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Editorial Team

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ORIGINAL ARTICLE

CONTENTS

Ag/Cu_2O nanocomposites as an alternative antimicrobial agent for multidrug-resistant bacterial infections

Igor Duzhyi[1], Petro Myronov[1,2], Tetiana Ivakhniuk[1], Roman Pshenychnyi[1], Oleksandr Pylypenko[1], Anatolii Opanasyuk[1], Viktoriia Holubnycha[1], Vladyslav Smiianov[1]

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ABSTRACT

Aim: To investigate the physicochemical properties and antibacterial and antibiofilm activity of nanocomposites Ag/Cu_2O synthesized by the polyol method.

Materials and Methods: The nanocomposites Ag/Cu_2O were synthesized using the polyol method, followed by a study of their physicochemical properties. The antibacterial and antibiofilm properties of the nanocomposites Ag/Cu_2O were investigated against multidrug-resistant clinical strains of bacteria *S. aureus*, *E. coli*, and *P. aeruginosa*.

Results: Nanocomposites Ag/Cu_2O, were mostly spherical in shape and had an average size of 135.6±65 nm. X-ray diffraction analysis confirmed the presence of Ag and Ag/Cu_2O phases. Energy-dispersive spectroscopy revealed the following elemental composition: 13.05% silver, 31.81% copper, and 55.14% oxygen. Ultraviolet-visible spectroscopy suggested the potential presence of silver oxides in the structure. The minimum inhibitory concentration and minimum bactericidal concentration values were as follows: for *S. aureus*, the minimum inhibitory concentration was 52.08±18.04 μg/ml, and the minimum bactericidal concentration – 72.92±47.74 μg/ml; for *P. aeruginosa* – 26.04±9.02 μg/ml, and 41.67±18.04 μg/ml, respectively; and for *E. coli* – 83.33±36.08 μg/ml, and 208.30±72.17 μg/ml, respectively. The nanocomposites Ag/Cu_2O NCs also demonstrated antibiofilm activity, being effective against *S. aureus* at 3 and 5 times the minimum bactericidal concentration, against *E. coli* at 1, 3, and 5 times the minimum bactericidal concentration, and against *P. aeruginosa* at 5 times the minimum bactericidal concentration.

Conclusions: The nanocomposites Ag/Cu_2O revealed high antibacterial and antibiofilm properties, which support their use as an alternative to conventional antibiotics in nanomedicine for treating multidrug-resistant infections.

KEY WORDS: Ag/Cu_2O nanocomposites, bacteria, biofilm, multidrug-resistance

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INTRODUCTION

Multidrug-resistant (MDR) bacteria are often the cause of various ranges of infectious diseases, their complications, and the death of patients [1]. Recently, there has been an exponential increase in the number of bacterial strains with multidrug resistance [2]. Each year, approximately 0.7 million people die from MDR bacteria, and the total number of deaths is projected to reach 10 million in 2025 [3]. In some cases, bacteria are resistant to almost all existing antibiotics [4]. In this regard, the World Health Organization has identified this problem as a major global health threat and called for an improved and coordinated global effort to contain antimicrobial resistance [5]. Even more problematic and difficult to treat are infectious diseases with persistent biofilm forms of MDR bacteria. Biofilm forms of bacteria exhibit a 10- to 1,000-fold increase in antibiotic resistance compared to planktonic forms of the same bacteria [3]. About 80% of chronic and recurrent microbial infections in humans are caused by biofilms of MDR bacteria, which lead to high mortality rates [3].

S. aureus, *E. coli*, and *P. aeruginosa* are ubiquitous bacteria that cause a variety of infectious diseases and are often responsible for hospital-acquired infections. *P. aeruginosa*, in particular, has been recognized as one of the most life-threatening bacteria and included by the World Health Organization in its list of priority pathogens for research and development of new antibiotics [6]. Methicillin-resistant *S. aureus* (MRSA) is a widespread pathogen that causes a spectrum of infections from superficial skin problems to severe conditions such as osteoarticular infections and endocarditis, resulting in high morbidity and mortality [7]. Biofilm production is a key aspect of MRSA's ability to invade, spread, and resist antimicrobial treatments [7]. In recent years, an increase in resistance of *E. coli* has also been noted:

the emergence of hypervirulent carbapenem-resistant strains has been reported [8].

One promising strategy for combating antimicrobial resistance is based on the use of nanostructured metals, the most powerful of which are silver and copper [9, 10]. However, resistance to them has already been noted [11]. The problem of the toxic effects of metal nanoparticles on human cells also remains unresolved, which limits their use in high concentrations [12, 13]. Taking into account the above, it is necessary to direct efforts to the study of new combined nanomaterials based on silver and copper.

AIM

To investigate the physicochemical properties and antibacterial and antibiofilm activity of nanocomposites Ag/Cu_2O (Ag/Cu_2O NCs) synthesized by the polyol method.

MATERIALS AND METHODS

SYNTHESIS OF AG/CU_2O NCS

The synthesis of Ag/Cu_2O NCs was carried out by the polyol method in a diethylene glycol medium. 1.2 g of copper (II) acetate monohydrate and 6.0 g of polyvinylpyrrolidone were dissolved in 50 ml at 50 °C in a 250 ml three-necked round-bottom flask on a flask heater with magnetic stirring for 10 min. The reaction mixture was kept at 220 °C for 1 hour under an argon flow. Then 0.68 g of silver nitrate was added to the reaction mixture cooled to 180 °C and kept for 2 h. After synthesis, the suspension was cooled, and 20 ml of isopropanol and 10 ml of distilled water were added to the flask. The resulting composite was centrifuged at 8000 rpm, followed by washing with distilled water 3 times the collected precipitate and centrifugation. The washed composite was dispersed in 30 ml of distilled water using an ultrasonic bath (Skymen JP-008, China) for 10 min, 40 kHz.

AG/CU_2O NCS CHARACTERIZATION

The morphology of synthesized Ag/Cu_2O NCs was examined by transmission electron microscopy (TEM) (electron microscope "PEM-125K", Ukraine). An X-ray diffraction (XRD) investigation of synthesized materials was carried out on the automated diffractometer DRON 4-07 connected to the computer-aided experiment control and data processing system. Energy dispersive spectroscopy (EDS) and scanning electron microscopy (SEM) of Ag/Cu_2O NCs were carried out on a scanning electron microscope JEOL JSM-6390LV (JEOL Ltd., Japan) with an Oxford INCA X-ray microanalysis detector. Ultraviolet-visible (UV-vis) spectroscopy was measured using a spectrophotometer Cary 4000 (Varian, USA). The concentration of Ag/Cu_2O NCs in the aqua solution was determined by the method of atomic absorption spectroscopy on a C-115-M1 spectrophotometer (NGO "Selmi", Ukraine).

ANTIBACTERIAL ACTIVITY OF THE AG/CU_2O NCS

To evaluate the antibacterial activity of Ag/Cu_2O NCs, MDR clinical isolates of *S. aureus*, *E. coli*, and *P. aeruginosa* were employed. The antibiotic resistance characteristics of the isolates have been previously reported [10]. The antibacterial efficacy of Ag/Cu_2O NCs was assessed by determining their minimum inhibitory concentration (MIC) using the tube serial dilution technique, following the guidelines of the Clinical and Laboratory Standards Institute [14]. Overnight bacterial cultures were adjusted in nutrient medium (HiMedia, India) to achieve a final concentration of 5×10^5 CFU/ml. Subsequently, 0.2 ml of serially diluted Ag/Cu_2O NCs suspensions were mixed with 1.8 ml of the bacterial inoculum, resulting in final concentrations ranging from 1000 to 15.63 µg/ml. Tubes containing only the growth medium and bacterial cultures served as positive controls, while tubes with medium and Ag/Cu_2O NCs acted as negative controls. All tubes were incubated aerobically at 37 °C for 24 h. The MIC was defined as the lowest concentration of Ag/Cu_2O NCs that completely inhibited visible bacterial growth. For determination of the minimum bactericidal concentration (MBC), 100 µl aliquots from each tube were plated on Mueller–Hinton agar (HiMedia, India) and incubated at 37 °C for 24 h. The lowest concentration that resulted in complete (100%) bacterial killing was recorded as the MBC. All experiments were performed in triplicate.

ANTIBIOFILM ACTIVITY OF AG/CU_2O NCS

The antibiofilm activity of Ag/Cu_2O NCs was evaluated by measuring their capacity to reduce biofilm biomass. Overnight bacterial cultures were adjusted to a concentration of 5×10^5 CFU/mL in Mueller–Hinton broth and inoculated into 96-well polystyrene microplates (200 µl per well). The plates were incubated at 37 °C for 72 h to allow biofilm formation. After incubation, the culture medium was carefully removed, and 200 µL of fresh broth containing Ag/Cu_2O NCs at concentrations equivalent to 1×, 3×, and 5× MBC was added to each well. Plates were then incubated for an additional 24 h at 37 °C. Wells containing untreated bacterial cultures served as positive controls, while wells

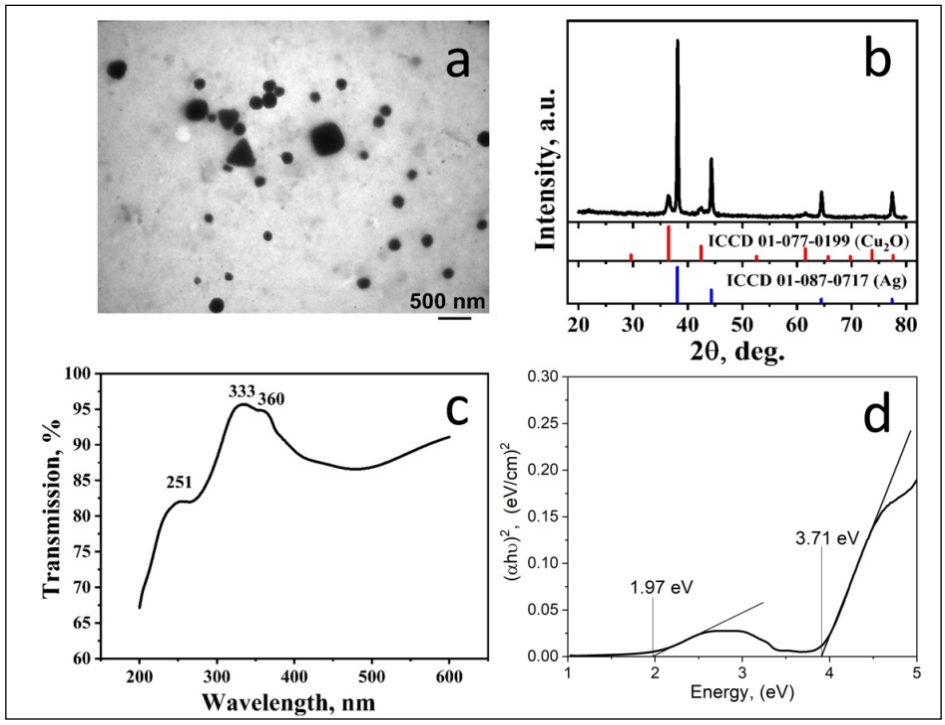

Fig. 1. Physico-chemical characteristics of Ag/Cu2O NCs: a – TEM, b – XRD, c – UV-vis, d – determination of the band gap width
Picture taken by the authors

with Ag/Cu_2O NCs (1×, 3×, and 5× MBC) but no bacteria, and wells with only Mueller–Hinton broth, served as negative and blank controls, respectively. Following treatment, the contents were removed, and the wells were washed three times with 0.9% saline solution. The remaining biofilms were stained with 0.1% (w/v) gentian violet solution. After staining, the wells were rinsed, air-dried, and 200 µl of 96% (v/v) ethanol was added to solubilize the bound dye. The absorbance of each well was measured at 595 nm using a Multiskan FC spectrophotometer (Thermo Fisher Scientific, USA). Each assay was performed six times, and the mean optical density (OD) values were calculated.

STATISTICAL ANALYSIS

MICs and MBCs data are presented as the mean ± standard deviation. In a study of antibiofilm activity, one-way ANOVA with multiple comparisons was used to assess the difference between groups using GraphPad Prism 9.0 software, where a p-value < 0.05 was considered statistically significant.

ETHICS

This article was written in accordance with ethical principles and industry standards.

FRAMEWORK

This work was supported by the National Research Foundation of Ukraine (grant No. 0124U004801); and Ministry of Education and Science of Ukraine (grant No. 0124U000540).

RESULTS

PHYSICO-CHEMICAL CHARACTERISTICS OF AG/CU_2O NCS

Ag/Cu_2O NCs were predominantly round in shape, with an average size of 135.6±65 nm, without a tendency to agglomerate (Fig. 1a). The peaks in the diffractograms according to reference data (JCPDS, card No. 01-077-0199 and 01-087-0717) correspond to the sum of reflections from the Ag and Cu_2O phases. The diffraction peaks at angles 36.41, 42.50, and 61.63 correspond to reflections from the crystallographic planes (111), (200), (220) of Cu_2O, and the peaks at 38.19, 44.37, 64.50, 77.54 correspond to reflections from the planes (111), (200), (220), (311) of Ag (Fig. 1b). UV-vis spectroscopy indicated the presence of peaks at wavelengths 251, 333, and 360 nm (Fig. 1c). To confirm the presence of oxide phases in the suspension, the band gap width of nanomaterials E_g was determined. For this purpose, dependences were constructed in the coordinates $(ahv)^2$ - hv (Fig. 1d). The linear section of these dependences is approximated by a straight line at the point of intersection with the energy axis, which allows the value of E_g to be determined. The suspension contains materials with a band gap E_{g1} = 1.97 eV and E_{g2} = 3.71 eV. The first value of the band gap width corresponds to copper dioxide Cu_2O, which is characterized by reference values of E_g = (2.1-2.6) eV. The second value may be associated with the

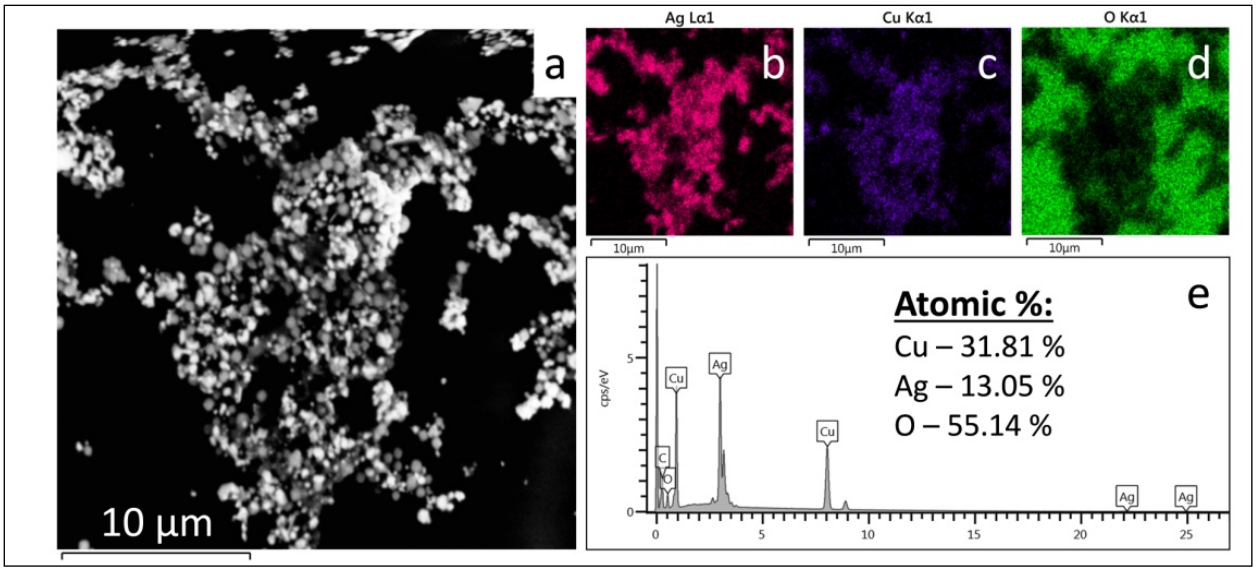

Fig. 2. SEM image (a), EDS (e) and EDS mapping of Ag/Cu20 NCs (b – Ag, c – Cu, d – O)
Picture taken by the authors

presence of silver oxide in the suspension. However, among several oxide phases, the most thermodynamically stable is the Ag_2O phase, which has a band gap width depending on the preparation method, stoichiometry, and oxidation temperature E_g = (1.2 - 3.4) eV. The increased band gap width of this oxide E_{g2} = 3.71 eV can be associated with quantum mechanical effects in the nanostructured state and its doping with copper. However, the content of this phase is relatively small because, unlike the other two, it is not recorded by a diffractometer.

This conclusion is confirmed by the data of determining the chemical composition of the nanomaterial. Fig. 2 shows SEM images of the surface of the film obtained by dripping a suspension of Ag/Cu_2O NCs onto the substrate after drying (Fig. 2a) and the results of mapping its chemical composition (Fig. 2b, 2c, 2d). EDS revealed that the nanomaterial contained 13.05 at. % silver, 31.81 at. % copper, and 55.14 at. % oxygen (Fig. 2e). The ratio of the concentrations of the components $\gamma_1 = C_{Ag}/C_{Cu}$ was 0.41, and $\gamma_2 = (C_{Ag} + C_{Cu})/C_O$ = 0,81. That is, there are no uncontrolled impurities in the material that could form an additional oxide.

ANTIBACTERIAL AND ANTIBIOFILM ACTIVITY OF AG/CU_2O NCS

Ag/Cu_2O NCs demonstrated the strongest antibacterial effect against the MDR *P. aeruginosa* (MIC – 26.04±9.02 µg/ml, and MBC – 41.67±18.04 µg/ml). For MDR *S. aureus*, MIC and MBC of Ag/Cu_2O NCs were higher – 52.08±18.04 µg/ml and 72.92±47.74 µg/ml, respectively. The highest concentration of Ag/Cu_2O NCs was required for the complete killing of MDR *E. coli* (MIC – 83.33±36.08 µg/ml, MBC – 208.30±72.17 µg/ml).

