen)

21. The person with B blood group and anti-A antibody adsorbing ability. The studied erythrocytes after adsorption led to the decreased expression of agglutination of group A erythrocytes by anti-A, B serum in 1:32 titre. Elution after adsorption of the studied erythrocytes with anti-A, B serum did not reveal the presence of anti-A antibodies. Ax antigen was determined. The citrated plasma agglutinated group B erythrocytes at 37 °C on the contrary to 4 °C.

22. The person with B blood group and anti-A antibody adsorbing ability. The studied erythrocytes after adsorption led to the decreased expression of agglutination of group A erythrocytes by anti-A, B serum in 1:32 titre. The studied serum led to the weak agglutination of group B erythrocytes after 30 minutes of contact at 4 °C, with no agglutination at 37 °C after 12 hours of contact. The studied serum agglutinated group B erythrocytes at 4 °C, group A erythrocytes at 4 °C and 37 °C (with decrease of their quantity). The studied serum did not inhibit agglutination of group B erythrocytes by anti-B serum (in 2:20 dilution). Ax antigen was determined.

23. Group B erythrocytes with anti-A antibody adsorbing ability. The studied serum in 1:2-1:4 titre after 15 minutes of contact inhibited agglutinating ability of anti-A, B serum with group A and B erythrocytes at 8 °C, as well as agglutination of group B erythrocytes by anti-B citrated plasma. The studied serum agglutinated group A erythrocytes at 4 °C and 37 °C after 12 hours of contact (without decrease of the quantity of erythrocytes) and group B erythrocytes at 37 °C and 4 °C (weakly). Ax antigen was determined.

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An interesting ability of the sera of the persons with A blood group and anti-B antibody adsorbing ability to inhibit agglutination of group A and B erythrocytes by anti-A, B serum was noted. The agglutination of group B erythrocytes by anti-A, B serum, previously contacted for 30 minutes with serum from A blood group person with anti-B antibody adsorbing ability was absent after adding the studied serum in 1:2 titre, however was demonstrated in 1:32 titre (Figures 2-5).

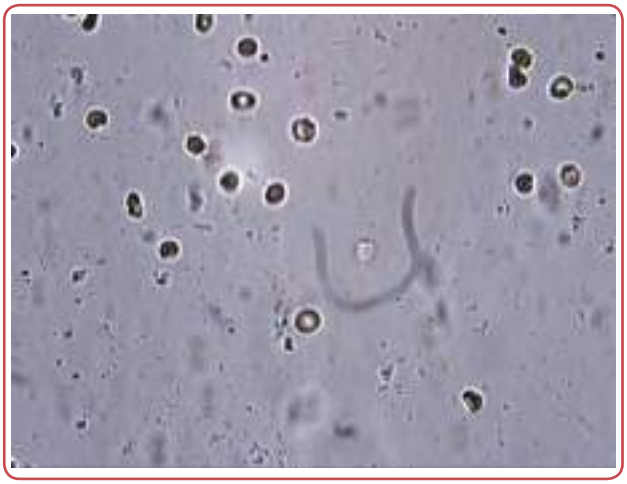

Figure 2: Group B erythrocytes with anti-A, B serum after the contact with serum from A blood group person with anti-B antibody absorbing ability in 1:2 titre (no agglutination)

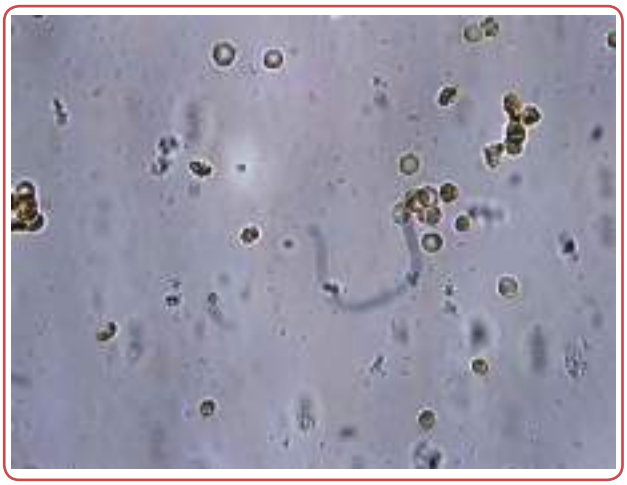

Figure 3: Group B erythrocytes with anti-A, B serum after the contact with serum from A blood group person with anti-B antibody absorbing ability in 1:4 titre

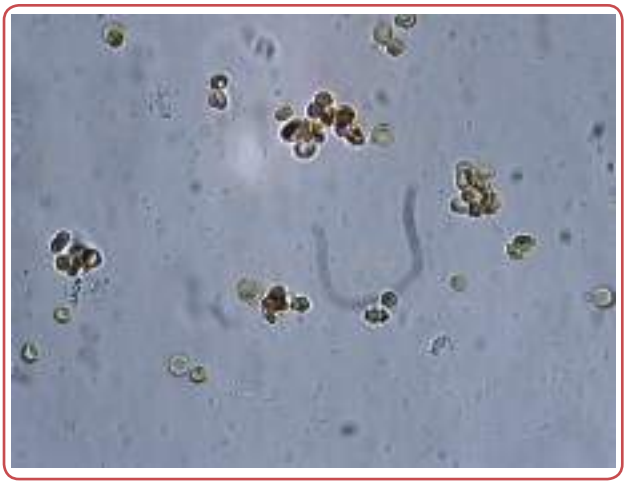

Figure 4: Group B erythrocytes with anti-A, B serum after the contact with serum from A blood group person with anti-B antibody absorbing ability in 1:8 titre

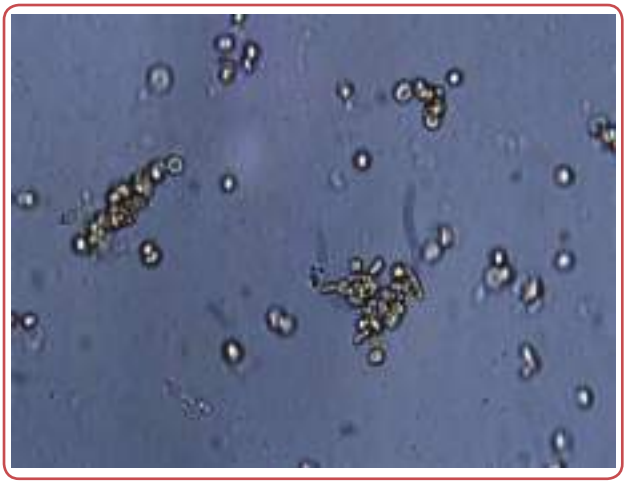

Figure 5: Group B erythrocytes with anti-A, B serum after the contact with serum from A blood group person with anti-B antibody absorbing ability in 1:32 titre (presence of agglutination)

24. The person with B blood group and anti-A antibody adsorbing ability. The studied erythrocytes after adsorption decreased the expression of agglutination of group A erythrocytes by anti-A, B serum (in 1:32 titre: from 2+ to 1+). Liquid chromatography of erythrocytes' sample revealed the presence of N-acetyl-D-galactosamine in 0.056 mg/mL concentration (Figures 6, 7). Importantly, the concentration of group A antigen correlated with the degree of haemagglutination inhibition (r = 0.32) (presence of weak antigen). II type of discrepancy with Ax antigen was determined.

25. The person with B blood group and anti-A antibody adsorbing ability demonstrated the presence of anti-B antibodies reactive at 37 °C.

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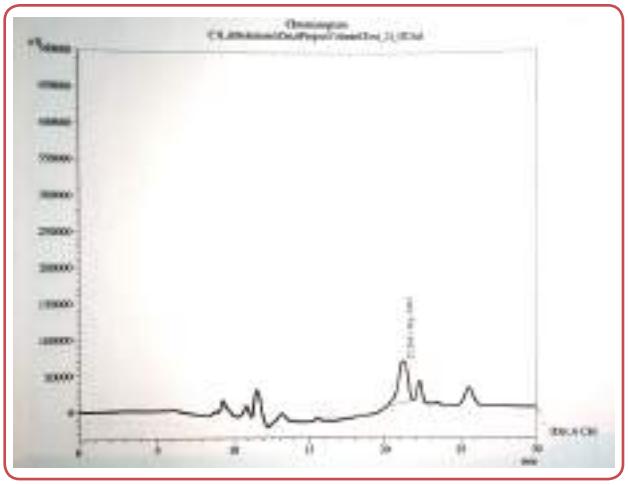

Figure 6: Chromatogram of the person with B blood group and weak Ax antigen

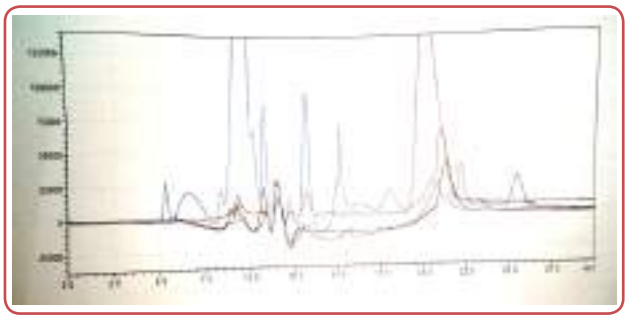

Figure 7: Chromatograms of the persons with A blood group and weak group Ax antigen

The studied erythrocytes after adsorption with anti-A, B serum at 4 °C for 12 hours decreased the titre of the serum (from 1:32 to 1:16) (type II discrepancy). The erythrocytes' sample showed presence of N-acetyl-D-galactosamine in 0.051 mg/mL concentration by liquid chromatography (Figure 7). The person's EDTA plasma inhibited agglutination of group A and B erythrocytes by anti-A, B serum. Ax antigen was determined.

Thus, the studied B blood group type persons with presence of serum anti-B antibodies reactive at 4 °C or 37 °C (type II discrepancy, Ax antigen) showed an ability of erythrocytes to adsorb anti-A antibody.

Discussion

The discrepancies in ABO typing are the reasons to investigate the mechanism of transfusion reactions. These discrepancies can be avoided through the analysis of the methods for the blood typing. The study demonstrated 20 cases of blood group discrepancy type II and 2 cases of blood group discrepancy type III. The persons showing unexpected antibodies in the serum demonstrated the presence of weak subgroup of A or B antigen on erythrocytes. The person's erythrocytes were analysed by commercial antisera for blood grouping, whereas adsorption studies were performed with polyclonal sera. The data of forward (known antisera) and reverse (known antigen) typing were not complimentary. The characteristics of the studied sera were explored with test erythrocytes of group A and B at 4 °C and 37 °C. Unexpected antibodies showed activity at 4 °C and 37 °C.