Ag/Cu_2O NCs showed antibiofilm activity against *S. aureus* at 3 and 5 MBC, against *E. coli* at 1, 3, and 5 MBC, and *P. aeruginosa* at 5 MBC (Fig. 3). There was no statistical difference between the antibiofilm activity of Ag/Cu_2O NCs using 1, 3, or 5 MBC, but it was observed compared to positive controls (biofilm growth without Ag/Cu_2O NCs). So, a stable antibiofilm effect was observed against all bacteria only when using 5 MBC of Ag/Cu_2O NCs.

DISCUSSION

Ag/Cu_2O NCs synthesized by the polyol method with a size of 135.6±65 nm showed antibacterial and antibiofilm activity against gram-positive and gram-negative MDR bacteria. Even though the Ag/Cu_2O NCs were most effective against planktonic *P. aeruginosa*, the study of antibiofilm properties showed that biofilms formed by *P. aeruginosa*, compared to other tested bacteria, are more resistant to destruction and require higher, fivefold bactericidal concentrations of Ag/Cu_2O NCs. At the same time, to kill planktonic *E. coli*, a significantly higher concentration of Ag/Cu_2O NCs (208.3±72.17 µg/ml) was initially required, but this concentration was already sufficient to destroy its biofilm. These gram-negative bacteria are particularly important to practicing clinicians and require further detailed research on their interaction with nanometal complexes.

For comparison, core-shell Ag-Cu nanostructures, 20-70 nm in size, prepared by S. F. Sabira et al. showed similar results to those described in the current study: MICs for *S. aureus* and *E. coli* were of 75 µg/ml, and against *P. aeruginosa*, MICs were lower – 20 µg/ml [15]. Significantly worse antibacterial properties were shown

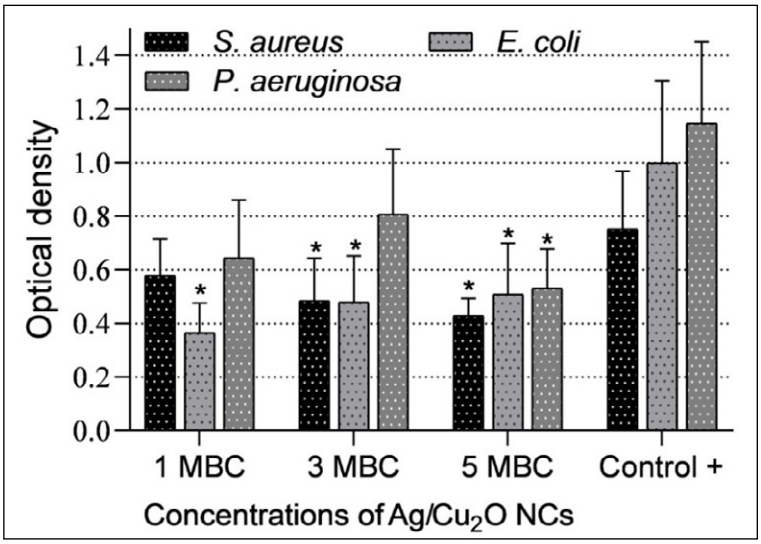

Fig. 3. Antibiofilm activity of Ag/Cu20 NCs against *S. aureus, E. coli, P. aeruginosa*. Control + – positive control. Note. Statistical difference compared to the positive control, $p < 0.05$
Picture taken by the authors

in the study of Ag/Cu nanoparticles (70 and 100 nm in size), where the MICs for *S. aureus* and *E. coli* were > 500 µg/ml, and for *P. aeruginosa* > 125 µg/ml [16]. M. Valodkar et al. reported the successful synthesis of silver-copper nanoparticles (size 20±5 nm) in a ratio of 1/1 and their antibacterial activity: for *S. aureus* the MIC was 0.33 mg/l, MBC – 1.32 mg/l, for *E. coli* the MIC was 0.23 mg/l, MBC – 0.71 mg/l [17]. However, as the authors themselves note, the stability of the solution of these nanoparticles is maintained only for several days. The difference in results can be attributed to various approaches to synthesis, characteristics, different research methods, and different strains of bacteria used.

The antibacterial effect of Ag/Cu_2O NCs is due to both the presence of Ag, which primarily affects the bacterial plasma membrane, and Cu, which can denature nucleic acids and other internal biomolecules and cellular structures. It has also been suggested that the released Cu ions enhance ROS production, leading to DNA damage and lipid peroxidation, which inactivates bacteria [18, 19]. Some authors indicate that Ag^+ ions, which also have a detrimental effect on bacteria, are released in significantly higher quantities in solutions with higher Cu^{2+} ion concentrations [20], explaining the enhanced synergistic antibacterial properties of the nanocomposites.

A more detailed study of the effect of oxide components on the interaction between composites and bacteria, as well as their biofilms, and the determination of the synergistic antibacterial potential of structural components, would be promising.

CONCLUSIONS

Ag/Cu_2O NCs are effective against planktonic and biofilm forms of MDR bacteria *S. aureus*, *E. coli*, and *P. aeruginosa*. Thus, Ag/Cu_2O NCs can be implemented as an alternative to antibiotics in various areas of nanomedicine to combat infections with multiple drug resistance.

COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Optimization and validation of an HPLC method for the quantification of Etodolac in liposomes and assessment of its pharmacokinetic profile

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ABSTRACT

Aim: To develop and evaluate high performance liquid chromatogram method to simultaneously quantify Etodolac in liposomes.

Materials and Methods: The chromatographic separation was carried out on the C18 column, mobile phase of methanol: acetonitrile: water at a ratio of (30:60:10) and a flow rate of (1ml.min^{-1}), with a detection wavelength of 225 nm. Liposomes were prepared by thin film hydrating method using Distearoylphosphatidylcholine and cholesterol.

Results: Linear concentration range of the assay was 2.5-25 µg.ml^{-1} with a regression coefficient factor $R^2 \geq 0.999$ with a retention time of Etodolac 4.11 ± 0.081 min. The method was rigorously validated for linearity, accuracy, precision, specificity, and stability, ensuring its reliability. The concentrations of Etodolac 2.5, 5, 7.5, and 10 µg.ml^{-1} were used, with the intraday n=6 and intraday n=6 precision and accuracy for Etodolac meeting the accepted criteria of the United States Food and Drug Administration guideline. The limited detection and limited quantification values for Etodolac were 0.42 µg.ml^{-1} and 1.3 µg.ml^{-1}, respectively. Conventional liposomes have characterization (PZ 88 ± 0.02, PDI 0.012 ± 0.01, %EE 89.5 ± 0.004, and Z-potential -10.2mV ± 0.001), while PEGylated liposomes (PZ 92 ± 0.003, PDI 0.01 ± 0.02, %EE 94.3% ± 0.01, and Z-potential -13.6mV ± 0.012).

Conclusions: The developed and validated method of HPLC was successfully applied to determine Etodolac in bulk and liposomes for routine quantitative analysis.

KEY WORDS: Etodolac, high performance liquid chromatogram, validation, analytical chemistry

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ABBREVIATIONS

HPLC: High Performance Liquid Chromatogram
BCS: Biopharmaceutical Classification System
DSPC: Distearoylphosphatidylcholine
FDA: Food and Drug Administration
LOQ: limit of quantification
LOD: Limit of Detection
RSD: Relative Standard Deviation
CCD: Central Composite Design
RBF: Round-Bottom Flask
PCS: Photon Correlation Spectroscopy
RSD: Injection Repeatability
k': Capacity Factor
T: Tailing Factor

INTRODUCTION

The bio-analytical method can be used to measure and identify the drug and its metabolite in biological matrices like blood, plasma, serum or urine, while the analytical method is used to determine the quantitative or qualitative of the drug in the sample. The chromatographic method represents a simple and accurate method that permits scientists to separate the closely related components in a complex mixture easily. The chromatographic process can be qualitative, and however, used to identify the active chemical substance, which establishes the amount of these given substances [1]. The Etodolac (ETO) drug belongs to the non-steroidal anti-inflammatory drug (NSAID) [2-3] with chemical formula 1, 8- diethyl-1, 3, 4, 9- tetrahydropyran (4, 4-b) –indole- 1- acetic acid [4-5]. Etodolac is widely used as an antipyretic [6], for patients with severe pain [7] or severe inflammation due to surgery, postoperative analgesia, rheumatoid arthritis, and osteoporosis [4, 8-9]. Etodolac is extensively metabolize in the liver to an inactive oxidative metabolite and a primary elimination renal route. Etodolac is a class II according to the biopharmaceutical classification system (BCS), with poor water solubility with limited dissolution, and poor absorption [10]. The recommended dose is 100-300 mg twice or three

times a day, which makes compliance and convincing for the patients difficult. Etodolac is a selective inhibitor for COX_2, and it has been reported that Etodolac is selective for COX_2 enzyme ten times as that for the COX_1 enzyme. Different methods were used to analyse Etodolac, either alone or in combination with other drugs, as HPLC with ultraviolet detection represents the most popular method used, but it is time-consuming and laborious [8], voltammetric [11], sequential injection analysis, other methods that can be used for the departure of Etodolac and its metabolite but not for determining the quantity like capillary electrophoresis [11-12] and spectrofluorimetric method [13-14] and high-performance thin-layer chromatography [15] furthermore was mention for identified Etodolac in bulk solution and in the pharmaceutical dosage form. In addition to that, several HPLC methods for Etodolac enantiomers and mechanism of depredate also have been reported [13, 16] but a fewer method to identify of Etodolac in liposome. Liposomes are artificial carriers with a spherical shape consisting of cholesterol and nontoxic phospholipid. Liposomes are biocompatible and biodegradable, having one or more phospholipid bilayers. Liposomes are an effective drug delivery system because they have the ability to load with hydrophilic or lipophilic drugs. In addition to that, liposomes can be targeted by a specific ligand to specific cells [17-20]. The aim of this study was a quantitative assay of Etodolac using the HPLC method. The method was developed and validated as a new, simple, sensitive, cost-effective, and accurate HPLC method for quantification of Etodolac as free and loaded in liposome. The method was developed using optimization parameters different from the literature. The analytical method was validated for sensitivity, selectivity, linearity, recovery, accuracy, precision, and stability according to the United States Food and Drug Administration (FDA) and the International Conference on Harmonization Guideline (ICH) [1, 21].

MATERIALS AND METHODS

MATERIALS

Etodolac and cholesterol 99%, were purchased from Shanghai Macklin Biochemical Co. Ltd/China. Phospholipid DSPC and DSPE-PEG were purchased from WEIHUA BIO. Acetonitrile 99%, methanol 99.8%, chloroform 99.8%, and water 100% are HPLC grade purchased from Hi Media Laboratories Pvt Ltd. in India. The used vials were purchased from CKlab in China. The C18 column and guard column were purchased from Novachrom and Wayeal in China.

METHOD

INSTRUMENTATION AND CHROMATOGRAPHIC CONDITIONS OF HPLC

A Knauer AZURA HPLC instrument from Germany was used with a UV-visible detector and a Clarity Chrom software version. The chromatography was performed isocratically at room temperature. The chromatographic separation was carried out using a C18 column, 250 mm × 4.6 mm, 5 µm particle size (Novachrom, Wayeal from China) with core-shell silica. The mobile phase used consisted of A methanol, B acetonitrile, and C water, which were mixed well and degassed by ultra-sonication in a water bath for 15 min using an HPLC pump that can be used to produce a mobile phase with a ratio of (60:30:10) delivered at a flow rate of 1 $ml.min^{-1}$ into the column, an opaque vial size 1.5 ml was used, and the sample size 100 µl put in the tray of the instrument and run. The sample size injection was 20µl, with a thermostat temperature of 30°C and a pressure of 15 MPa. The Clarity Chrome software was used to calculate the area under the peaks. Before each analysis, the column was rinsed with mobile phase at a flow rate of one millilitre per minute for 10 minutes, after each analysis, the column was cleaned with methanol for 5 minutes.

DETERMINATION WAVELENGTH OF DETECTION

In order to determine the wavelength of Etodolac, accurately weight 10 mg of Etodolac in 100 ml of methanol and dilute with a mobile phase to get to 10 µg/ml of Etodolac solution. The solution was scanned 200-400 nm by a UV spectrophotometer (Shimadzu 1700).

PREPARATION OF STOCK AND STANDARD SOLUTIONS

A stock solution of Etodolac was prepared by dissolving an accurate amount of Etodolac 10 mg in 100 of a mobile phases (methanol: acetonitrile: water) at ratio of (60:30:10). The working solution for calibration were prepared from serial dilution for stock solution with mobile phase to yield ten standard solutions 2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20, 22.5 and 25 $\mu g.ml^{-1}$, however, to ensure the accuracy of the processes, its repeated six times within a single day (intraday) and once over six days (intraday); for each repetition, a new stock solution was prepared.

METHOD VALIDATION

The HPLC validation process focus on accuracy, precision, recovery, linearity, stability studies, LOQ and LOD.

These parameters were assessed following guideline established by Food and Drug Administration FDA and the International Conference on Harmonization Guideline (ICH).

SUITABILITY OF HPLC FOR QUANTIFYING ETODOLAC

The HPLC suitability system was determined to study the accuracy and precision of the quant method. It was achieved by measuring the injection repeatability RSD, tailing factor T, capacity factor k', and theoretical plates N to analyse system suitability. The concentration 2.5 $\mu g.ml^{-1}$ of Etodolac dissolved in methanol was used. The optimized chromatogram system should pass the accepted limited value before being used for further studies with the development of the HPLC method.

PRECISION AND ACCURACY OF THE DEVELOPING METHOD OF ETODOLAC DETECTION

Precision is represented as closeness of agreement among a series of measurements. However, this involved running the analyses six times in the same day (intraday) and once day for six days (intraday) at four different concentrations of analyse: 2.5, 5, 7.5 and 10 micrograms per millilitre. It was determined by measuring the percentage of relative standard deviation (% RSD) using the following equation:

$$\%RSD = \frac{\text{Observed Concentration}}{\text{Nominal Concentration}}$$

While accuracy explained as the closeness of agreement among the theoretical and actual value, it was measured by using twelve repeated and determining the % RE (percentage of residual error), according to the following equation:

$$\%R = \frac{\text{Observed Concentration - Nominal Concentration}}{\text{Nominal Concentration}} \times 100$$

The percentage of residual error should not exceed 2%, four standard solutions were used for precision and accuracy analyses: 2.5, 5, 7.5 and 10 $\mu g.ml^{-1}$.

LINEARITY OF HPLC DEVELOPED PROCESS OF ETODOLAC DETECTION

Linearity can be detected depending on the least square method of the calibration curve (peak area as a function of Etodolac concentration) when adding regression. To prepare the calibration curve, ten standard solutions of Etodolac were used in the range 2.5-25 $\mu g.ml^{-1}$.

LOD AND LOQ SPECIFICITY

The LOD and LOQ were detected through HPLC validation to measure the lowest detectable and measurable concentration of Etodolac. The LOD and LOQ can be directly calculated from the calibration plot. The LOD was 3.3 σ/S, while LOQ was 10 σ/S, wherever (σ) represented the standard deviation of the intercept, (S) represented the slope of the calibration plot.

STABILITY AND RECOVERY STUDY FOR ETODOLAC

To study the stability of Etodolac during handling and lab formulation like liposome, the stability test was detected. Etodolac sock solution was tested under three scenarios, which include room temperature for one day (short term), -20 °C for ten days (long term), and freeze-thawing cycle (for 3 times), in which the solution was frozen at -20 °C for one day, thawed at room temperature for one day, and this cycle was repeated 3 times. For evaluation, the recovery of Etodolac from liposome, they compared the amount recovered with standard solution at four concentrations 2.5, 5, 7.5, and 10 $\mu g.ml^{-1}$, repeated six times.

PREPARATION OF LIPOSOME

Liposomes were prepared using the central composite design (CCD) (Design Expert version 13 software) by the thin film hydrating method. In this method, Etodolac (8 mg/ml), DSPC (20 mg), cholesterol (5 mg), or Etodolac (8 mg/ml), DSPC (20 mg), cholesterol (5 mg), and DPSE-PEG (1 mg) were dissolved in 4 ml at a 1:1 ratio of chloroform to methanol to prepare conventional and PEGylated liposomes, respectively. The component was put in a round-bottom flask (RBF) 1000 ml and put in a rotary evaporator (from Heidolph Instruments GmbH & Co. KG, Germany) and set at rotary evaporator 65°C and 100 rpm and allowed the solvent to evaporate, and a thin film was formed in the wall of the RBF and left aside for one hour in a vacuum desiccator to stabilize the film and remove the trace amount of organic solvent. Follow that the thin film was hydrated using phosphate buffer (pH 7.4), and the formation of liposomes was then retained in the rotary evaporator for 10-15 min at 65°C and 100 rpm [22]. The liposome suspension formed consists of multilayer vesicles that underwent three cycles of freezing and thawing. Consequently, liposome suspension extruder (10 times) through 0.2 µm polycarbonate membrane using hand extruder (Avanti Polar lipids Inc.). The polycarbonate filter was soaked in phosphate buffer at pH 7.4 prior to extrusion. The process was repeated with a smaller pore size of

0.1 µm and the final suspension of liposome appeared clear and transparent.

PARTICLE SIZE, POLYDISPERSE INDEX, AND ZETA POTENTIAL FOR LIPOSOME PREPARATION

The average diameter and PDI were measured by photon correlation spectroscopy (PCS) using a nanosizer at a 25°C and 90° scattering angle; hence, 3 ml of liposome suspension was put in the glass cell of the instrument without dilution.

DRUG ENTRAPMENT EFFICIENCY (EE)

Entrapment efficiency refer to amount of drug that embedded in side liposome as compare to the entire amount of drug that applied in preparation of formula. Ultracentrifugation method was used to calculate %EE. Liposome suspension put in centrifuge at 20000 rpm for 25-30min.at 5C (remi cooling centrifuge), the supernatant withdrawal that contain a free drug by indirect method [23], calculated by following equation:

$$E = \frac{W_t - W_f}{W_t} \times 100\%$$

W_t : Total amount of drug use

W_f : Un-capsulated drug

MORPHOLOGY OF LIPOSOME BY FESEM

The morphology of surface liposome for liposomal Etodolac was observed by field emission electron microscope (Inspect TM F50 FEI Company). A few drops of the sample were deposited on a carbon-coated copper grid and then allowed to dry at room temperature and observed by microscope with different magnifications.

IN VITRO RELEASE OF ETODOLAC FROM LIPOSOME

The Etodolac release from liposome was measured by the dialysis membrane diffusion method with some modification. Sample 2 ml of pure Etodolac, liposome-loaded Etodolac, and conventional and PEGylated liposomes were put in a dialysis bag with a molecular weight cut (12000-14000 Da), the membrane soaking overnight in media before using. After that dissolution media consist from either 250ml of phosphate buffer saline pH7.4 at 37^0C at 100rpm, at predetermine time 3ml was taken and replace with fresh media to maintenance the volume of media constant. The amount of Etodolac released was assayed by HPLC, and the cumulative percentage of the drug release profile was recorded.

IN VIVO PHARMACOKINETICS

Pharmacokinetic studies were done on male rabbit weighting 2-2.5kg were randomly divided into three groups (each group n=6). The PK was done for free ETO, ETO loaded in conventional liposomes F1, and PEGylated liposome F2. The studies were done according to ethics approved by the Ethics Committee of the Experimental Animal Teaching and Research Center, University of Kufa. The rabbits were obtained from the College of Science, University of Kufa, Iraq. The rabbits were kept in standard conditions of temperature and humidity and a natural cycle of dark and light in a room at a house animal at a temperature of 20-25°C and RH of 60-68 for two weeks, with access to free water and food [24-25]. For three groups of rabbits: the first group (n=6) received free ETO solution, the second group (n=6) received ETO loaded in conventional liposomes, and the third group (n=6) received ETO loaded in PEGylated liposomes. The dose of 8 mg/kg of rabbit's body weight was given as a suspension by IV administration route through the jugular vein under anaesthesia. At predetermined times of 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, and 24 hr. after dosing. The blood samples 500 µl were withdrawn from the jugular vein and rapidly centrifuged at 4000 rpm at 4°C for 10 min., and the plasma was collected in 2 ml. To prepare the sample for HPLC analysis, allow thawing at room temperature, add 500 µl of methanol, and shake well. Follow that with centrifugation at 10000 rpm for 10 min to precipitate protein, and the sample is ready to be analysed [25].

STATISTICAL ANALYSIS

The quantitative variables were present as average value mean ± SD, calculated using Microsoft Excel 2016 and SPSS ANOVA test $p<0.5$ was consider statistically significant. PK solver software for calculate the pharmacokinetic parameters.

RESULTS

REVERSED-PHASE HPLC METHOD DEVELOPMENT AND OPTIMISATION

The HPLC method was developed to quantify Etodolac, in which the optimization is based on the guidelines of both the ICH (International Conference of Harmonization) and FDA (Food and Drug Administration). A number of preliminary trials were conducted using various combinations of solvents and different ratios of methanol, acetonitrile, and water. The optimum resolution, the low retention time of the peak, the proper run time of the analytic, and good peak shape were achieved by

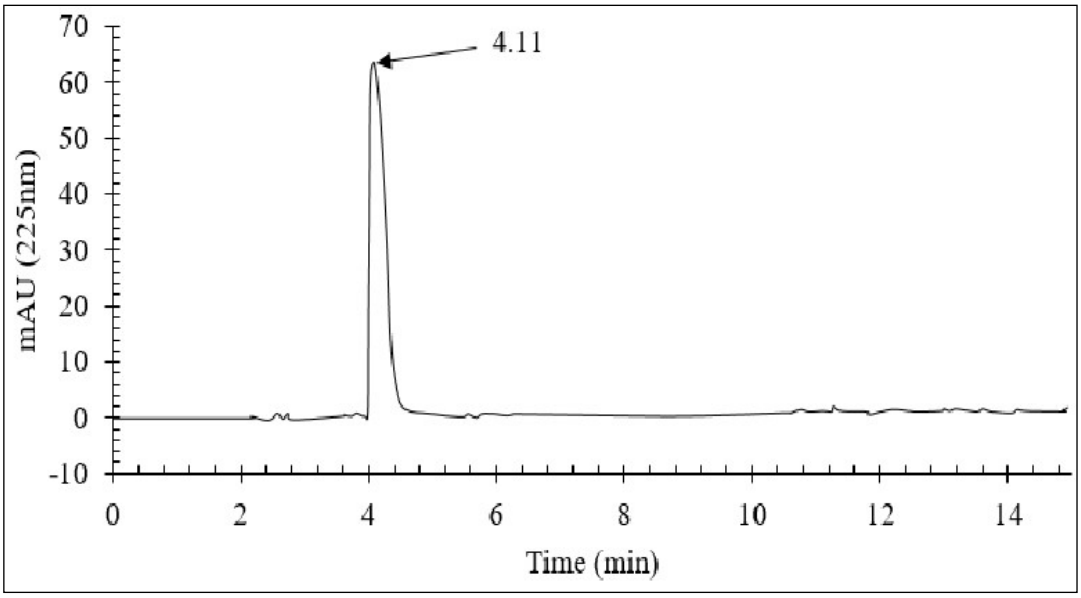

Fig. 1. HPLC Chromatographic of Etodolac (2.5 µg/ml) was analysed using a C18 column (250 mm × 4.6 mm, 5 µm particle size) at room temperature
Source: Own materials

Table 1. System suitability tests for Etodolac with accepted ranges.

Parameters	Etodolac	Accepted ranges
Injection repeatability (RSD)	0.04	<1
Capacity factor (K')	2.422	>2
Tailing factor (T)	1.09	~ 1
Number of theoretical plates (N)	4793	>2000

using a mobile phase (methanol: acetonitrile: water, at a ratio of 60:30:10). The wavelength adjustment was investigated for optimum response; 225 nm showed the optimum response [26]. The retention time was detected at 4.11 ± 0.081 min, appearing as a sharp peak with no tailing observed, meaning a good peak symmetry, figure (1); the run time was totally 15 min.

VALIDATION OF HPLC METHOD FOR ETODOLAC QUANTIFICATION

SUITABILITY TESTS OF SYSTEM

To ensure that the system is sensitive, specific, and reproducible for the analyses, a system suitability of the chromatographic method was conducted. An injection of 2.5 µg/ml of Etodolac was used to study the suitability of system parameters for the HPLC method. Repeatability, column efficiency, and resolution were determined for HPLC detection of Etodolac. The result, shown in Table 1, revealed acceptable parameters

The HPLC instrument performance was evaluated by measuring how well it could detect small amounts of Etodolac (sensitivity) and how consistent its results were (precision). The % RSD (relative standard deviation) was calculated to determine the limitations of the analysis.

THE LINEARITY, LIMITED QUANTIFICATION, AND LIMIT OF DETECTION OF THE HPLC METHOD

The linearity of the analytical method can be defined as the ability of the technique to get test results in which the proportion is directly related to the concentration of the material to be analysed in the sample; the range can be expressed as an interval between the lower and upper concentrations of the substance to be analysed, for which the method can detect a better level of linearity, precision, and accuracy. The standard curve of ETO was used to establish the linearity (LOD) representing the lowest Etodolac concentration that can be measured. In contrast, (LOQ) represents the lowest Etodolac amount that can be measured with suitable accuracy and precision. The Etodolac calibration curve was determined by plotting the peak of its area against the known concentration of the sample, figure (2).

The calibration curve n = 12, with a correlation coefficient R^2 (0.999 ± 0.0007), indicates a highly linear relationship between peak area and concentration within the tested range (2.5 - 25 µg/ml). The LOD and LOQ were measured according to the coefficient of slope and standard error of intercept; the HPLC method was used to measure them. They were found to be 0.42 µg/ml and 1.3 µg/ml, respectively.

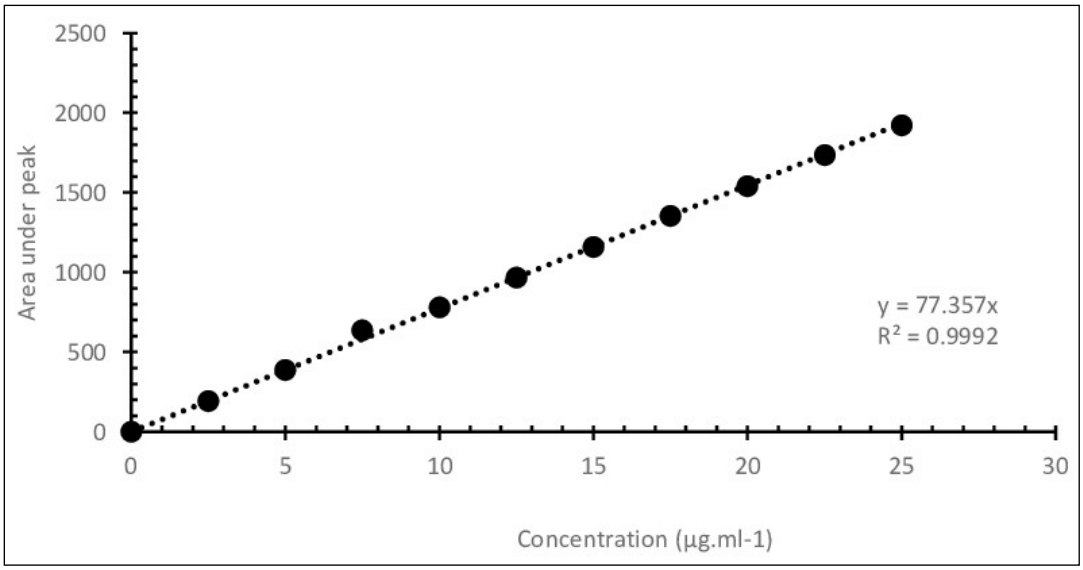

Fig 2. Linearity plot of Etodolac over conc.2.5-25 µg.ml^{-1} (n=12)
Source: Own materials

Table 2. Accuracy and precision within single day and between days

Within-day (n=6)			
Etodolac concentration in sample (µg.ml^{-1})	**HPLC detection (µg.ml^{-1})**	**Accuracy %RE**	**Precision %RSD**
2.5	2.50±0.0052	99.87-100.13	0.2
5	5.01±0.0440	99.77-100.23	0.87
7.5	7.49±0.0103	99.91-100.09	0.1
10	10.01±0.0427	99.87-100.13	0.04
Between-day (n=6)			
Etodolac concentration in sample (µg.ml^{-1})	**HPLC detection (µg.ml^{-1})**	**Accuracy %RE**	**Precision %RSD**
2.5	2.50 ± 0.0137	99.87-100.13	0.5
5	5.01 ± 0.0427	99.73-100.27	0.8
7.5	7.51 ± 0.0456	99.87-100.13	0.6
10	10.06 ± 0.0852	99.37-100.63	0.847

Source: Own material

ACCURACY AND PRECISION OF DEVELOPED HPLC METHOD

Intraday and intraday HPLC analysis methods were used for measuring the repeatability of the developed method and if the investigative method was appropriate for additional studies to determine the accuracy and precision. The accuracy and precision of the HPLC method were assessed both within a single day (intraday) and between days (Intraday) to determine if the developed method was a reliable result and if the analytical method was suitable for future studies. The accuracy was measured as a percentage of residual error (RE), while precision was represented as a percentage of residual standard error (RSD), as shown in Table 2

STABILITY ANALYSES OF ETODOLAC

Three conditions were used to assay the stability of Etodolac: short-term storage, long-term storage and freeze-thawing cycle. These studies are critical in determining the ability of Etodolac to withstand during preparation and in plasma. The results obtained from these studies are in three situations, as shown in Table 3.

For short-term stability, the percentage of accuracy ranges from (99.72-100.33%); for long-term stability, % the accuracy is (97.92-99.42%) and for freezing-thawing (98.93-99.93%).

INTERFERENCE STUDY OF ETODOLAC

It's essential to detect Etodolac in liposomes to study if there is any effect or interference of components of liposomes

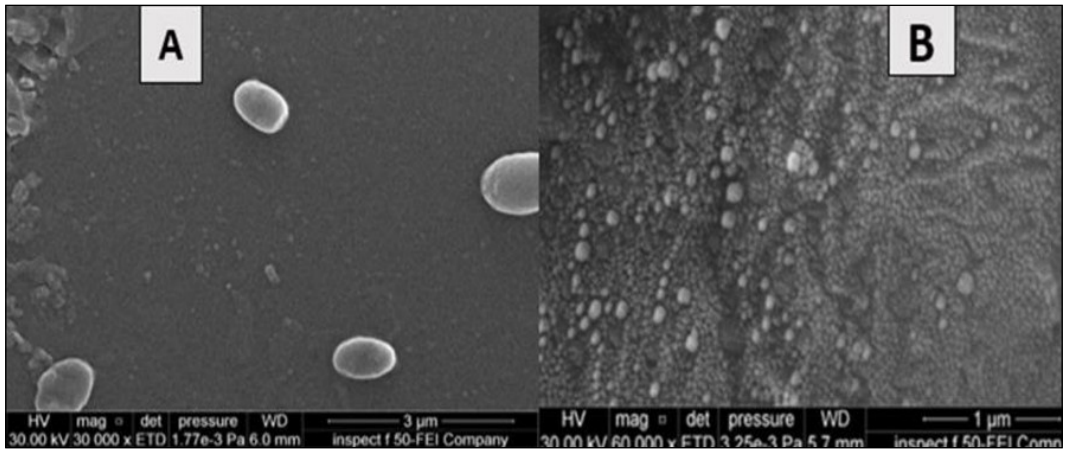

Fig. 3. FESEM photograph for A: Etodolac loaded conventional liposome, B: Etodolac loaded PEGylated liposome
Source: Own materials

Table 3. Short-term storage, long- term storage and freezing-thawing cycle stability of Etodolac (n=6)

Experimental conditions	actual concentration (µg.ml^{-1})			
	2.5	5	7.5	10
Short term				
Average conc. (µg.ml^{-1})	2.49±0.03	5.02±0.065	7.51±0.046	9.97±0.042
Accuracy %	99.73	100.33	100.11	99.72
Long term				
Average conc. (µg.ml^{-1})	2.48±0.018	4.92±0.037	7.46±0.036	9.79±0.24
Accuracy %	99.33	98.47	99.42	97.92
Freezing-thawing				
Average conc. (µg.ml^{-1})	2.47±0.016	4.99±0.056	7.48±0.02	9.99±0.018
Accuracy %	98.93	99.77	99.76	99.93

Source: Own material

Table 4. Recovery of Etodolac from liposome (n=6)

Etodolac	Liposome	
µg.ml^{-1}	HPLC detection	Recovery (%)
2.5	2.48±0.038	99.33
5	4.92±0.037	98.47
7.5	7.52±0.045	100.22
10	9.99±0.029	99.85

Source: Own material

(lipid and cholesterol) on the detection of Etodolac by HPLC. Liposome formulas consist of four concentrations of stock Etodolac prepared (2.5, 5, 7.5, and 10 µg.ml^{-1}) separately mixed with DSPC and cholesterol and analysed by HPLC. The result shown in Table 4 is that retention time was 4.1 min. The recovery percentage was in the range (98.47-100.22%).

CHARACTERIZATION OF LIPOSOME LOADED WITH ETODOLAC

Liposomal preparation containing Etodolac was developed using the thin film hydrated method with extrusion. Conventional liposome that prepared using DSPC and cholesterol, the PZ diameter 88 nm±0.02, with narrow size of distribution 0.012±0.01, while PEGylated liposome prepared using DSPC-Chol-DSPE-PEG 2000 has PZ 92 nm ± 0.003 and a PDI 0.01±0.02,. PDI was less than 0.1 mean particle size well controlled and narrow disperse. The Z-potential was (-10.2mV±0.001, -13.6mV±0.012) for conventional liposome and PEGylated liposome, respectively. The entrapment efficiency %EE (89.5±0.04, 94.3%±0.01) for conventional liposome and PEGylated liposome, respectively. The FESEM analysis explain the spherical shape of vesicles and confirm diameter of liposome, figure (3).

The *in vitro* drug release kinetic profile shows there is a significant difference between the release of drug from free Etodolac and liposomal Etodolac ($p < 0.05$) as shown in Fig 4. As seen from the figure, the % release of

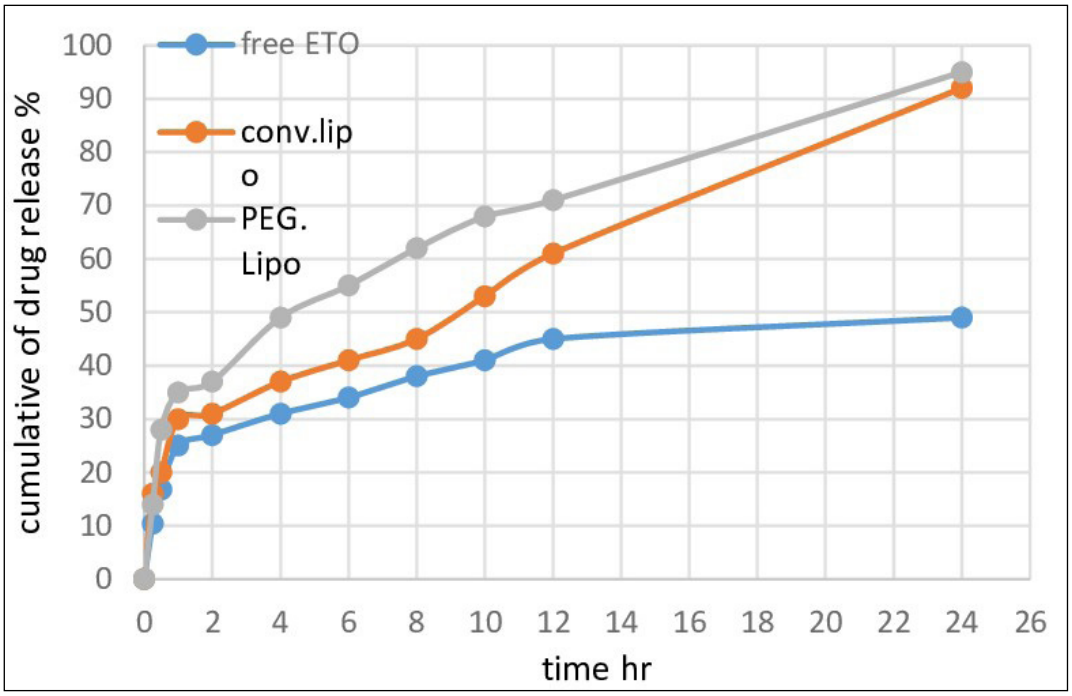

Fig. 4. *In vitro* release of free Etodolac, Etodolac loaded conventional liposome and Etodolac loaded PEGylated liposome
Source: Own materials

Table 5. Pharmacokinetic parameter for pure ETO, conventional liposome and PEGlytated liposome follow IV administration (n=6)

Parameters	pure ETO	Conventional liposome	PEGylated liposome
T_{max} hr.	0.25	0.25	0.25
C_{max} µg/ml	10.49	15.32	19.43
K_{el} hr^{-1}	0.138	0.103	0.084
$t_{½}$ hr.	6.5	8.12	10.74
$AUC_{0-\infty}$ µg/ml*h	69.12	111.17	162.03
$AUMC_{0-\infty}$ µg/ml*h^2	743.04	1172.54	2447.91
MRT hr.	4.37	7.04	7.82
CL (mg)/(µg/ml)/h	0.173	0.107	0.07
Vd (mg)/(µg/ml)	2.034	1.29	1.14

Source: Own material

Etodolac occurs in two patterns from conventional lip and PEGylated liposome as a burst release within (2 hr.) as 31.4% and 37.8% for conventional lip and PEGylated liposome, respectively, followed by a sustained release until 24 hr., in which it reaches 92.1% and 96.3% for conventional lip and PEGylated liposome, respectively, while pure Etodolac is 49%.