Thus, a person with B_3 antigen, 7 persons with Bm and 7 persons with Bel antigen and 5 persons with Ax antigen were described. Two persons of O blood group and Bel antigen were revealed. The analysis demonstrated the presence of unexpected antibodies at prolonged incubation period (12 hours) at 4 °C and 37 °C with the presence of adsorbing group specific antigens by adsorption with polyclonal sera. This demonstrates the necessity to perform blood group typing in type II-III of discrepancy with prolonged incubation time both at 4 °C and 37 °C and importance of the use of polyclonal sera for adsorption reaction to avoid mistyping. Activity of the antibodies at 37 °C indicated the presence of IgG antibodies and in all studied samples we used prolonged incubation at 4 °C and 37 °C.

The unusual ability of the studied A and B blood group persons to agglutinate erythrocytes of the same specificity was determined at the incubation at 4 °C and at 37 °C. The studied erythrocytes showed additional ability to adsorb anti-A and anti-B antibodies at prolonged incubation at cold temperature (4 °C). Group II ABO discrepancy among the studied persons was the predominant type.

The described observations have been reported in various studies. The discrepancies are known to be divided into four major types. Type I discrepancies are revealed in reverse typing due to weakly reacting or missing antibodies. Type II discrepancies are observed in forward grouping due to weakly reacting or missing antigens. Type III discrepancy is observed due to excess plasma proteins. Type IV discrepancies were demonstrated in miscellaneous causes like cold auto-antibodies, cold alloantibodies and Bombay

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phenotype. Type III discrepancy were observed in persons with Hodgkin lymphoma, cord blood and plasma exchange with dextran or polyvinyl pyrrolidone.

In plasma cell myeloma the discrepancy was observed due to the loss of isoagglutinin. B blood group was determined, however anti-A antibodies were absent in serum.[20] Washing erythrocytes with saline has been found to resolve discrepancy.[21, 22] A case of a person with O blood group and weak anti-A1 as compared to anti-B antibodies was reported.[23] The case of the person with B blood group without anti-A antibodies in the serum due to the low level of gamma-globulin has been described.[19, 24]

Type II discrepancy was reported, when anti-B antibodies were revealed by serum grouping, however erythrocytes were not agglutinated by anti-A antibody at room temperature and 37 °C, nevertheless erythrocytes were agglutinated by anti-A1 lectin.[20, 21] Similarly, the researchers reported of a sample, that on cell typing demonstrated O blood group type, whereas the serum contained anti-A antibodies with weak anti-B antibodies revealed at 4 °C. ABO genotyping showed an O/O genotype and the serum showed reduced levels of IgG and IgM.[19] Group II ABO discrepancy was found in stage IV Hodgkin lymphoma person. The person showed AB Rh positive blood group, however there was weak (1+) reaction with anti-A antibody and anti-A1 lectin. The sample was incubated at 40 °C for 30 minutes and 3+ reaction was revealed.

The researchers reported of the person of A blood group, however the serum agglutinated group A, B and O erythrocytes (type III discrepancy).[25]

Group B positive sample and unexpected agglutination of group B erythrocytes (Ewing sarcoma)[6] after recent group O Rh positive whole blood transfusion from relative was demonstrated. The antibodies transfused with group O plasma were responsible for the spurious result and both IgG and IgM anti-A and anti-B antibodies were detected.

The microtube column agglutination technique using anti-IgG and anti-C3d helped to find additional group B antigen in A group type: AB+ (the forward type: A cell 4+, B cell 2+; the reverse type: absence of anti-A and anti-B antibodies). The erythrocytes were negative for both Coombs test and irregular antibody screening.[20, 22]

Although blood typing techniques have been well described, occurrence of weak variants of erythrocyte antigens causes enigma for transfusiologist. The detection of weak antigens has been reported to be achieved by increasing the time of incubation, modifying the temperature of reaction, processing of erythrocytes with enzymes (stronger interaction of group A and B antigens with antibodies), molecular analysis, adsorption-elution methods.[14, 24, 25]

Usually ABO discrepancies in forward and reverse typing are revealed at room temperature. Nevertheless, the authors underlined the necessity of using different temperatures in blood typing.

Warm auto-antibodies directed against person's antigens may complicate the identification of erythrocyte allo-antibodies (type IV discrepancy).[26] The person demonstrated O blood group type and presence of anti-A antibodies at room temperature typing, whereas forward typing at cold temperature revealed the presence of weak anti-B antibodies.[27]

In accordance with these studies, unexpected antibodies was found in the sera of the persons with ABO discrepancies active at 37 °C and 4 °C on reverse typing.

The conducted study revealed, the serum of the persons with A blood group and presence of weak subgroup of B antigen agglutinated group A erythrocytes at 37 °C and 4 °C after 12 hours of contact. The contact of the studied sera (containing unexpected anti-A antibodies) with group A erythrocytes at 37 °C did not lead to their decreased quantity on the contrary to the usual serum from O blood group (containing usual anti-A antibodies), able to haemolyse group A erythrocytes. Moreover, the sera of the studied persons agglutinated group B erythrocytes at 4 °C and 37 °C, decreasing their quantity at 37 °C. Therefore, in persons of group A with B subgroup, II type of ABO blood group discrepancy, anti-A non-haemolysing and anti-B haemolysing antibodies were revealed. Thus, persons with type II discrepancy should be investigated on adsorbing ability of erythrocytes and agglutinating and haemolysing ability of the sera at 4 °C and 37 °C at prolonged incubation with erythrocytes. The studied sera are recommended to be analysed on agglutination inhibiting ability with other sera.