IN VIVO PHARMACOKINETIC STUDIES

In vivo pharmacokinetics was done for pure drugs, conventional and PEGylated liposomes using PK Solver software, and a non-compartment model was used to fit the data. All formulas were subject to a sterilization process by filtration method using 0.22 µm PVDF filters. The free ETO is rapidly removed from the blood as compared with ETO loaded with liposome; these results might be due to metabolism or distribution to other organs. The result shows the pharmacokinetic profile of liposomes loaded with ETO significant differences ($p<0.05$) from free ETO; the PEGylated formula shows the higher plasma concentration as compared with the conventional and free drug after IV administration; the pharmacokinetic parameters obtained from the plasma-time profile are summarized in Table (5) and figure (5).

AUC, which involves AUC_{0-24}, $AUC_{24-\infty}$, and total $AUC_{0-\infty}$ measured by the trapezoidal method for each animal of these data, in the group (n=6), and take the average of these data as shown in Table 6 and Fig 6. The total AUC for PEGylated liposome shows a significant difference from both pure ETO and conventional liposome ($p < 0.05$). PEGylated liposome had a higher AUC_{0-24} and

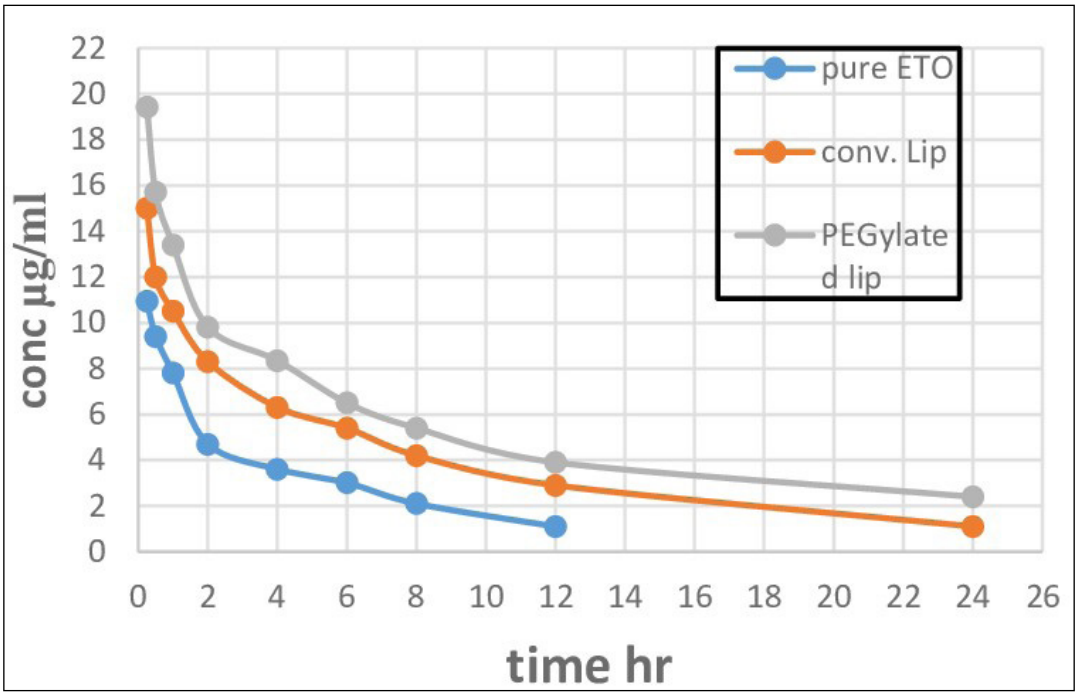

Fig. 5. *In vivo* pharmacokinetic profile for pure ETO, conventional liposome (F1) and PEGylated liposome (F2) follow IV administration (8mg/kg per rabbit). The data show mean± SD (n=6). The ETO loaded in liposome profile has a significant difference from pure ETO ($P<0.05$)
Source: Own materials

Table 6. Area Under Curve AUC_{0-24}, $AUC_{24-\infty}$ and total AUC (n=6)

Formula type	AUC_{0-24} µg/ml*h	$AUC_{24-\infty}$ µg/ml*h	AUC_{total} µg/ml*h
Pure ETO	46.85	22.26	69.12
Conventional lip	97.93	13.24	111.17
PEGylated lip	124.82	37.21	162.03

Source: Own material

AUC_0-∞ (124.82, 162.03 µg/ml*h), respectively, while (97.93, 111.17 µg/ml*h) for the conventional liposome formula and (46.85, 69.12 µg/ml*h) for pure Etodolac.

The maximum plasma concentration Cmax and Tmax were obtained from the plasma concentration versus time curve. Cmax is equal to 10.84, 15.32, and 19.34 µg/ml for pure ETO, conventional liposome, and PEGylated liposome, respectively, while t_{max} 0.25 hr. The results show Cmax of PEGylated liposome significant differences as compared with conventional liposome and pure ETO at any time. AUMC0-∞ for pure ETO, conventional lip, and PEGylated lip were found to be 743.04 µg/ml*h^2, 1172.54 µg/ml*h^2, and 2447.91 µg/ml*h^2, respectively. The result shows the AUMC0-∞ for pure ETO the lowest while AUMC0-∞ for PEGylated lip is the highest, there is a significant difference p< 0.05) between conv. lip and PEGylated lip as compare with pure ETO. The t1/2 was found to be 6.5 hr., 8.12 hr., and 10.74 hr. for pure ETO, conventional liposome, and PEGylated liposome, respectively. The MRT (mean resident time) equal to 7.82 hr., for PEGylated liposome as compare with 7.04 hr. for conventional liposome and 4.37hr for pure ETO.

DISCUSSION

The HPLC method was developed to quantify Etodolac, in which the optimization is based on the guidelines of both the ICH (International Conference of Harmonization) and FDA (Food and Drug Administration). The mobile phase, which consists of methanol, acetonitrile, and water in a ratio of 60:30:10, was pumped through the column at a flow rate of 1 millilitre per minute. The sample was injected at a volume of 20 µl, and Etodolac was detected at a wavelength of 225 nm. The calibration curve was validated over the range of 2.5 - 25 µg/ml. The flow rate was, and the investigation was conducted in isocratic mode, with a flow rate of 1 ml/min, the thermostat temperature is 30°C, and the injection volume is 20 µl. The results are consistent with the result found by Akman et al. (2019), who show the retention time of Etodolac 4.21 min when using a mobile phase containing acetonitrile: water at a ratio of (80:20), a flow rate of 1 ml/min, and a wavelength of 272 nm [27], also, these results were compatible with Mohan *et al.* , 2011, who stated a retention time for Etodolac was 4.8 min using a mobile phase containing phosphate buffer 5.5 and acetonitrile

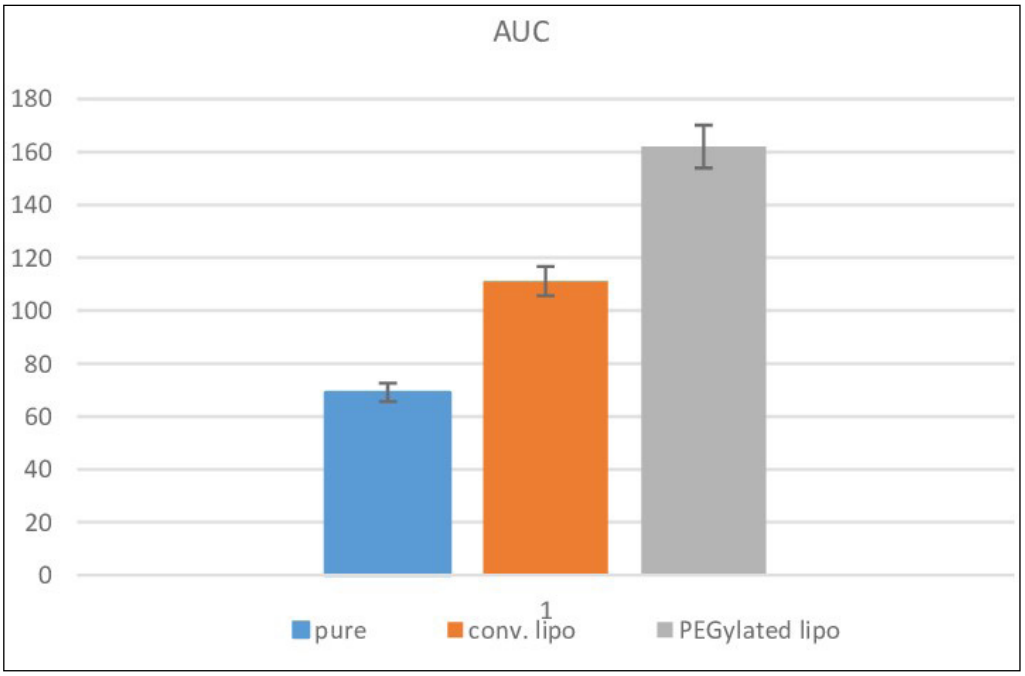

Fig. 6. AUC $_{0-\infty}$ for pure ETO, conventional liposome and PEGylated liposome by trapezoidal method
Source: Own materials

at a ratio (60:40) [28]. The %RSD was less than 1%; indicate that the chromatographic conditions were optimal and that the injections were highly repeatable. To measure resolution, the capacity factor k' was detected, and the result revealed $k' > 2$, which indicates a good resolution. The tailing factor T also measures the asymmetry of a peak; the result appears T = 1.09 close to one, reproducing a good symmetry of the peak. The theoretical plate number N > 2000 indicates a good efficiency of column, according to the Centre for Drug Research and Evaluation. The results of the system suitability test were comparable to those reported by Patel et al. (2011), which included theoretical plates N greater than 2000, capacity factor and resolution k' greater than 2, and asymmetry 1.03, all factors within accepted and limiting ranges [29]. Additionally, the finding was consistent with those of Siddiraju et al. (2013), who confirmed the system suitability of Etodolac using a tailing factor (T=1.04) and theoretical plates N greater than 2000, capacity factor and resolution k' greater than 2 within accepted ranges [30]. The calibration curve n = 12, with a correlation coefficient R^2 (0.999 ± 0.0007), indicates a highly linear relationship between peak area and concentration within the tested range (2.5 - 25 µg/ml). However, these results demonstrate a good fit between the data and linear regression model, confirming a strong relationship across the chosen range of concentration, and this finding aligns with previous research by Lee et al. (2008), who reported the linear calibration curve of Etodolac across a range of concentrations (0.1-25 µg/ml), with an R^2 correlation coefficient (0.9975 ± 0.0018) [31]. So this indicate the method was sensitive and easy quantify method for Etodolac. The results obtained are consistent with the finding of Thomas et al. (2023), who determined Etodolac succinic acid co-crystals in spiked rabbit plasma to be 0.370 µg/ml and 1.121 µg/ml, for LOD and LOQ, respectively [32]. LOD and LOQ were measured according to the coefficient of slope and standard error of intercept; the HPLC method was used to measure them. They were found to be 0.42 µg/ml and 1.3 µg/ml, respectively, refer the method was sensitive to determine a minimal quantity of Etodolac in a bulk sample. For precision and accuracy, the outcomes of the method show that accuracy and precision, are within the accepted limited range (85–115%) as mention via the analytical method of the FDA, while for % RSD less than 2. The results are consistent with the findings of Biswal et al. (2019); that finding appeared, the % RSD for a precision of less than 2% [33]. Results were also consistent with Patel *et al.,* 2011, who informed an %RSD of < 2%, Results were also consistent with Patel et al. (2011), who reported an %RSD of < 2% , and also results were reliable with the result of Dhiware et al. (2012), who reported a calculated % RSD of less than 2% [34]. The results obtained from the stability test study proved that the sample that contains Etodolac can be handled under standard laboratory conditions and stored in the refrigerator at -20°C. The experiment's results are compatible with Patel *et al.*, 2011, who reported that Etodolac can be stable for two days at ambient temperature [29]. The result confirmed no interferences

between Etodolac and a component of liposome in detection by HPLC, which is compatible with the result of Shaikh and his colleagues, 2017, that informed there is no interference was observed between Etodolac and excipient present in tablet dosage form [21]. the liposomes successful formulation by thin film method, the results show, PZ of PEGylated liposome higher PZ than conventional liposomes due to the PEG formed a thick layer at the surface of liposome make it large PZ. The Z-potential of PEGylated liposome more than conventional liposome because of present of PEG give a charge on the surface due to the DSPE-PEG2000 has a negative charge and prevent the aggregation of particles by electric repulsive effect. The PEGylated liposome shows a higher %EE than the conventional liposome, and this may be result from that the PEG enhancing the solubility of drug lead to improve its %EE, the pH of the liposome a round 7.4, so the addition of PEG does not affect the pH of liposome make it suitable for IV administration [35]. This result consistence with the finding of Sara Pereira et al. (2016), who reported that the increase of %EE of Docetaxel in DSPC-Chol-DSPE-PEG 2000 liposome than DSPS or DPPC base formulation [36]. Etodolac is a hydrophobic drug with poor water solubility 0.016mg/ml and $\lg_{P}$ 2.5, so can be partition between lipid layers and aqueous phase. The in-vitro study show, the fast release of the drug result can be explained as the drug being present near the head of the phosphate group, followed by a slow or sustained release due to the entrapment of the drug in the lipid by layer [37]. Effect of PEG2000 which prolongs the release of Etodolac from liposomes and also improves solubility and stability of Etodolac. The results show the PEGylated liposome shows a higher % release reach 95% within 24hr.The release of Etodolac from liposomes shows a biphasic pattern, and these findings are in agreement with the finding of Vivek et al. 2019, who reported that the release of celecoxib from PEGylated liposomes was a biphasic. for in-vivo study, the lowest half-life of pure ETO and conventional liposome due to the opsonization process by Kupffer and RES was responsible for elimination from systemic circulation. In addition to that, the volume of distribution (Vd) and elimination rate constant (Kel) were found to be less for PEGylated liposomes as compared to conventional and pure ETO, as listed in Table 5, reflecting the long circulation time of liposomes due to the PEGylated liposome containing PEG 2000 with stearic properties, making it a stealth liposome that has a long circulation time. The modification the surface of liposomes with polyethylene glycol (PEG) has emerged as a prominent strategy for enhancing their stability and extending circulation time. The inherent hydrophobicity of PEG molecules, upon grafting onto the liposomal surface, facilitates the formation of a robust hydration layer. This hydration shell effectively mitigates nonspecific interactions between the liposome and plasma components, consequently diminishing the formation of the protein corona—the adsorption of plasma proteins onto the liposome surface. A reduced protein corona minimizes recognition and clearance by the reticuloendothelial system (RES). Furthermore, PEG's steric hindrance properties impede particle aggregation and confer protein-repellent characteristics, further contributing to decreased RES uptake and a prolonged drug half-life. These attributes have earned PEG the designation of a "stealth polymer" in liposomal drug delivery, leading to its widespread adoption in liposome modification [38].

CONCLUSIONS

The HPLC process was developed and evaluated for its ability to rapidly measure and quantify Etodolac in a pure bulk sample and liposomes from the outcomes that obtain from the validation parameter, it has been established that the HPLC method was simple, reliable, sensitive, rapid, and reproducible with a short runtime. Etodolac was stable during handling and formulation. The method fully meets FDA guidelines. It can be used for quality control assays of Etodolac in test laboratory samples and formulation of pharmaceutical industries, and in vivo pharmacokinetic. The liposome loaded with Etodolac was successful prepared using thin film hydrate technique

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Environmental microbial contamination as potential risk for healthcare associated infections after dental procedures: a multicenter study

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ABSTRACT

Aim: Aim this study to the microbial and molecular analysis of environmental (air, water and surfaces) microbial contamination in Ukrainian dental clinics.

Materials and Methods: This multicenter observational study was conducted between January 1, 2022, to December 31, 2024 in fifteen dental clinics located in five regions of Ukraine. Evaluation of the environmental contamination was performed according to microbial and molecular methods.

Results: During study period in dental clinics tap water, dental unit water systems, and water from the bottles attached to the dental chair units (before clinical activity and during clinical activities), and surfaces of dental equipment, and air (during clinical activities) was heavily contaminated with potential pathogens of healthcare associated infections (HAIs). The bacteria identified were mainly of the Gram-positive and Gram-negative species. The main potentially HAI-pathogenic of bacteria were *Escherichia coli* (13%), *Pseudomonas aeruginosa* (9.1%), *Enterococcus faecalis* (4%), *Klebsiella pneumoniae* (3.6%), *Acinetobacter lwoffii* (3.5%), *Acinetobacter baumannii* (3.3%), *Klebsiella oxytoca* (3.2%), *Serratia marcescens* (3.2%), *Stenotrophomonas maltophilia* (3.2%), *Staphylococcus aureus* (3.2%), *Enterococcus faecium* (3.1%), *Enterobacter cloacae* (3%), *Enterobacter aerogenes* (2.9%), *Burkholderia cepacia* (2.6%), *Providencia stuartii* (2.5%), *Proteus mirabilis* (2.2%), *Proteus rettgeri* (2.1%), and *Streptococcus pneumoniae* (2.1%).

Conclusions: During study period in dental clinics tap water, dental unit water systems, and water from the bottles attached to the dental chair units (before clinical activity and during clinical activities), and surfaces of dental equipment, and air (during clinical activities) was heavily contaminated with potential pathogens of HAIs. Patients are exposed during dental practice to an infective risk.