In II type of discrepancy of the persons with B

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blood group and anti-A antibody adsorbing ability the ability of the sera to agglutinate group B erythrocytes at 37 °C and 4 °C after 12 hours of contact was revealed.

These findings indicate the importance to perform prolonged incubation at reverse typing at 4 °C and 37 °C in persons with B_3, Bm, Bel and Ax antigen (II and III types of ABO discrepancy). The clinical significance of unexpected agglutination at 37 °C by the studied sera deserves attention in transfusion therapy.

In addition, the sera of the studied persons with ABO discrepancies showed an ability to inhibit haemagglutination of erythrocytes by specific antibodies. Thus, the sera from A blood type with presence of anti-A antibodies in 1:2-1:4 titre showed the ability to inhibit agglutination of group A erythrocytes under the influence of anti-A, B serum. The studied sera of group B persons with anti-A antibody adsorbing ability (Ax antigen) in 1:2-1:4 titre decreased agglutination of group B erythrocytes under the influence of anti-A, B serum.

This observation was mentioned by Brazilian scientists. Thus, Subramanian et al concluded that rarely patients with carcinoma and lymphoma can develop excess serum blood group substances, able to deactivate the typing antisera.[28, 29]

Since the pretransplant antibody removal and standard immunosuppressive drug medicine as the only additional pretreatment was postulated to be insufficient when using group A1 and B donors,[30] the use of donor plasma able to inhibit agglutination and haemolysis of erythrocytes (as was revealed in the present study) might be important in ABO incompatible transplantation. Whether the use of the plasma of the donors with type II-III of ABO discrepancy will increase the long-term graft survival in ABO incompatible transplantation remains to be shown.

The use of the plasma of the persons with blood group discrepancies able to inhibit haemagglutination of erythrocytes by anti-A and anti-B antibodies (from the persons with blood group discrepancies) might be important for the treatment of autoimmune pathology, especially in autoimmune haemolytic anaemia, when the auto-antibodies of the person's serum react with all normal erythrocytes.

Blood group antigens are known as an expression of human individuality and in pathological conditions a reduction of the expression of group A and B antigens is accompanied by an increase of the expression of precursor molecules.[14, 28, 29] Type III discrepancies were found in persons with modification of blood group type: a person of O blood group with anti-A (1:8 titre) and anti-B antibodies (1:4 titre) after allogeneic group AB transplantation for juvenile myelomonocytic leukaemia showed modification of blood group type: the forward typing demonstrated conversion to AB blood group (anti-A and anti-B reagents showed weak mixed-field agglutination).[17, 30] Type III discrepancy was observed in persons with Hodgkin lymphoma, cord blood and plasma exchange with dextran or polyvinyl pyrrolidone.

After receiving a liver transplant from B group in the forward typing the recipient demonstrated AB, Rh (D) positive blood type, however reverse typing demonstrated group O with anti-E antibodies.[31]

The investigators reported, erythrocytes of A blood type person showed lost group A antigen and were agglutinated by anti-H lectin. Anti-H IgM cold autoantibody agglutinated group O adult cells, however not cord blood cells, only a weak agglutination was noted with group A erythrocytes. Liver transplantation resulted in appearance of ABO discrepancy.[32]

Heal and coworkers have noted, transfusion of plasma across ABH molecules can lead to generation of immune complexes of anti-A and anti-B antibodies with plasma glycoproteins or glycolipids bearing group A and B antigens.[33-35] These can bind to both transfused cells and cells of the recipient. The fact that glycoproteins IIIa, Ia/IIa, IV, PECAM and Ib are constitutively expressed in tissues, other than megakaryocytes (in endothelium) could have implications for transplantation of bone marrow, where ABH compatibility between donor and recipient is not usually observed.[36]

The studied cases of type II-III of ABO discrepancy in forward and reverse typing were associated with additional ability of erythrocytes to adsorb other specificity of antibody as revealed by prolonged incubation at 4 °C temperature. The use of 4 °C and 37 °C temperatures while reverse blood typing increased the possibility of revealing these cases. Methods of inhibition of aggluti-

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nation revealed the agglutination inhibiting ability of the serum of the studied persons with type II ABO discrepancy, that might be useful for the management of autoimmune haemolytic disorders. Liquid chromatography may help to reveal the specific erythrocyte antigen.

Conclusion

Identification of adsorbing erythrocyte antigens is important since persons might be mistyped and show decreased survival due to presence of unexpected anti-A and anti-B antibodies.

Investigation of the sera for anti-A and anti-B antibodies at 37 °C and 4 °C with 12 hours of incubation with test erythrocytes increased the possibility of detection of type II-III discrepancy. Adsorption of erythrocytes with polyclonal serum at 4 °C helped to reveal weak erythrocyte antigen and further heat elution allowed to obtain specific antibodies. Inhibition of haemagglutination helped to reveal agglutination inhibiting ability of the serum in persons with type II-III of discrepancy, whereas liquid chromatography allowed to determine the presence of erythrocyte antigen.

Acknowledgement

None.

Conflict of interest

None.

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CASE REPORT

Rare Benign Median Nerve Angiogenetic Lipofibromatous Hamartoma: A Case Report

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Abstract

Only a few cases of lipofibromatous hamartoma (LFH) of the median nerve have been described in the literature. LFH is a rare and low growing benign fibro-fatty tumour. It is characterised by the proliferation of mature adipocytes within the epineurium and the perineurium of the peripheral nerves. The median nerve is most frequently affected in the upper extremities. Carpal tunnel syndrome, paraesthesia, numbness and pain are frequently the results of involvement of the median nerve. In presented case, in addition to fibrolipoma, there was also new blood vessel formation noted, which was attributed to the chronicity of the lesion. Diagnosis was missed by ultrasound and MRI due to the presence of blood vessels. Histopathological diagnosis was confirmed. Surgical treatment with carpal tunnel release and neurolysis were performed. This resulted in favourable outcome in 3 months.

Key words: Lipofibromatous hamartoma (LFH); Median nerve; Angiogenesis; Carpal tunnel syndrome; Short tau inversion recovery (STIR).

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Introduction

The condition known as lipofibromatous hamartoma (LFH) is characterised by the widespread infiltration of peripheral nerves by infrequent fibrous and adipose tissues. Emmett referred to this tissue development as a hamartoma.[1] Although the specific cause of LFH is still unclear, often proposed aetiology include congenital malformation and trauma.[2] The median nerve is primarily affected by LFH in 80 % of cases, however studies have also included the buccinators, sciatic, plantar, superficial peroneal and posterior interosseous nerves associated with nerve territory overgrowth of bone and soft tissue accounting for 62 % of cases. It was found that the female-to-male ratio was 2:1 in cases with macrodactyly and 1:1 in those without. The majority of these incidences are in infants, while children and teenagers have them less frequently.[3] Patients frequently exhibit symptoms of carpal tunnel syndrome and nerve compression associated with an expanding mass along the median nerve territory in the area between the distal forearm and fingers. Although no exact guidelines have been established for the diagnosis and treatment of LFH, asymptomatic patients may be monitored or treated prophylactically with a carpal tunnel release, either with or without neurolysis, in the case of large tumours.

Case history

Written informed consent was taken from the patient. A 31-year-old female patient complained

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CONTENT

of right wrist swelling for the last seven years, discomfort for the past six months and paraesthesia in the thumb, index and middle finger for the past three months. The swelling, which started off small in size, steadily grew until it covered the whole distal third of the right forearm's flexor area. Her daily tasks were challenging due to the dull aching pain, which was mild to moderate and steadily deteriorated.

When palpated, the swelling was widespread and reached the distal portion of the forearm from the palmar crease (Figure 1). Swelling ranged in consistency from soft to firm. It wasn't reducible or compressible. The swelling was pulsatile without affecting any vessels. The patient's first three digits also showed motor impairment and sensory loss. Additionally, there was muscle atrophy in the thenar and hypothenar areas. Carpal tunnel syndrome was clinically diagnosed. Ultrasound imaging indicated a soft tissue mass with many blood vessels entangling the median nerve and compressing it. An X-ray of the right wrist and hand revealed a soft tissue enlargement without any bone abnormalities. Nerve conduction tests were not done. According to an ultrasound scan, the median nerve was being compressed by an arteriovenous malformation.

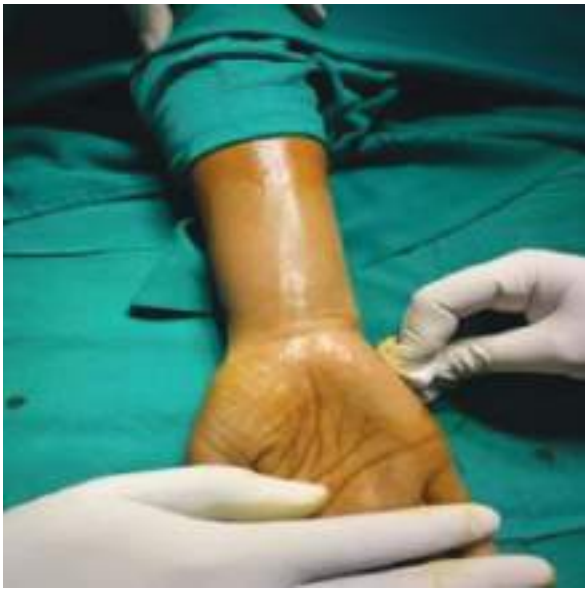

Figure 1: Diffuse swelling over the flexor aspect of the right wrist, just below the palmar crease extending up to distal third of forearm

Magnetic resonance imaging (MRI) study of the wrist joint revealed thickened and enlarged segment of the median nerve at the level of the wrist joint with a maintained fibrillar pattern. Fat content was seen in the lesion on T1 and T2 sequences. MRI scan on sagittal images showed a typical "spaghetti-like" appearance of the thickened median nerve at the level of the wrist (Figure 2: A, B).