KEY WORDS: dental clinics, environmental contamination, microorganisms, infective risk, dental unit water systems, dental equipment surfaces, Ukraine

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INTRODUCTION

Patients and healthcare workers are exposed during dental practice to an infective risk, which derives from microorganism suspended in aerosols. Therefore, environmental microbiological monitoring is crucial to control in dental settings and represents a good instrument to detect critical situations [1, 2].

Monitoring of microbial contamination in the air, water and surfaces has been carried out for over 100 years and its importance has been widely pointed out, and more recently the use of molecular genetic tools has been implemented [3]. According to the literature, research interests include various fields from the environmental point and human health perspective [4] due to pathogens such as bacteria, fungi and viruses. However, up to now there are no established international protocols or standards for detecting diversity of these microorganisms.

According to the literature, research interests include various fields from the environmental point and human health perspective due to pathogens such as bacteria, fungi and viruses. Recent publications have showed the possibility to collect microbial DNA through air [3, 5], water [6, 7] and surfaces [8] sampling, providing insight into microorganism diversity. Given its health implications, a primary focus of studies is the analysis of microbiological contamination in the air, water and surfaces in healthcare setting. Modern epidemiological studies from various countries indicate that currently the most hospitals have high level of bacterial contamination of surfaces.

Most epidemiological studies from various countries indicate that currently the most hospitals have high level of bacterial contamination of surfaces, with even higher rates observed in dental clinics. One of the leading causes of worldwide healthcare-associated infections (HAIs) in dental clinics are bacteria. Some of these pathogens are transmitted through the air and are opportunistic while others are host specific. Monitoring is therefore of importance in programs mitigating further spread of HAIs. However, monitoring airborne fungi and bacteria can be very challenging, as many are very difficult to identify accurately. Presently only using DNA analysis makes it possible to accurately distinguish different opportunistic microorganisms' species in air, water and surfaces samples.

Currently, in Ukraine the microbiological contamination of the environment in dental healthcare facilities was poorly studied. A previous study has focused on the prevalence of HAIs and antimicrobial resistance of the responsible pathogens [9] and nosocomial transmission of multi-drug-resistant organisms in Ukrainian hospitals [10], and on the evaluation of bacterial contamination in the inanimate environment surfaces in acute care hospitals [11]. However, these results do not provide sufficient information about the factors contributing to the spread of the incidence of HAIs in Ukrainian dental clinics, which requires further study.

AIM

The aim of this study was to the microbial and molecular analysis of environmental (air, water and surfaces) microbial contamination in Ukrainian dental clinics.

MATERIALS AND METHODS

STUDY DESIGN AND SETTING

This multicenter observational study was conducted between January 1, 2022, to December 31, 2024 in fifteen dental clinics located in five regions (Lviv, Kharkiv, Kyiv, Zhytomyr, Odessa) of Ukraine. The clinics were recruited on a voluntary basis. All dental chair and ancillary equipment in these clinics are used for routine general oral healthcare procedures. These procedures include oral examinations, dental extractions, endodontics, prosthodontic procedures, restorations and prophylaxis among others. In dental clinics water is sourced from the mains municipal supply, and stored in secondary reservoirs such as large tanks from where water is then supplied to dental unit water systems (DUWLs). All dental clinics used a closed system, where the reservoir bottles were filled with distilled water and attached to the dental chair units. In all dental clinics surfaces and the handpieces are routinely treated with disinfectants between patients or are autoclaved. Wet cleaning was carried out once a day - after the occupancy period in all dental offices. Concerning ventilation, 90% of offices had an air-conditioning system, and 95% had mechanical ventilation. The ventilation of the operating areas for at least 10–15 min was implemented between each patient. All dental operating areas, from the least critical to the most critical, were adequately cleaned and disinfected. Inclusion criteria: all dental clinics willing to partake in the study and had to be perform routine dental procedures. Exclusion criteria: dental clinics that did not sign a consent letter to participate in this study.

SAMPLING STRATEGIES

Microbial contamination of air, water, and environmental surfaces, was assessed in each dental clinic. Microbial contamination was evaluated for the following types of dental treatments: (a) orthodontics, (b) periodontic, (c) oral surgery, and (d) prosthodontic. Samples were collected in the closed dental operatory. In this study, water and surfaces were sampled before clinical activity and after clinical activity. Air was sampled 5 min before starting clinical activity, during, and immediately after the clinical activity. Evaluation of the microbial contamination of water, air and surfaces was performed according to previously reported methods. All samples were transported to the Zarifa Aliyeva International Center of Medical Science Laboratory (Ukraine) and processed (for microbial and molecular analysis). Evaluation of the microbial contamination of water, air and surfaces was performed according to microbial and molecular methods, which are briefly described below.

MICROBIAL ANALYSIS

AIR SAMPLING

In this study, microbial contamination in air was evaluated through active sampling, to measure the concentration of microorganisms in the air, and by passive sampling, to measure the rate at which viable particles settle on surfaces. Microbial contamination in air was evaluated by DSTU ISO 14698-1:2008 [12]. TVC was performed both for active and passive sampling using Slit-to-Agar biological air sampler. The air sampler was placed at the average working distance of the clinicians involved in the study (and from the aerosol source), 30 cm, at the height of 1.5 m above the floor, which corresponded to the area where the patient was breathing. Passive sampling was performed to deter-

mine the Index of Microbial Air Contamination (IMA) who corresponds to the number of CFU counted on a Petri dish with a diameter of 9 cm placed according to 1 m above the floor, about 1 m away from dental chair units or the patient's mouth for 1 h. After incubated period all colonies were counted using the ScanStation Automatic colony counter (interscience) and reported as colony forming units. The total numbers of colony-forming units (CFU) were determined, and the data were expressed as the number of CFU per cubic meter of air sampled. Passive sampling was performed to determine the Index of Microbial Air Contamination (IMA) Microbial species identification was performed with standard microbial methods.

WATER SAMPLES

Tap water, DUWS water, and distilled water from the reservoir bottles attached to the dental chair units were sampled to determine the total viable count (TVC), and the presence of *Legionella* spp. *Pseudomonas aeruginosa, Escherichia coli*, intestinal enterococci, and other microorganisms. In this study for DUWS, the TVC was measured on every handpiece (ablator, air–water syringe, cup filler, microengine, turbine). The detection of the bacteria was performed according to the international standards for water quality: E DSTU EN ISO 6222:2002 for total viable count [13], DSTU EN ISO 11731:2022 for *Legionella* [14], EN ISO 16266 for *P. aeruginosa* [15], DSTU EN ISO 9308-1:2022 for *Escherichia coli* and coliform bacteria [16], and DSTU EN ISO 7899-2:2022 for intestinal enterococci [17].

SURFACE SAMPLES

Six surface samples were collected in each dental operatory. Flat surfaces were sampled by agar contact plates through an applicator. Non-flat surfaces were sampled using swabs with tubes, which contains a disinfectant inhibitor. All petri dishes were incubated for bacteria and fungi. The results were expressed as CFU/cm^2. Bacteria and fungi were isolated and identified using aVitek-2 automated system.

MOLECULAR ANALYSIS

Genomic DNA was extracted from a single colony of each isolate using QIAmp® DNA Mini kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. The quality of DNA extraction was measured by agarose gel electrophoresis. In this study DNA was quantified by ultraviolet spectrophotometer. Extracted genomic DNA was amplified using a set of primers (341F: CCTACGGGNGGCWGCAG; 806R: GGACTACHVGGGTATCTAAT) targeting bacterial 16SrRNA genes [18]. Primers (ITS3_KYO2: GATGAAGAACGYAGYRAA; ITS4: TCCTCCGCTTATTGATATGC) were used for the fungal ITS2 gene amplification [19]. The purified amplification products were pooled and sequenced on an Illumina platform following the manufacturer's recommendations.

ETHICS

The Ethics Board of the Ukrainian Association of Infection Control and Antimicrobial Resistance has approved the protocols of this study. All dental clinics and patients in the study signed a letter of consent.

STATISTICAL ANALYSIS

Microbiological data were collected in Microsoft® Excel (Microsoft Corporation, Redmond, WA, USA). Descriptive statistical analysis was performed to provide mean, standard deviation, 95% confidence interval. In this study all the data are presented as numbers and percentages. The microbial contamination was summarised using descriptive statistics separately by type of pathogen and location. Statistical significance in this study was assumed for p values lower than 0.05.

RESULTS

MICROBIAL ANALYSIS

Water contamination. A total of 2,520 water samples were collected, of which 76,7% (95% CI: 75.9-77.5) samples did not meet microbial parameters to Ukrainian National Standard DSTU 7525:2014 (2015) and the State Sanitary Norms and Rules of the DSanPiN 2.2.4-171-10, section Indicators of Epidemiological Safety of Drinking Water (TVC<100 CFU/mL). During study period (2020-2022) the share of non-standard TVC for tap water, DUWSs, and water from the reservoir bottles attached to the dental chair units was 62.5% (224/360), 87.4 % (1,573/1,800), and 38.1% (137/360), respectively. In 2022, compared to the 2020 for the tap and DUWSs water quality there was an increase in the number of non-standard water samples for TVC indicators. In the largest specific weight of non-standard (on TVC) water samples was detected in Kyiv, Zhytomyr and Kharkiv region of Ukraine.

The TVC values for tap water and DUWSs, varied markedly between the 15 dental clinics, and significantly decreased between before clinical activity and after clinical activity ($p < 0.0012$). The TVC median values for

Table 1. Distribution of main microorganisms (>1%) isolated from water samples in Ukrainian dental clinics (2020-2022)

Microorganism	All isolates (n=3,176)		Source of water samples: Tap (n=965)		DUWS (n=1,295)		Bottle (n=595)	
	n	%	n	%	n	%	n	%
Gram-positive cocci								
Enterococcus faecalis	394	12.4	153	15.9	184	14.2	57	9.6
Enterococcus faecium	311	9.8	128	13.3	139	10.7	44	7.4
Staphylococcus aureus	96	3.0	-	-	51	3.9	45	7.6
Staphylococcus haemolyticus	36	1.1	14	1.5	15	1.2	7	1.2
Streptococcus pneumoniae	53	1.7	13	1.3	28	2.2	12	2.0
Gram-negative bacilli								
Acinetobacter lwoffii	107	3.4	39	4.0	47	3.6	21	3.5
Acinetobacter baumannii	92	2.9	0	0	54	4.2	38	6.4
Burkholderia cepacia	117	3.7	36	3.7	57	4.4	24	4.0
Enterobacter cloacae	124	3.9	42	4.4	51	3.9	31	5.2
Enterobacter aerogenes	121	3.8	43	4.5	51	3.9	27	4.5
Escherichia coli	491	15.5	189	19.6	221	17.1	81	13.6
Klebsiella pneumonia	99	3.1	30	3.1	48	3.7	21	3.5
Klebsiella oxytoca	112	3.5	35	3.6	48	3.7	29	4.9
Providencia stuartii	88	2.8	29	3.0	35	2.7	24	4.0
Pseudomonas aeruginosa	372	11.7	131	13.6	166	12.8	75	12.6
Stenotrophomonas maltophilia	117	3.7	39	4.0	47	3.6	31	5.2
Serratia marcescens	125	3.9	44	4.6	53	4.1	28	4.7

Source: compiled by the authors of this study

tap water collected pre-clinical activity ranged from 3.6×10^4 CFU/mL to 7.8×10^4 CFU/mL. While at the end of the clinical activity the corresponding median values ranged from $3{,}8 \times 10^3$ CFU/mL to 6.8×10^3 CFU/mL. The TVC median values for DUWSs pre-activity ranged from 12.6×10^4 CFU/mL to 19.8×10^4 CFU/mL. Post-activity median values ranged from 3.9×10^4 CFU/mL to 9.7×10^4 CFU/mL. Of the 1,800 handpieces sampled, the cup filler had the lowest median contamination values before (3.1×10^3 CFU/mL) and after (2.2×10^3 CFU/mL) clinical activity. The air–water syringe and ablator showed the highest microbial contamination before clinical activity (TVC was 8.7×10^5 CFU/mL and 5.3×10^5 CFU/mL, respectively). While the turbine had the highest contamination values after clinical activity (3.1×10^5 CFU/mL).

A total of 3,176 isolates were detected from 2,520 water samples. Among the isolates, gram-negative bacteria and gram-positive cocci was 85.8% and 12.1%, respectively. Fungi was 2.1%. The main microorganisms isolated from water samples are shown in Table 1.

Air contamination. A total of 1,080 air samples were collected in dental clinics. When the air samples were analyzed at before the start of the dental procedure, a low number of bacterial colonies was detected. The species identified in dental clinics were mainly Gram-positive, including *Staphylococcus* sp. (45–85 CFU/m^3), *Bacillus* spp., and fungi (10–20 CFU/m^3). For the microbial contamination in the air, active and passive sampling median values showed a significant increase between before clinical activity and during dental treatments ($p<0.0014$), followed a decrease once the clinical activity was over ($p<0.0012$). During clinical activity median values ranged, for in dental clinics, from 396 CFU/m^3 to 2,735 CFU/m^3. No *Legionella* spp. were isolated in any Ukrainian dental clinic. The bacteria identified were mainly of the Gram-positive species and included *Staphylococcus* sp. and *Bacillus* sp. with a greater number of colonies. The most abundant species belonged to the *Staphylococcus* sp. (*S. aureus, S. epidermidis,* and *S. haemolyticus*) and *Streptococcus* sp. family, including *Streptococcus salivarius* and *Streptococcus mutans*, among the main species in the oral cavity. In this study there was a significant correlation between active

Table 2. Distribution of microorganisms isolated from environmental samples in Ukrainian dental clinics (2020-2022)

Microorganism	All isolates (n=17,482)		Water				Air				Surfaces			
			BCP (n=4,223)		ACP (n=1,384)		BCP (n=124)		ACP (n=987)		BCP (n=1,286)		ACP (n=9,478)	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Gram-positive cocci														
Enterococcus hirae	73	0.4	5	0.1	2	0.14	-	-	2	0.2	32	2.49	32	0.4
Enterococcus faecalis	703	4.0	208	5.2	71	5.13	-	-	38	3.85	38	2.95	348	3.7
Enterococcus faecium	549	3.1	127	3.2	41	2.96	-	-	35	3.55	35	2.72	311	3.3
Kocuria varians/rosea	85	0.5	7	0.2	2	0.14	-	-	1	0.1	37	2.88	38	0.4
Micrococcus sp.	152	0.9	16	0.4	3	0.22	6	4.8	12	1.22	52	4.04	63	0.7
Staphylococcus aureus	553	3.2	71	1.8	9	0.65	7	5.6	59	5.98	49	3.81	358	3.8
Staphylococcus capitis	77	0.4	29	0.7	4	0.29	4	3.2	4	0.41	14	1.09	22	0.2
Staphylococcus epidermidis	486	2.8	29	0.7	3	0.22	5	4.1	19	1.93	19	1.48	411	4.4
Staphylococcus haemolyticus	208	1.2	44	1.1	13	0.94	7	5.6	33	3.34	33	2.57	78	0.8
Staphylococcus hominis	143	0.8	33	0.8	9	0.65	12	9.7	28	2.84	28	2.18	33	0.4
Staphylococcus sciuri	55	0.3	11	0.3	2	0.14	2	1.6	4	0.41	18	1.4	18	0.2
Strptococcus viridans	200	1.1	5	0.1	7	0.51	4	3.2	4	0.41	9	0.7	171	1.8
Streptococcus pneumoniae	360	2.1	76	1.9	25	1.81	9	7.3	9	1.91	13	1.01	228	2.4
Streptococcus pyogenes	316	1.8	66	1.7	20	1.45	5	4.1	5	0.51	11	0.86	209	2.2
Streptococcus sp.	105	0.6	49	1.2	7	0.51	4	3.3	4	0.41	12	0.93	29	0.3
Other Gram-positive cocci	40	0.2	11	0.3	3	0.22	3	2.4	3	0.3	5	0.39	15	0.2
Gram-negative bacilli														
Acinetobacter lwoffii	612	3.5	122	3.1	42	3.03	4	3.3	28	2.84	18	1.4	398	4.2
Acinetobacter baumannii	574	3.3	114	2.9	39	2.82	5	4,1	26	2.63	19	1.48	371	3.9
Acinetobacter sp.	81	0.5	44	1.1	15	1.08	1	0.8	3	0.3	9	0.7	9	0.1
Bacillus cereus	127	0.7	32	0.8	10	0.72	4	3.3	5	0.51	18	1.4	58	0.6
Bacillus sp.	85	0.5	24	0.6	7	0.51	3	2.4	6	0.61	11	0.61	34	0.4
Burkholderia cepacia	453	2.6	144	3.6	49	3.54	-	-	33	3.34	33	2.57	194	2.1
Enterobacter cloacae	522	3.0	137	3.4	47	3.4	1	0.8	34	3.44	34	2.64	269	2.8
Enterobacter aerogenes	506	2.9	124	3.1	43	3.11	-	-	31	3.14	31	2.41	277	2.9
Enterobacter sp.	253	1.5	22	0.6	10	0.72	-	-	7	0.71	17	1.32	197	2.1
Escherichia coli	2,266	13.0	812	20.4	281	20.3	-	-	162	16.4	22	1.71	989	10.4
Klebsiella pneumonia	631	3.6	196	4.9	69	4.99	3	2.4	41	4.15	11	0.86	311	3.3
Klebsiella oxytoca	565	3.2	161	4.1	56	4.05	2	1.6	37	3.75	12	0.93	297	3.3
Legionella pneumophila	19	0.1	11	0.3	4	0.29	2	1.6	2	0.2	-	-	-	-
Moraxella sp.	278	1.6	43	1.1	14	1.01	1	0.8	9	0.91	19	1.48	192	2.1
Providencia stuartii	435	2.5	125	3.1	44	3.18	2	1.6	28	2.84	19	1.48	217	2.3
Proteus mirabilis	384	2.2	41	1.1	27	1.95	-	-	7	0.71	18	1.4	291	3.1
Proteus rettgeri	373	2.1	46	1.2	25	1.81	-	-	9	0.91	17	1.32	276	2.9
Pseudomonas aeruginosa	1,596	9.1	501	12.6	155	11.2	-	-	121	12.7	21	1.63	798	8.4
Pseudomonas luteola	69	0.4	12	0.3	4	0.29	-	-	-	-	28	2.18	25	0.3
Pseudomonas sp.	58	0.3	19	0.5	6	0.43	-	-	3	0.3	14	1.09	16	0.2
Stenotrophomonas maltophilia	558	3.2	144	3.6	50	3.61	-	-	31	3.14	6	0.47	327	3.5
Serratia marcescens	561	3.2	161	4.1	56	4.05	-	-	38	3.85	9	0.7	297	3.1
Other Gram-negative bacilli	41	0.2	9	0.3	3	0.22	3	2.4	2	0.2	12	0.93	12	0.1
Fungi														
Aspergillus flavus	88	0.5	19	0.5	5	0.36	3	2.4	3	0.3	19	1.48	39	0.41
Aspergillus niger	73	0.42	22	0.6	4	0.29	4	3.3	3	0.3	15	1.17	25	0.26
Aspergillus ochraceus	45	0.26	9	0.2	-	-	-	-	-	-	13	1.01	23	0.24
Candida albicans	347	1.98	72	1.8	25	1.81	1	1.8	12	1.22	18	1.4	219	2.31
Candida glabrata	344	1.97	58	1.46	20	1.45	-	-	11	1.11	21	1.63	234	2.47
Candida krusei	290	1.66	43	1.08	15	1.08	1	0.8	1	0.1	19	1.48	211	2.23
Penicillium sp.	91	0.52	19	0.48	1	0.07	1	0.8	3	0.3	19	1.48	48	0.51
Other fungi	40	0.23	5	0.13	3	0.22	2	1.6	2	0.2	14	1.09	14	0.15

BCP, before clinical practice; *ACP*, after clinical practice

Source: compiled by the authors of this study

Table 3. Distribution of main potential human pathogenic microorganisms(n=17,483) isolated from environmental samples of Ukrainian dental clinics (2020-2022)

Microorganisms	n	%	95% CI
Escherichia coli	2,266	13.0	12.71 – 13.21
Pseudomonas aeruginosa	1,596	9.13	8.91 – 9.35
Enterococcus faecalis	703	4.02	3.87 – 4.17
Klebsiella pneumonia	631	3.61	3.47 – 3.75
Acinetobacter lwoffii	612	3.5	3.36 – 3.64
Acinetobacter baumannii	574	3.28	3.15 – 3.41
Klebsiella oxytoca	565	3.23	3.1 – 3.36
Serratia marcescens	561	3.21	3.08 – 3.34
Stenotrophomonas maltophilia	558	3.19	3.06 – 3.32
Staphylococcus aureus	553	3.16	3.03 – 3.29
Enterococcus faecium	549	3.14	3.01 – 3.27
Enterobacter cloacae	522	3.0	2.86 – 3.12
Enterobacter aerogenes	506	2.89	2.76 – 3.02
Staphylococcus epidermidis	486	2.78	2.66 – 2.9
Burkholderia cepacia	453	2.59	2.47 – 2.71
Providencia stuartii	435	2.49	2.37 – 2.61
Proteus rettgeri	373	2,13	2.02 – 2.24
Proteus mirabilis	384	2.2	2.09 – 2.31
Streptococcus pneumoniae	360	2.06	1.95 -2.17
Candida albicans	347	1.98	1.87 – 2.09
Candida glabrata	344	1,97	1.86 – 2.08
Streptococcus pyogenes	316	1.81	1.71 – 1.91
Candida krusei	290	1.66	1.56 – 1.76
Moraxella sp.	278	1.59	1.5 – 1.68
Enterobacter sp.	253	1.45	1.36 – 1.54
Staphylococcus haemolyticus	208	1.19	1.11 – 1.27
Strptococcus viridans	200	1.14	1.06 – 1.22

Source: compiled by the authors of this study

and passive air sampling ($p<0.0013$). The air samples collected after the clinical activity showed a decrease in bacterial load compared to the previous sampling.

Contamination of surfaces. During study period 2,160 samples were collected from six dental equipment surfaces (light, dental spittoon, countertop, head board, chair arm rest, and air/water syringe) of the 15 dental clinics. The dental equipment surfaces were chosen due to their frequent interaction with the patient or the dental doctor, being the most likely surfaces to accumulate bacteria. Bacteria and fungi isolated from samples were cultured, then counted and identified. There was no statistical difference in microbiological contamination between dental clinics. In dental equipment surfaces before the start of the dental procedure was a poor microbiological contamination with rare pathogenic microorganisms. The only significant difference was a higher fungal contamination of surfaces in prosthodontic dental rooms compared with oral surgery, periodontic, and orthodontics rooms ($p < 0.03$). Oral surgery, periodontic, and orthodontics offices with daily wet cleaning had surfaces significantly less contaminated by fungi but similarly contaminated by bacteria ($p < 0.002$). The most prevalent microorganisms in oral surgery, orthodontics, periodontic, and prosthodontic surface samples before clinical activity were Gram-positive cocci, mainly *Micrococcus, Staphylococcus*, and *Kocuria*, followed by filamentous fungi, mainly *Cladosporium* and *Penicillium*; Gram-negative bacilli, mainly endospore-forming Gram-positive bacilli with *Bacillus*. The surfaces samples collected after the clinical activity showed a significant increase in bacterial load compared to the previous sampling ($p<0.001$). On the surfaces, concentrations of bacteria and fungi

were from 6,287 to 29,756 CFU/100 cm^2 and from 136 to 778 CFU/100 cm^2, respectively. During study period in dental equipment surfaces samples was detected 27 bacterial species from 8 genera, and 10 filamentous fungal species from 5 genera. Species of *S. aureus, S. epidermidis, E. faecalis, E. faecium, A. lwoffii, B. cepacia, E. cloacae, E. aerogenes, E. coli, K. oxytoca, P. aeruginosa, S. maltophilia*, and *S. marcescens* were found after clinical activity in all orthodontics, periodontic, oral surgery, prosthodontic room. The highest microbial diversities were found in surfaces of air/water syringe and light (orthodontics, periodontic, oral surgery, prosthodontic room) for bacteria and in the prosthodontic rooms for fungi and yeasts.

MOLECULAR ANALYSIS

In this study the 16S rDNA gene sequencing showed that the bacterial communities of all samples (air, water and environmental surfaces) covered 29 classes, 61 orders, 122 families, 143 genera, and 411 species. The distribution of microorganisms isolated from environmental samples in Ukrainian dental clinics in Ukraine is shown in table 2. The highest number of microorganisms was found in water samples and dental equipment surfaces. Potential human pathogens were detected in water, air and surface samples, including 7 genera of bacteria and 5 genera of fungi. The main potentially human-pathogenic genera of bacteria with relative abundance over 1% were *E. coli* (13%), *P. aeruginosa* (9.1%), *E. faecalis* (4%), *K. pneumoniae* (3.6%), *A. lwoffii* (3.5%), *A. baumannii* (3.3%), *K. oxytoca* (3.2%), *S. marcescens* (3.2%), *S. maltophilia* (3.2%), *S. aureus* (3.2%), *E. faecium* (3.1%), *E. cloacae* (3%), *E. aerogenes* (2.9%), *B. cepacia* (2.6%), *P. stuartii* (2.5%), *P. mirabilis* (2.2%), *P. rettgeri* (2.1%), *S. pneumoniae* (2.1%), *C. albicans* (2%), and other microorganisms (Table 3).

DISCUSSION

The environmental microbial contamination in Ukrainian dental setting was never studied. Therefore, the present study aimed to assess microbial contamination in the dental environment, identify the microorganisms involved, and determine their count in dental clinics. To the authors' knowledge, this study is the first to microbial and molecular analysis the microbiological contamination of air, water and dental equipment surface in dental setting. Environmental microbial contamination in dental clinics was comprehensively evaluated, with more abundance of bacterial communities using standard microbial methods and high-throughput sequencing technology. The findings of this study revealed tap water, DUWLs, and water from the bottles attached to the dental chair units (before clinical activity), and surfaces of dental equipment, and air (during clinical activities) are heavily contaminated with potential human pathogens. These results are consistent with previous studies [8, 20, 21]. Our previous study showed that the microbial load in dental rooms air is highly influenced by the ventilation and number of patients and health care workers, who can be a potential source of microorganisms as they shed the microorganisms from the skin squames and the respiratory tract [22].

The 16S rDNA gene sequencing showed that the main potentially human-pathogenic species of bacteria with relative abundance over 3% were *E. coli* (13%), *P. aeruginosa* (9.1%), *E. faecalis* (4%), *K. pneumoniae* (3.6%), *A. lwoffii* (3.5%), *A. baumannii* (3.3%), *K. oxytoca* (3.2%), S. marcescens (3.2%), *S. maltophilia* (3.2%), *S. aureus* (3.2%), *E. faecium* (3.1%), and *E. cloacae* (3%). The highest numbers of these pathogens were found in DUWLs water. There was noticeable contamination of air and dental equipment in surfaces the active dental treatment area. The bacterial aerosols were able to spread into areas where there was dental activity.

The present study showed that *P. aeruginosa*, *E. coli*, and *Enterococcus* sp. is the most important and difficult to eliminate pathogens commonly found in DUWLs. These pathogens originated from DUWLs may generate healthcare-associated infections (HAIs) [9], including like dental abscesses among patients [23]. The results this study highlights the need to perform microbiological analysis of DUWLs water routinely. However, to date, there is no standard about microbial water quality control of DUWL systems in dental healthcare setting. The water quality monitoring by sample analysis is recommended in the USA in dental setting to detect of pathogenic bacteria [24, 25]. In a previous study showed that P. aeruginosa could survive in DUWLs despite a disinfection, and the resistance of this strains to several biocides has been confirmed by antimicrobial susceptibility tests [26].

According to the literature, various regulations have been formulated to control clinical DUWLs contamination. The American Dental Association (ADA) set a limit of ≤ 200 CFU/mL on the TVC in the output water of the dental unit. In some EU countries and China, the drinking water standard of < 100 CFU/ml is referenced, clarified in the standard for prevention and control of HAIs in dental setting that the water from DUWLs should meet the drinking water standard (< 100 CFU/ml) [27]. In Ukraine there are no legislated parameters relating to the presence of P. *aeruginosa, E. coli, Enterococcus* sp., and other bacterial species in water from DUWLs.

According to the Ukrainian State Sanitary Norms and Rules of the DSanPiN 2.2.4-171-10, these bacterial species should not be listed to national statistical reports under water quality. In Ukraine there are two standards at the same time: SanPiN 2.2.4-171-10 and State standards of Ukraine DSTU 7525: 2014 (Drinking water). Currently, SanPiN 2.2.4-171-10 is a binding normative legal act. However, National standards of Ukraine DSTU 7525:2014 (2015) are optional. The Ministry of Health of Ukraine currently has no explicit requirements for the microbial parameters of water, which is supplied to DUWLs. Also, not any regulations related to aspects to curb the transmission of HAI or for infection control issues related to the dental setting.

STRENGTHS AND LIMITATION

This study is the first to environmental microbial contamination in dental clinics was comprehensively evaluated, with more abundance of bacterial communities using standard microbial methods and high-throughput sequencing technology. A limitation of this study is the absence of microbiological analysis the disinfection to evaluate the efficiency of the disinfectants used. The quantity of bacteria detected in water and dental equipment surfaces significantly. Further studies are required to assess the contamination rate as risk factor of HAIs and mechanisms of antimicrobial resistance these pathogens. In addition, further studies should integrate data on microbial water, air and environmental surfaces contamination with microbiologically examined pathogens and oropharyngeal swabs from patients with dental HAIs to identify pathogens from the oral cavity and perform the appropriate patient-related risk assessment for airborne and aerosol-transmitted infections in a dental setting.

CONCLUSIONS

The findings of this study revealed during study period in dental clinics tap water, DUWLs, and water from the bottles attached to the dental chair units (before clinical activity and during clinical activities), and surfaces of dental equipment, and air (during clinical activities) was heavily contaminated with potential pathogens of HAIs. Patients are exposed during dental practice to an infective risk. The primary sources of environmental contamination in Ukrainian dental clinics are bacteria from DUWLs water. The water output microbial contamination from DUWLs depends on the feed water of the supply unit, which are the main municipal water supply, external storage tanks, or isolated bottled reservoirs. The bacterial aerosols were able to spread into areas where there was dental activity. Therefore, environmental microbiological monitoring is crucial to infection control in dental settings. To control the environmental contamination, an internal control plan in each dental clinic is necessary, including an water and dental equipment surfaces disinfection a followed by a microbiological analysis to control its efficiency. Microbiological monitoring of DUWLs water should be performed routinely to control the microbial contamination of water used for dental care and to prevent of HAIs. This is necessary not only to assess the efficiency of preventive measures but also to guide the implementation of corrective strategies.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Serological detection of measles virus in association with IL-10 and IL-17 levels in aborted women

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ABSTRACT

Aim: To investigate immunological markers that may be associated with measles viral infections in aborted women, specifically cytokines IL-10 and IL-17.

Materials and Methods: From October 2024 to the end of December 2024, the study included 352 participants, comprising 176 females with a history of abortions and 176 females with normal pregnancies, who acted as a control group. Serum samples were analyzed for IL-10 and IL-17 using the enzyme-linked immunosorbent assay (ELISA) method.

Results: The serum level of IL-10 was significantly lower in women with abortion compared to the control group, with a median of 216.02 pg/ml versus 329.79 pg/ml, respectively ($p<0.001$). Similarly, the serum level of IL-17 was significantly higher in women with abortion compared to the control group, with a median of 85.24 pg/ml versus 51.76 pg/ml, respectively $p<0.001$).

Conclusions: The main clinical features of a viral infection, such as measles, during pregnancy are fever. Correlation of IL-10 and IL-17 to measles infection in the control group: Measles IgM and IgG showed no significant correlation to IL-10 and IL-17 ($p>0.05$). In the abortion group, measles IgM and IgG showed significant positive correlation to IL-10 and IL-17 $p<0.001$, finally, family history is a significant risk factor for abortion.

KEY WORDS: measles virus, Interlukein-10, Interlukein-17, enzyme-linked immunosorbent assay, abortion

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INTRODUCTION

Measles virus (MV) is a single-stranded, negative-sense RNA virus that belongs to the *Morbillivirus* genus and the *Paramyxoviridae* family. Its genome encodes at least six structural proteins, causing an acute, highly contagious infection primarily seen in children. Recovery is typically successful, but severe complications may occur [1]. It disseminates through the respiratory system via respiratory droplets, with humans being the only known reservoirs for MV [2]. MV infection during pregnancy is linked to a heightened risk of preterm labor, spontaneous abortion, and low birth weight in the neonate [3]. When measles appears late in pregnancy, it may lead to congenital infection, which can present in numerous ways and raise the risk of severe encephalitis in newly born infected mothers. Furthermore, pregnant individuals are at a higher risk of complications, such as pneumonia, compared to non-pregnant women [3-4]. Interleukin is a cytokine that facilitates communication between leukocytes and other cell types, exhibiting various stimulatory activities that regulate immune, inflammatory, and hematopoietic responses [5]. IL-10 is an important cytokine that reduces inflammation. It is produced by immune cells like macrophages, natural killer (NK) cells, dendritic cells (DCs), CD8 T cells, B cells, and dermal keratinocytes [6]. It regulates cellular immunity by limiting cell-mediated responses and reducing proinflammatory cytokines during infections, thereby mitigating their negative effects. In particular, IL-10 suppresses responses by weakening APC antigen presentation [6-7]. The IL-17 cytokine family comprises IL-17A through IL-17F. Interleukin-17 is produced by CD4 and Th17 cells [8]. Interleukin-17 (IL-17) is known as an inflammatory cytokine that mainly affects myeloid and Mesenchymal cells. It does this by encouraging the production of certain chemokine that attract neutrophils to infection sites [6, 9]. Cytokines play an important role in reducing the detrimental effects of viral infections, as well as pro-inflammatory reactions [8, 10].

AIM

Aim of this study is to investigate immunological markers that may be associated with measles viral infections in aborted women, specifically cytokines IL-10 and IL-17.

MATERIALS AND METHODS

PARTICIPANTS AND STUDY DESIGN

From October 2024 to the end of December 2024, the research was conducted. Samples were collected from Al-Rumaitha General Hospital and the Gynecology and Pediatrics Teaching Hospital in the Al-Muthanna Governorate. A total of 352 participants were included in the study, comprising 176 women who had previously experienced abortions and 176 women in a control group with normal pregnancies without abortions. All participants were between the ages of 18 and 45.

SAMPLES COLLECTION

According to the aim of this study, which focused on detecting each rubella and measles virus, the most relevant women with miscarriage were collected in the first trimester, particularly before 12–16 weeks, and extended to the second trimester within 18–20 weeks of pregnancy. This is because of a high risk of getting infection with these viruses, especially rubella virus infection with serious consequences for fetuses.

Miscarriages occurred between the first and twentieth weeks as follows:

In the first trimester, there were 108 women (23 in weeks 1–6, 33 in weeks 7–9, and 52 in weeks 10–13).

In the second trimester, the number of women was 68 (from the 14th to the 15th week, there were 12 women, and from the 16th to the 20th week, there were 56 women).

All women in the patient group had experienced a spontaneous miscarriage, while the control group included pregnant women who had not experienced any type of miscarriage.

This study includes 4 ml of whole blood samples from both control and patient groups. The samples are centrifuged for 5 minutes at 4000 rpm. Serum samples are then analyzed to detect virus antibodies and cytokine markers such as IL-10 and IL-17. ELISA can identify measles through the presence of IgG and IgM antibodies.

INCLUDED CRITERIA

I grouped patients based on specific criteria. All patients participating in this study are using specific data pertaining to their needs. The following patients were enrolled in the study and had their information gathered, as detailed in the questionnaire: The family has a medical history. Date of birth, place of residence, clinical picture, and profession are all pieces of demographic data. Through the review of women with miscarriage in hospitals, all clinical signs were examined and recorded, and their general health condition was confirmed. Based on the comparison between the aborted and non-aborted cases regarding specific symptoms, such as rash, fever, and other general symptoms related to the general health of these individuals.

EXCLUDED CRITERIA

The study established specific inclusion criteria for patients based on age groups, and it excluded patients 46 years old or older. The study also excluded patients with autoimmune diseases or chronic diseases.