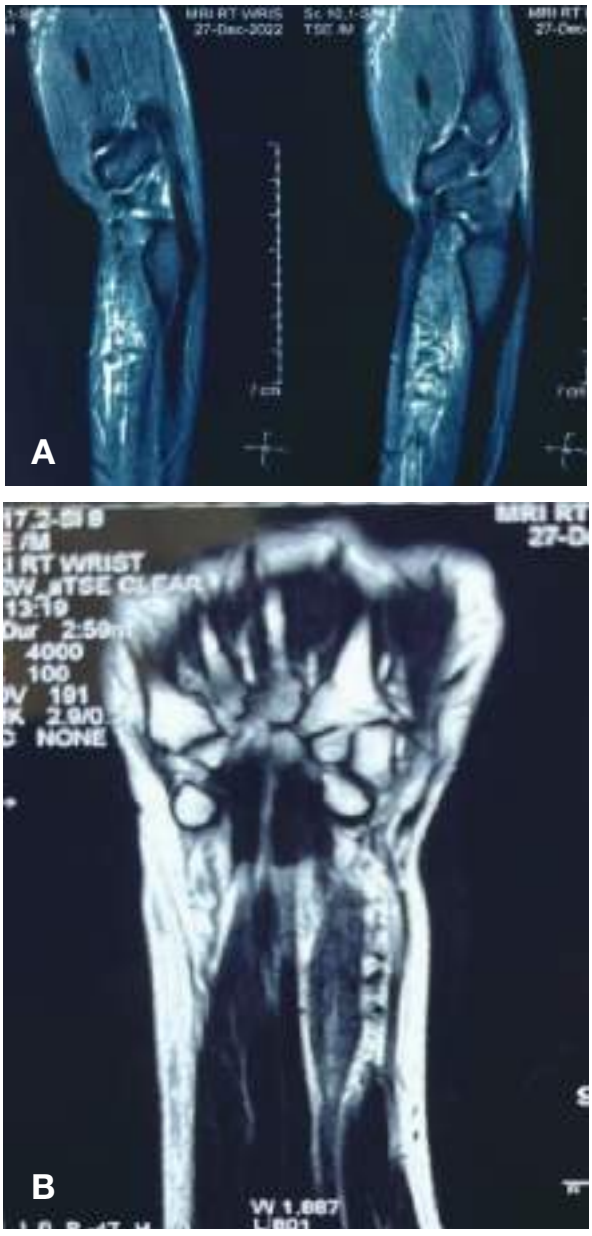

Figure 2 (A, B): Magnetic resonance imaging (MRI) scan. Sagittal short tau inversion recovery (STIR) images showing typical "spaghetti-like" appearance of the thickened median nerve at the wrist level. Surrounding oedema was seen due to pressure effects

MRI scan on axial short tau inversion recovery (STIR) images showed a "cable-like" appearance of the nerve fibres at level of the wrist with the thickened nerve and maintained fibrillar pattern. Surrounding oedema was seen due to pressure effects. MRI scan of T1 axial images showed fibrofatty intensity within the enlarged thickened nerve (Figure 3: A, B).

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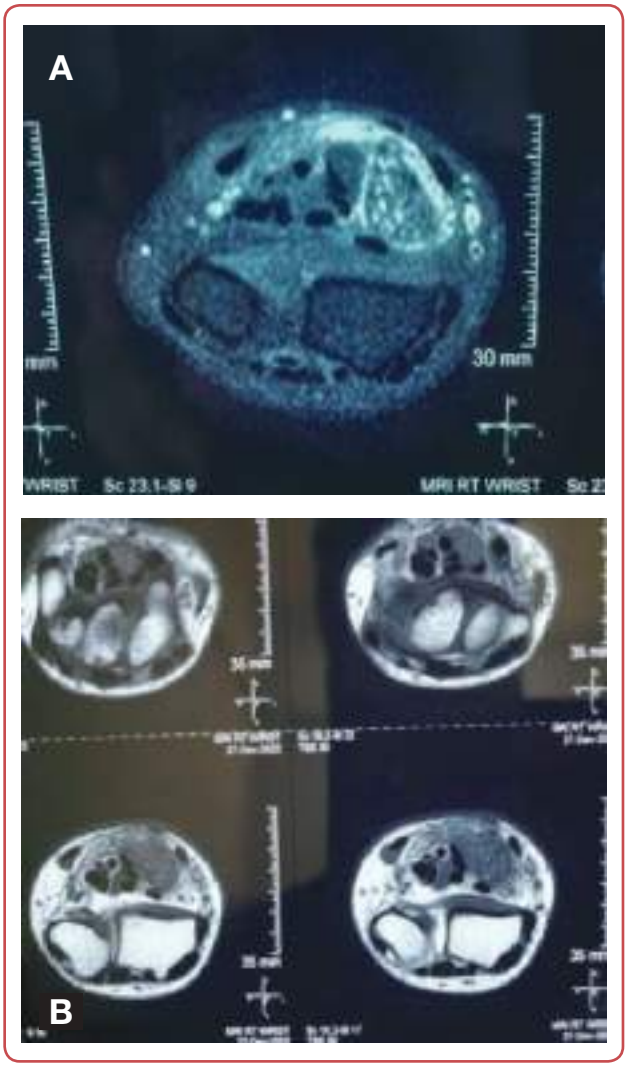

Figure 3 (A, B): Magnetic resonance imaging (MRI) scan. Axial sagittal short tau inversion recovery (STIR) images showing "cable-like" appearance of the nerve fibres at level of the wrist with the thickened nerve and maintained fibrillar pattern. Surrounding oedema was seen due to pressure effects. MRI scan of T1 axial images showing fibrofatty intensity within the enlarged thickened nerve

Based on the symptoms and investigations, a tentative diagnosis of "space occupying lesion causing severe carpal tunnel syndrome of right median nerve" was determined. Carpal tunnel release surgery was scheduled under general anaesthesia, placed tourniquet on the arm. The skin was cut off to remove the swelling. The distal section of the median nerve was discovered to be thickened and swollen intraoperatively. Multiple engorged veins were seen entangling the nerve (Figure 4A). After the tumour was debulked, the thickened median nerve was subjected to fascicular neurolysis (Figure 4B). It was decided to send a tissue biopsy for histological analysis. The histological analysis indicated mature adipose tissue and fibro-collagenous tissue infiltrating interlacing fas-