IL-10 AND IL-17 AND MEASLES VIRUS IGG AND IGM INVESTIGATION

A human interleukin ELISA kit was used for the test. IL-10, IL-17 (Bioassay Technology Laboratory), and measles virus IgG and IgM were measured and detected according to the ELISA kit instructions (SunLong Biotech Co., LTD).

STATISTICAL ANALYSIS

Microsoft Office Excel 2010 and the Statistical Package for the Social Sciences (SPSS) version 23 were used for data collection, summarization, analysis, and presentation. To express normally distributed numerical variables as mean (a measure of central tendency) and standard deviation, quantitative (numerical) variables were first assessed for normal distribution using the Kolmogorov-Smirnov test. Quantitative (numerical) variables were represented by means and standard deviations, while qualitative (categorical) variables were represented by numbers and percentages (a measure of dispersion). The following statistical tests were used in this investigation: The Fisher exact test was used in situations where the chi-square test proved unreliable. A significance threshold was considered reached when the P-value was 0.05 or below.

RESULTS

Clinical features contrasted between the abortion group and the control group are shown in Table (1). A rash was seen in a single case in the abortion group, and fever was reported in a substantial proportion of patients in the abortion group. The rash symptom showed no significant variation ($p = 1.000$); whereas, fever showed a significant association with abortion $p<0.001$.

The risk association between current abortion and family history of abortion is shown in Table (2). It was observed that a positive family history of abortion was reported in 25.6%

Table 1. Clinical features contrasted between abortion group and control group

Characteristic	Abortion group n = 176	Control group n = 176	p
Rash			
Positive	1 (0.6%)	0 (0.0%)	1.000 F NS
Negative	175 (99.4%)	176 (100.0%)	
Fever			
Positive	45 (25.6%)	10 (5.7%)	<0.001 C ***
Negative	131 (74.4%)	166 (94.3%)	

n: number of cases; F: Fischer exact test; C: chi-square test; NS: not significant; ***: significant at p≤ 0.001
Source: compiled by the authors of this study

Table 2. Risk association between current abortion and family history of abortion

Characteristic	Abortion group n = 176	Control group n = 176	p	OR	95%CI
Family history of abortion					
Positive	45 (25.6%)	10 (5.7%)	<0.001 C ***	5.70	2.77 -11.74
Negative	131 (74.4%)	166 (94.3%)			

n: number of cases; OR: odds ratio; CI: confidence interval; C: chi-square test; ***: significant at p≤0.001
Source: compiled by the authors of this study

Table 3. Comparison of serum IL-10 and IL-17 levels between abortion and control groups

Characteristic	Abortion group n = 176	Control group n = 176	p
IL -10			
Median (IQR)	216.02 (65.55)	329.79 (323.88)	<0.001 M ***
Range	101.91 -504.65	18.54 -7858.60	
IL -17			
Median (IQR)	85.24 (52.37)	51.76 (19.45)	<0.001 M ***
Range	9.52 -1239.57	11.29 -950.62	

IQR: inter-quartile range; IL-10: interleukin-10; IL-17: interleukin-17; M: Mann Whitney U test; ***: significant at p ≤ 0.001.
Source: compiled by the authors of this study

Table 4. Correlation of IL-10 and IL-17 to measles and *Rubella* infection

Infection	Statistic index	Control group		Abortion group	
		IL -10	IL -17	IL-10	IL-17
Measles IgM	*r*	-0.121	-0.036	0.292	0.382
	p	0.111	0.632	<0.001 ***	<0.001 ***
Measles IgG	*r*	-0.003	-0.004	0.496	0.376
	p	0.973	0.960	<0.001 ***	<0.001 ***

*: significant at p ≤ 0.05; **: significant at p ≤ 0.01; ***: significant at p≤0.001
Source: compiled by the authors of this study

of women in the abortion group and in only 5.7% of women in the control group. Thus, the proportion is significantly higher in women with abortions than in the control group p<0.001. In terms of risk, the reported odds ratio was 5.7 (95% CI of 2.77-11.74); therefore, a woman with a positive family history of abortion is at approximately six times higher risk compared to women with a negative family history.

Comparison of serum IL-10 and IL-17 levels between the abortion and control groups is shown in Table (3) and Figures (1-2). The serum level of IL-10 was significantly lower in women with abortions compared to the control group, with a median of 216.02 pg/ml versus 329.79 pg/ml, respectively (p < 0.001). The serum level of IL-17 was also significantly higher in women with abortions compared to the control group, with a median of 85.24 pg/ml versus 51.76 pg/ml, respectively; p<0.001.

The correlation of IL-10 and IL-17 to measles infection is shown in Table (4). In the control group, measles IgM and IgG showed no significant correlation to IL-10 and IL-17 (p>0.05). In the abortion group, measles IgM and IgG showed a significant positive correlation to IL-10 and IL-17 (p<0.001).

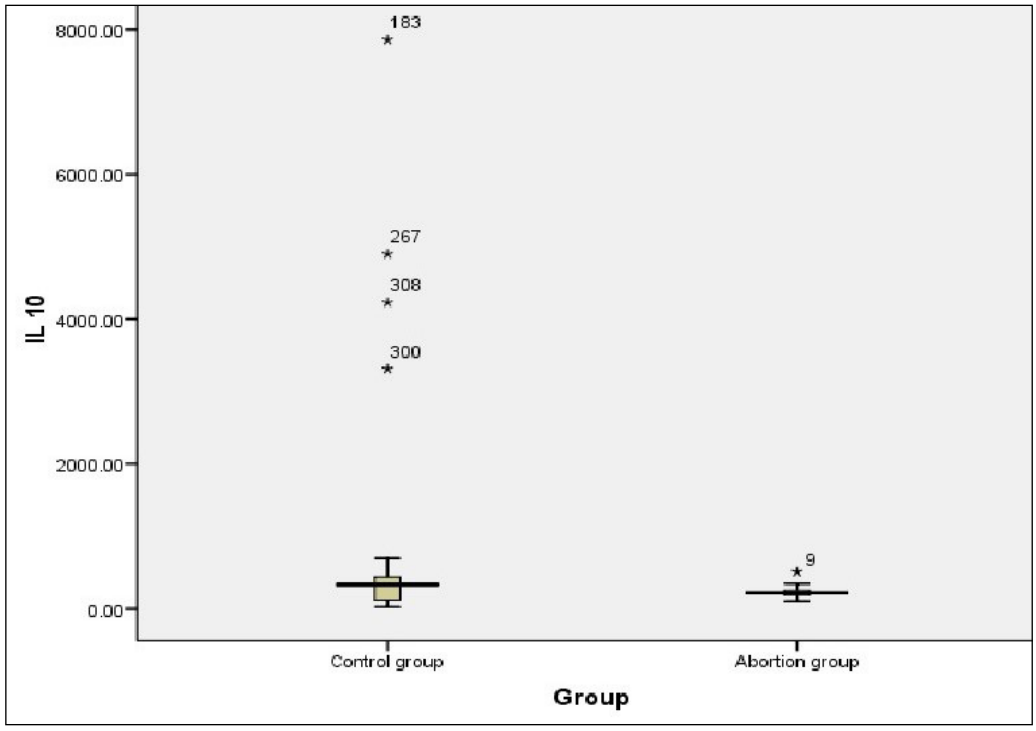

Fig. 1. Box plot showing comparison of serum IL-10 levels between abortion and control groups
Source: compiled by the authors of this study

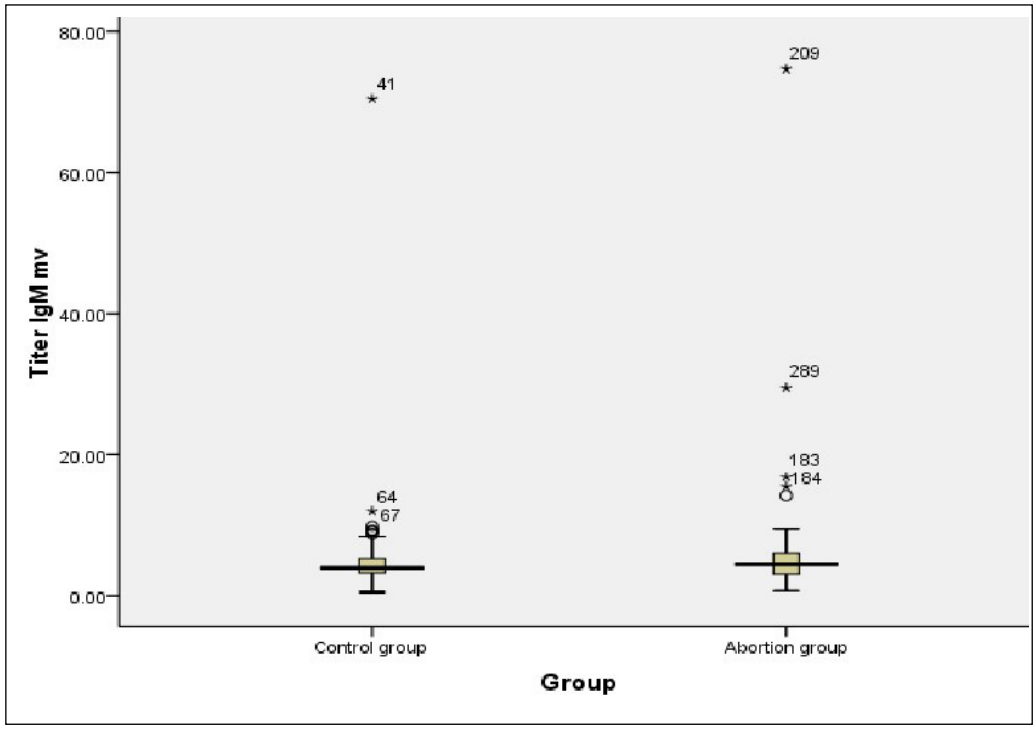

Fig. 2. Box plot showing comparison of serum IL-17 levels between abortion and control groups
Source: compiled by the authors of this study

Comparison of titer levels of IgM and IgG for measles virus between the patient group and the control group is shown in Figures 3 and 4. Regarding measles, there was no significant difference in the levels of IgM and IgG between the abortion group and the control group ($p>0.05$).

DISCUSSION

Regarding the association between fever and abortion in this study, the results align with previous reports [11-13]. One study reported that a significant proportion of women with fever 8% experienced abortion early in pregnancy [13]**.** Fever during early pregnancy is often a clinical sign of maternal viral or bacterial infection [14]. Among well-known viral infections, measles and rubella viruses are implicated as risk factors for adverse pregnancy outcomes. In a study of 55 pregnant women with measles, this viral infection was identified as a significant contributor to miscarriage p=0.011[15]. Additionally, this study found that a positive family history

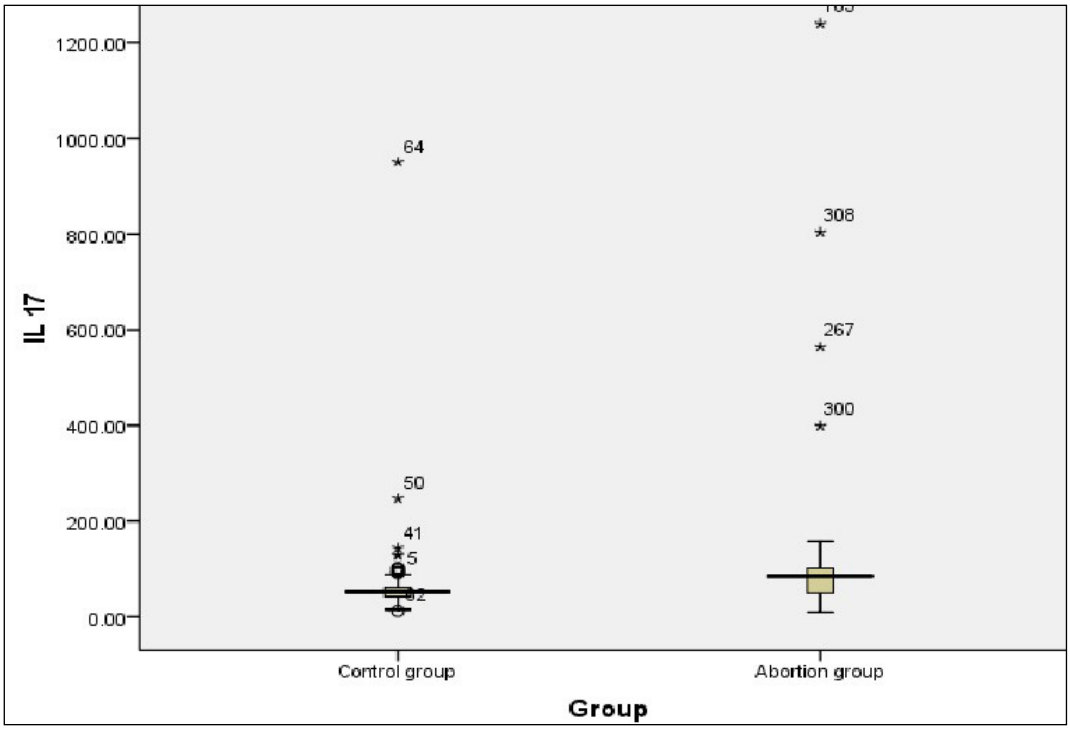

Fig. 3. Box plot showing comparison of IgM antibody titers for measles virus between abortion group and control group
Source: compiled by the authors of this study

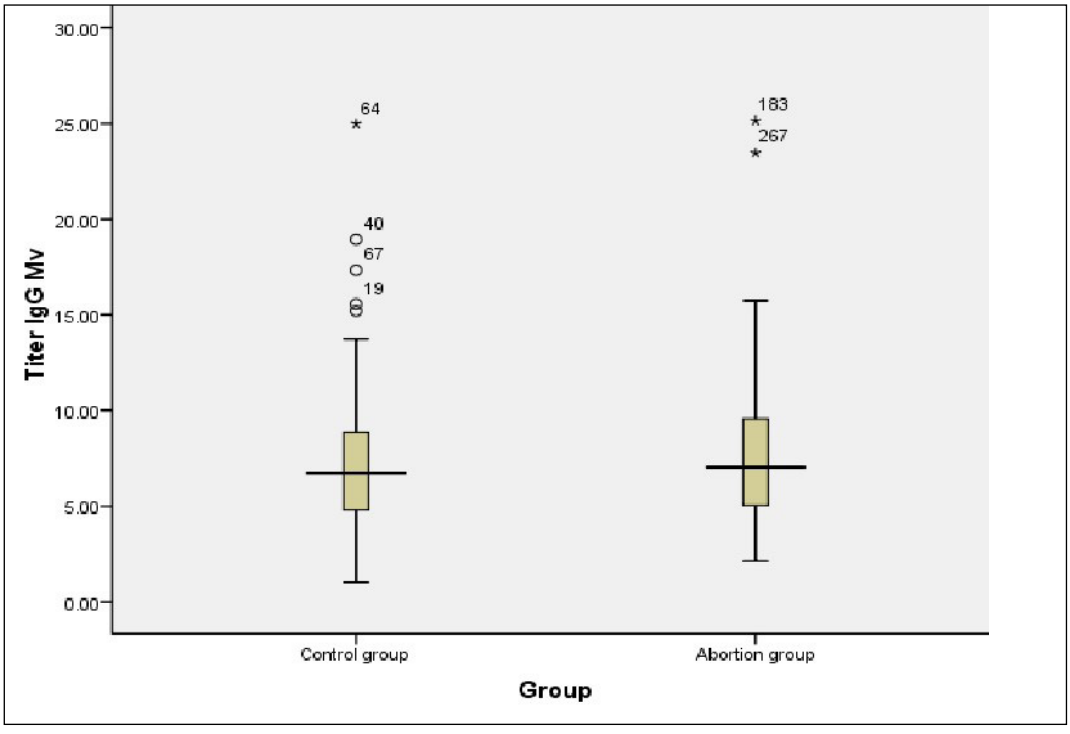

Fig. 4. Box plot showing comparison of IgG antibody titers of measles virus between abortion group and control group
Source: compiled by the authors of this study

of abortion is a significant risk factor. These findings are consistent with previous reports by several authors who examined the association between a positive family history and the current status of abortion [16-17]. Parental genetic polymorphisms related to immunological responses, coagulation processes, metabolic functions, and angiogenesis have been linked to idiopathic or recurrent miscarriage [18-19]. It is estimated that over 5% of couples experiencing recurrent miscarriage may carry chromosomal anomalies, which could increase the risk of chromosomal irregularities in the fetus, leading to miscarriage or recurrent miscarriage [20]. If genetic determinants, such as specific gene polymorphisms or chromosomal rearrangements, play a role in miscarriage or recurrent miscarriage, it is plausible that this genetic susceptibility could be transmitted through familial lineages. Numerous environmental and lifestyle factors have also been implicated in miscarriage, including exposure to ionizing radiation, environmental heavy metals, chemical substances, tobacco use, caffeine con-

sumption, extremes of body mass index, and illicit drug use [17, 21]. Additionally, it is conceivable that these factors may be inherited across generations within the same family, potentially explaining the familial patterns of miscarriage observed among relatives [17]. According to [22], the level of IL-10 is reduced in cases of abortion. Human IL-10, which has a variable immunologic function that can be either stimulatory or counter-regulatory, is located on chromosome 1q32. IL-10 is referred to as a Th2 cytokine and has anti-inflammatory effects against the cytokines produced by the Th1 subset [22]. Reports have also linked reduced IL-10 levels to premature birth [23]. Based on the observation of [24], median levels of IL-10 are statistically significantly lower in pathological conditions compared to matching gestational ages of normal pregnancy. Successful pregnancy depends on the mother's immune system's ability to undergo immunoregulation to tolerate the fetus and to create and sustain a nurturing environment throughout all stages of pregnancy. Several reports indicate that interleukin 10 (IL-10) is vital for normal pregnancy, and low IL-10 levels are associated with pregnancy complications [24]. A study conducted by Sharief et al., (2014) showed that serum IL-10 levels are reduced in cases of abortion [25]. On the contrary, Bakir et al., [26] proved that IL-10 was higher in control group than in recurrent spontaneous [26]. IL-17 expression has been evaluated in various pregnancy-related situations, although the data from these studies are often controversial. For instance, one study reported higher IL-17 levels in patients with unexplained recurrent spontaneous abortion (URSA) compared to women with normal early pregnancies. Importantly, in this study, samples were obtained before artificial miscarriage [27]. Conversely, Hosseini et al., [28] found high IL-17 levels in the menstrual blood of healthy fertile women but not in URSA patients, suggesting that high IL-17 levels may create an optimal environment for successful embryo implantation [28]. Additionally, the invasion of maternal tissues by the fetus can be compared to an allograft, and Th17 cells have been reported to play a significant role in allograft rejection [29]. The proposal of a Th1/Th2 balance to a favorable pregnancy outcome, in which a Th2- type cytokine response is predominant, was reinforced by a Th1 prevalence in various pregnancy complications. However, this dichotomy has been challenged by recent findings regarding the role of Th17 cytokines both in normal and pathological pregnancies [30]. Consistent with other research, this study has not found any evidence that measles causes abortions [31-32]. During viral infections, levels of interleukin-10 (IL-10) and interleukin-17 (IL-17) are significantly altered, reflecting both proinflammatory and anti-inflammatory immune responses. For instance, IL-10, which works as an anti-inflammatory cytokine, is commonly elevated in many viral diseases, including papillomavirus infection as cutaneous or mucosal warts, COVID-19, human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV). Its elevation is often associated with disease severity, immunosuppression, and poor clinical outcomes. For example, in both *Papillomaviral* infection and COVID-19, IL-10 levels are significantly higher in severe and critical cases, contributing to immune dysfunction and cytokine storms [33-35].