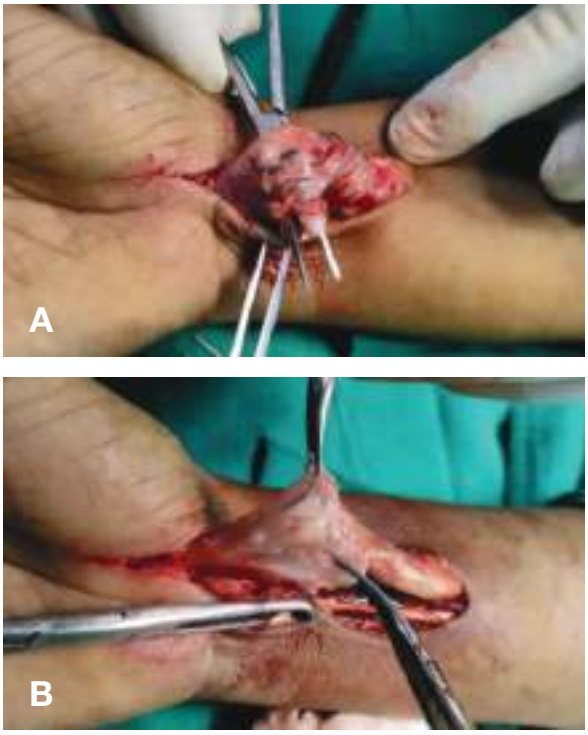

Figure 4 (A, B): Intra-operative view of the enlarged right median nerve with fibrofatty proliferation and a cluster of entangled blood vessels and intra-operative view of neurolysis of the right median nerve

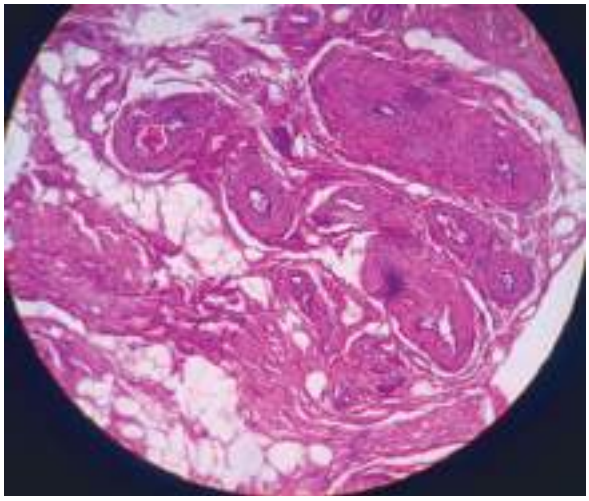

Figure 5: Histopathology of the tissue biopsy showed interlacing nerve fascicles infiltrated by adipose tissue and fibrous tissue. Numerous thickened blood vessels were seen

cicles of nerve bundles in extra neuronal diffuse LFH. Additionally, several thickened blood vessels were seen (Figure 5). This supported the diagnosis of angiogenetic LFH of the median nerve. After a three months of following up, patient restored the sensory and muscular strength in the right thumb, index and middle finger.

CONTENT

Discussion

A rare and benign fibro-fatty tumour called a LFH is characterised by the growth of mature adipocytes inside the peripheral nerves epineurium and perineurium. Although the exact aetiology of LFH is unknown, the great majority of the cases involve children, suggesting that it likely has a congenital origin.[4] Men are frequently affected to a greater extent than women. Macrodactyly is more common in females.[5] In the third or fourth decade, symptoms often start to appear. MRI can be used to differentiate between plexiform neurofibroma and LFH as the findings from MRI indicate neuronal growth instead of fat. Both of the tumours might also differ in histology: although tumefaction is associated with neurofibromatosis, the formation of fibrofatty tissue that penetrates between nerve fibres is the main cause of LFH.

Radiography, ultrasound and MRI are some of the frequently utilised imaging modalities. An X-ray of the afflicted region may reveal soft tissue oedema. There may also be osteoarthritic alterations and bone enlargement in LFH. A fusiform tumour is seen on ultrasound as having longitudinal nerve bundles and alternating bands of hypoechoic and hyperechoic tissue. On the coronal section of the LFH MRI, the median nerve is enlarged in a fusiform or hourglass-shaped pattern and transverse section images reveal displacement of the flexor retinaculum. MRI appearances for LFH of the median nerve include "coaxial cable-like" on an axial plane and "spaghetti-like" on coronal or sagittal slices.[6,7] The flexor retinaculum and tendons are displaced and a large bulging mass in the carpal tunnel is shown on computed tomography (CT). The density of the bulk reveals fibroblastic elements.

Both the nerve conduction studies (NCS) and the electromyogram (EMG) of the median nerve exhibit abnormal results, such as reduced sensory and motor conduction, fibrillations in the muscles of the extremities, signs of chronic denervation and findings consistent with compressive neuropathy. Examining the tissue's histology reveals collagen interlacing, fibroblasts, mature adipocytes and sporadic capillaries that split nerve fascicles and colonise the region between the epineurium and the perineurium. No signs of myelin degradation, inflammation or abnormal looking nerve fibres exist. Routine nerve biopsy is not advised due to the possibility of functional impairment.

MRI provides pathognomonic characteristics that enable reliable lesion identification without requiring extra invasive procedures. Malignancy-suggestive traits may include an aggressively expanding firm mass and invasion of nearby structures. Malignant peripheral nerve sheath tumours are suspected when there are indications of central haemorrhage or necrosis, which show up as inhomogeneous intensity on an MRI.

The four main treatment objectives for LFH with or without macrodactyly are function improvement, aesthetics, symptom prevention and symptomatic relief. There is no known treatment for LFH of the median nerve and the treatment of patients varies from case to case. Full tumour excision is traditionally carried out to completely remove the possibility of malignancy.[8] Patients frequently have painful neuromas along with debilitating deficiencies in sensations and powers. There is no importance for medical care management. Several authors described the neural sheaths decomposition and debulking.[9,10] Elbayer et al described median nerve carpal tunnel release plus covering the median nerve with dermal substitute and pronator quadratus flaps, an effective new modality of successful surgical management for LFH.[10]

Conclusion

Angiogenetic LFH in the median nerve is extremely rare. Observation or prophylactic carpal tunnel release is recommended for big tumours in asymptomatic patients. Carpal tunnel decompression with or without neurolysis is one of the neurological indications.

Acknowledgement

None.

Conflict of interest

None.

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LETTER TO THE EDITOR

Health Professionals and War

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World has been engulfed in the flames of two military conflicts and they may spread. There are only a few who try to extinguish those and force the warring parties to negotiate. Many countries help one side or the other because they hope to benefit if they are on the winning side. Can medicine help to bring the participants to the peace talks? Here is what I did as a refugee and a medical doctor, due to the war in Bosnia and Herzegovina which lasted from March 1992 to December 1995. Some 140,000 Muslims, 97,000 Serbs and over 28,000 Croats were killed and more than two million people were displaced.[1]

In 1998, I sent a letter and a few attachments to leading medical journals with the following suggestion: let us consider whether medicine can contribute to preventing war or ending an existing one. I mentioned the words of Rudolph Virchow, a German pathologist and politician, who stated that medicine must participate in political decisions in order to prevent diseases, sufferings and deaths – that was the birth of preventive medicine. Dudley Hrschbach's article "The dolphin oracle" was also attached; this American Nobel laureate points out a plea for communication.[2]
On 1 November 1998, Richard Smith, Editor-in-Chief of the British Medical Journal (BMJ), wrote to me:[3]

"Thank you for your letter and fascinating enclosures. I was particularly interested to read the article on The Dolphin Oracle.

I must admit that my immediate reaction to your letter suggesting that we may try to find a way to prevent it was to think the letter grandiose. But on reflections – and particularly after reading your enclosures – I began to think that perhaps we could do something. After all, I constantly carry Einstein's advice that it is better to light a candle than to live in darkness.

What I would like to do is to discuss with my editorial colleagues whether there is any way that we might contribute to your mission. One of these colleagues is Fiona Godlee, the President of WAME.

We will be meeting on November, 2nd, and I'll get to you as soon as possible after that meeting".

On 9 February 1999 Richard Smith wrote, again: "*The outcome of your letter for us was the enclosed editorial, which we published in our Christmas issue. It was posted - like everything else in the BMJ – on our website (www.bmj.com), and we have had a number of responses".*

Yusuf, Anand and MacQueen published an editorial entitled: "Can medicine prevent war? Imaginative thinking shows that it might".[4] The authors stated that for every combatant killed in war, one non-combatant is also killed directly and 14-15 civilians lose their lives from loss of shelter, food and water or epidemics - and several times these numbers are physically or psychologically wounded. Medical professionals should join others to establish international agreements that would prevent certain weapons from being used,

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in addition to nuclear and biological weapons. They also mentioned Virchow›s words: "Medicine is a social science and politics is nothing but medicine on a grand scale."

Despite the fact that usage of cluster munitions have been banned by over 120 countries, two superpowers Russia and the USA did not sign the treaty and now the USA *via* Ukraine are using them in the Ukraine war. Cluster munitions disperse in dozens or hundreds smaller submunitions across a broad area, the size of a city block. This causes civilian casualties, as well. Some weapons do not explode immediately, but after months or years afterwards, like landmines. The voice of medical associations should be hired in Russia, the USA, Ukraine and Europe against using such deadly weapons.

Russia and Ukraine are both major global producers of grains. They are suppliers of wheat, barley, sunflower oil and other food that developing nations rely on. This war shall damage grain production and export. The Russians already occasionally block navy shipments of Ukrainian grain. How many people shall die of hunger in the poorest countries?

The wars are now burning on a much larger scale than in Bosnia and Herzegovina. Medicine cannot turn its head away from seeing the carnage of soldiers, sufferings of civilians and destructions in these wars. The most dangerous thing is if any of these military conflicts spreads and if Russia or Israel find herself on the verge of defeat, the Russians and Israelis can use atomic weapons. This would be a catastrophe for humanity and preventing that black scenario is the most important current task of health associations, as well as prominent medical individuals.[5]

Doctors and other healthcare workers should influence citizens in the countries involved in war to contribute to the general anti-war mood. That is how medicine can increase the pressure on the sides that participate in war and countries that help to maintain war. Medical professionals can develop "group health mind" and with medical associations turn politicians to the negotiations and peace.

Acknowledgement

None.

Conflict of interest

None.

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