CONCLUSIONS

The main clinical features of a viral infection, such as measles, during pregnancy are fever. Correlation of IL-10 and IL-17 to measles infection in the control group: Measles IgM and IgG showed no significant correlation to IL-10 and IL-17 ($p>0.05$). In the abortion group, measles IgM and IgG showed significant positive correlation to IL-10 and IL-17 ($p<0.001$).

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ETHICAL STATEMENT

The Research Committee of Al-Muthanna Health Directorate (No. 1627 on 27/10/2024) approved this study, and participants' consent was obtained verbally and in written form, printed for consent in hospitals.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Volitional qualities development in future law enforcement officers during various types of motor activity

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ABSTRACT

Aim: To investigate the impact of various types of motor activity on the development of cadets' indicators of volitional qualities during their training at higher educational institutions.

Materials and Methods: The research, conducted in the 2024-2025 academic year, involved 352 male cadets, who were divided into three groups: the HHC Group (n=54), whose cadets participated in hand-to-hand combat (HHC) during their sporting and mass participation events; the SC Group (n=91), whose cadets participated in various sports clubs of the academy; the K Group (n=207), whose cadets did not participate in additional sports. Research methods: analysis and generalization of literary sources, methods of assessing volitional qualities, and methods of mathematical statistics.

Results: It was found that the cadets of the HHC Group showed significantly better results in terms of volitional qualities in their senior training years than those in the K group, which indicates a more pronounced effect of HHC training sessions on the development of volitional qualities in future law enforcement officers. At the same time, no significant difference was found between the indicators of cadets in the HHC and SC groups, which indicates the effectiveness of additional sports activities in developing cadets' volitional qualities.

Conclusions: The comprehensive impact of hand-to-hand combat training sessions on the volitional qualities of cadets has been proven. This emphasizes the advisability of wider introduction of hand-to-hand combat techniques into the physical training of cadets during their studies at higher educational institutions to improve the effectiveness of their future service and professional activities.

KEY WORDS: volitional qualities, motor activity, hand-to-hand combat, cadet, war

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INTRODUCTION

The specific nature of modern law enforcement service and combat activities, which are accompanied by the negative impact of various psycho-traumatic factors, places high demands not only on the physical readiness of future specialists, but also on their psychological readiness, since it is the level of physical and psychological readiness of law enforcement officers that determines not only the effectiveness and results of their activities, but also their personal safety [1, 2]. Scientists [3-5] argue that psychological readiness, as a conscious reflection of the existing potential and current state of the "personality-activity" system, ensures the formation of the personal content of a law enforcement officer's participation in service and combat activities, the timeliness and expediency of current operational actions, their adequacy to the conditions of situations, situational stability and regulation of actions when tension rises, and the restoration of the initial characteristics of the psychological structure of activity after the law enforcement officer has performed service and combat missions. Among the components of the psychological readiness of law enforcement officers for their service and combat activities, scientists [6, 7] highlight volitional readiness, which involves the development of qualities such as purposefulness, self-confidence, courage, determination, self-control, endurance, and readiness to take risks.

Scientists [8] note that, being the active side of the mind and moral sense willpower manifest itself in

voluntary actions aimed at achieving a set goal. Each volitional manifestation contains: intellectual components, because willpower is the active side of the mind; emotional (motivational) components, because willpower is the active side of moral feelings; operational components (skills), because volitional manifestations are associated with overcoming obstacles [9, 10].

A summary of the data from literary sources [11-13] allows us to conclude that motor activity (exercise and sports) plays an essential role in the formation of the psychological (volitional) readiness of law enforcement officers to act in extreme conditions of their service and combat activities and in increasing their psychological resistance to stress. This is because motor activity (MA) ensures the development and improvement of not only the physical but also the mental aspects of a person [14]. When used correctly, MA can significantly improve all components of law enforcement officers' moral and psychological readiness: moral state, volitional qualities, emotional stability, and mental abilities [15]. Experts argue that MA can increase the body's resistance to the effects of psycho-traumatic factors of service and combat activities by 30-40 % [16].

According to scientists [17], the most significant impact of MA is on the willpower and emotional stability of law enforcement officers (the emotional and volitional components of psychological preparedness). Experts [18] identify the following volitional qualities that are most important for law enforcement officers' actions in extreme situations: courage, determination, initiative, ingenuity, resourcefulness, perseverance, persistence, endurance, self-control, and self-confidence. Courage and determination are developed during physical exercises that contain elements of novelty, risk and danger; initiative, ingenuity and resourcefulness are most effectively developed during exercises that require independent decision-making; perseverance and persistence are developed through the use of physical exercises, during which law enforcement officers experience significant and prolonged physical exertion and mental stress; endurance and self-control are practiced in the course of performing precision movement exercises under conditions of physical loads and mental stress that are constantly changing and becoming more complex. Therefore, it is timely and relevant to study the impact of various types of MA on the development of volitional qualities of cadets – future law enforcement officers during their training at higher educational institutions with specific learning environments (HEI with SLE).

AIM

The aim is to investigate the impact of various types of motor activity on the development of cadets' indicators of volitional qualities during their training at higher educational institutions.

MATERIALS AND METHODS

PARTICIPANTS

The research, conducted in the 2024-2025 academic year, involved 352 male cadets from the National Academy of Internal Affairs (NAIA, Kyiv, Ukraine) majoring in "Law Enforcement" and "Law" specialties. To study the impact of various types of motor activity on the development of cadets' volitional qualities indicators, we formed three groups of cadets based on the results of a survey: the HHC Group (n = 54) included cadets who, in addition to attending compulsory special physical training sessions during their studies at the HEI with SLE, also systematically participated in a hand-to-hand combat sports club during their sporting and mass participation events (SMPEs) (the 1st training year – 14 people, the 2nd training year – 16 people, the 3rd training year – 13 people, the 4th training year – 11 people); the SC Group (n = 91) included cadets who, like the cadets in the HHC Group, additionally participated in sports during their SMPEs hours in various sport clubs of the HEI with SLE in polyathlon, CrossFit, multi-event competitions, orienteering, sports games, and powerlifting (the 1st training year – 24 people, the 2nd training year – 26 people, the 3rd training year – 23 people, the 4th training year – 18 people); the K Group (n = 207) included cadets who, during their studies, attended only compulsory training sessions in special physical training and did not engage in additional sports activities; their SMPEs were conducted according to standard approved options (the 1st training year – 53 people, the 2nd training year – 45 people, the 3rd training year – 54 people, the 4th training year – 55 people). The amount of physical activity per week (in hours) did not differ between the study groups. The primary variable was the content of the MA training sessions across the groups. Inclusion criteria: male cadets, willingness to engage in a particular sport during training (determined by a survey at the beginning of the academic year), no health contraindications to sports; exclusion criterion – the cadet's desire to withdraw from the research at any convenient time.

RESEARCH METHODS

Analysis and generalization of literary sources, psychodiagnostic methods, methods of mathematical statistics. Analysis and generalization of literary sources

Table 1. The level of volitional qualities development in cadets of the HHC Group (n = 54), the SC Group (n = 91), and the K Group (n = 207), M ± m, in points

Training year	Number of people	HHC Group	Number of people	SC Group	Number of people	K Group	Level of significance, p		
							HHC-SC	HHC-K	SC-K
Methodology "Willpower Study"									
1	14	14.5±1.43	24	15.0±1.17	53	13.6±0.79	>0.05	>0.05	>0.05
2	16	18.1±1.32	26	18.9±1.08	45	16.5±0.76	>0.05	>0.05	>0.05
3	13	22.6±1.24	23	21.8±0.99	54	19.1±0.68	>0.05	<0.05	<0.05
4	11	26.1±1.19	18	25.7±0.94	55	22.3±0.71	>0.05	<0.05	<0.05
Methodology "Impulsivity Study"									
1	14	48.2±2.81	24	47.8±1.31	53	48.6±0.57	>0.05	>0.05	>0.05
2	16	44.7±2.79	26	45.3±1.19	45	46.9±0.66	>0.05	>0.05	>0.05
3	13	41.6±2.41	23	42.1±1.22	54	45.1±0.55	>0.05	>0.05	<0.05
4	11	38.8±2.28	18	40.3±1.37	55	43.7±0.52	>0.05	<0.05	<0.05
Methodology "Volitional Self-Regulation Study"									
1	14	14.8±1.34	24	15.1±1.08	53	14.6±0.73	>0.05	>0.05	>0.05
2	16	16.3±1.29	26	16.3±1.04	45	15.4±0.69	>0.05	>0.05	>0.05
3	13	18.9±1.22	23	17.9±1.01	54	16.3±0.67	>0.05	>0.05	>0.05
4	11	20.6±1.27	18	19.6±0.98	55	17.5±0.64	>0.05	<0.05	<0.05
Methodology "Patience Self-Assessment"									
1	14	11.2±1.09	24	10.9±0.79	53	10.7±0.52	>0.05	>0.05	>0.05
2	16	13.5±1.04	26	12.7±0.75	45	12.1±0.59	>0.05	>0.05	>0.05
3	13	15.9±1.03	23	14.8±0.73	54	13.5±0.51	>0.05	<0.05	>0.05
4	11	17.8±1.07	18	16.9±0.81	55	14.8±0.48	>0.05	<0.05	<0.05

Notes: M – arithmetic mean; m – error of the arithmetic mean; p – reliability of the difference between the indicators of the cadets of the studied groups, which was determined using Student's t-test

Source: compiled by the authors of this study

were used to conduct an analytical review of scientific sources on the outlined range of issues (23 sources from PubMed, Scopus, Web of Science, and Index Copernicus were analyzed).

Psycho-diagnostic methods involved the use of several methodologies for assessing the volitional qualities of cadets: "Willpower Study" (according to M. M. Obozov); "Impulsivity Study" (according to V. A. Losenkov); "Volitional Self-Regulation Study" (according to A. V. Zvierkov and Ye. V. Eidman); "Patience Self-Assessment" (according to Ye. P. Iliin and E. K. Feshchenko) [19, 20].

METHODS OF MATHEMATICAL STATISTICS

The methods of mathematical statistics were used to process the data obtained. The compliance of the sample data distribution with the Gauss' law was assessed using the Shapiro-Wilk W test. The reliability of the difference between the indicators was determined using the Student's t-test. The reliability of the difference was set at $p<0.05$. All statistical analyses were performed using SPSS software, version 10.0, adapted for medical and biological research.

ETHICS

The procedure for organizing the study and the topic of the article were previously agreed with the Committee on compliance with Academic Integrity and Ethics of the NAIA. Also this study followed the regulations of the World Medical Association Declaration of Helsinki. Informed consent was received from all participants who took part in this study.

FRAMEWORK
This scientific article was carried out according to the plan of the research work of the National Academy of Internal Affairs for 2020-2026 "Psychological, pedagogical and sociological support of law enforcement officers» (state registration number 0113U008196).

RESULTS

Using the "Willpower Study" methodology, we studied the ability of cadets to apply volitional efforts in overcoming obstacles. The analysis of the results shows that in the 1st and the 2nd training years, the indicators of cadets in all three groups do not differ significantly ($p > 0.05$). In the 3rd training year, the level of cadets' volitional qualities in the HHC Group (22.6 points) and the SC Group (21.8 points) was significantly ($t = 2.47$; $t = 2.25$; $p < 0.05$) better than that of the K Group (19.1 points) by 3.5 points and 2.7 points, respectively. At the same time, the indicators of cadets in the HHC Group and SC Group were significantly the same ($p > 0.05$). In the 4th training year, the ratio of indicators did not change – the cadets who practiced HHC and other sports had a significantly ($t = 2.74$; $t = 2.89$; $p < 0.05$) higher level of their volitional qualities than the cadets who attended their SMPEs using traditional methods. Moreover, in the 4th training year, cadets in the HHC Group had the highest level of development of their volitional qualities – 26.1 points. This value is significantly better than in the K Group, by 3.8 points ($t = 2.74$; $p < 0.05$) and significantly the same as the indicators of the SC Group ($p > 0.05$) (Table 1).

The analysis of the dynamics of indicators of volitional qualities development showed that in all groups there was a significant improvement in volitional qualities: in the HHC Group – by 11.6 points ($t = 6.24$; $p < 0.001$), in the SC Group – by 10.7 points ($t = 7.13$; $p < 0.001$), in the K Group – by 8.7 points ($t = 8.19$; $p < 0.001$). Instead, the most significant changes were observed in the indicators of cadets of the HHC Group, which emphasizes the effect of HHC training sessions on the development of volitional qualities of cadets – future law enforcement officers.

The analysis of the results of the study of cadets' impulsivity shows that in the 1st and the 2nd training years, between the studied groups of cadets, there was no significant difference ($p > 0.05$). In the 3rd training year, the level of impulsivity in the HHC Group and the SC Group was lower than in the cadets of the K Group by 3.5 and 3.0 points, respectively, but a significant difference was found only between the SC Group and the K Group ($t = 2.24$; $p < 0.05$). In the 4th training year, in the HHC Group (38.8 points) and the SC Group (40.3 points), the level of impulsivity was also significantly lower than in the K Group (43.7 points), by 4.9 points ($t = 2.10$; $p < 0.05$) and 3.4 points ($t = 2.32$; $p < 0.05$), respectively. In the 4th training year, the HHC Group showed the lowest level of impulsivity, which indicates a positive effect of HHC training sessions on the development of volitional qualities of future law enforcement officers; however, the level of impulsivity of cadets of the HHC Group and the SC Group is significantly the same ($p < 0.05$). The analysis of the mean values characterizing cadets' impulsivity, regardless of their training years, shows that the average value (43.3 points) for the HHC Group is the highest among the three groups under study. Still, the indicators of impulsivity do not differ significantly ($p > 0.05$) in the HHC Group and the SC Group. In the K Group (46.1 points), the indicators are considerably worse than in the groups of cadets who were engaged in HHC and other sports. In the process of training at the HEI with SLE, the level of impulsivity in cadets of all three groups significantly decreased: in the HHC Group – by 9.4 points ($t = 2.60$; $p < 0.05$); in the SC Group – by 7.5 points ($t = 3.96$; $p < 0.001$); in the K Group – by 4.9 points ($t = 6.35$; $p < 0.001$).

The analysis of the results using the methodology referred to as "Volitional Self-Regulation Study" shows that, in the 1st, 2nd, and 3rd training years, the level of volitional self-regulation among cadets who were additionally engaged in sports (HHC and SC) though was higher than in the K Group, but there is no significant difference between the obtained indicators ($p > 0.05$). In the 4th training year, the level of volitional self-regulation in the HHC Group was the best (20.6 points); in the SC Group (19.6 points), the indicators were worse than in the HHC Group by 1.0 points, but no significant difference with the HHC Group was found ($p > 0.05$). Instead, in both the HHC Group and the SC Group, the level of volitional self-regulation in the 4th training year is significantly better than in the K Group, by 3.1 points ($t = 2.18$; $p < 0.05$) and 2.1 points ($t = 2.05$; $p < 0.05$), respectively (Table 1). The comparison of the mean values of the level of volitional self-regulation of cadets of the three groups under study confirmed the positive effect of additional sports activities during training in the HEI with SLE and HHC, in particular, on the formation of volitional qualities of future law enforcement officers: in the HHC Group (17.7 points) and the SC Group (17.2 points), the average value is significantly better than in the K Group (15.9 points) by 1.8 points ($t = 2.16$; $p < 0.05$) and 1.3 points ($t = 2.69$; $p < 0.05$), respectively. Between the HHC Group and the SC Group, despite a difference of 0.5 points, no significant difference was found ($p < 0.05$). The research also found that during the course of training at the HEI with SLE, the level of volitional self-regulation of cadets of all

three groups significantly improved: in the HHC Group – by 5.8 points ($t = 3.14$; $p < 0.01$), in the SC Group – by 4.5 points ($t = 3.29$; $p < 0.01$); in the K Group – by 2.9 points ($t = 2.99$; $p < 0.01$).

The level of cadets' patience was assessed using the self-assessment of patience methodology proposed by Ye. P. Iliin and Ye. K. Feshchenko. The maximum number of points that could be scored using this methodology is 18 points. The results of the study on the level of patience show that in the 1st and 2nd training years, there was no significant difference in the indicators of cadets across all three groups ($p > 0.05$). In the 3rd training year, the level of patience in the HHC Group was the best (15.9 points) and significantly better than in the K Group by 2.4 points ($t = 2.09$; $p < 0.05$). In the 4th training year, the ratio of indicators has not changed – in the HHC Group, the level of patience (17.8 points) is the best among the study groups and is significantly better than in the K Group (14.8 points) by 3.0 points ($t = 2.56$; $p < 0.05$). In the SC Group (16.9 points), the level of patience in the 4th training year was also significantly better than in the K Group, by 2.1 points ($t = 2.23$; $p < 0.05$). However, no significant difference was found between the HHC Group and the SC Group in the 3rd and 4th training years ($p < 0.05$) (Table I). The analysis of the mean values by groups of cadets showed that the best level of patience was found in cadets of the HHC Group (14.6 points), and the worst in cadets of the K Group (12.7 points). The difference between the HHC Group, the SC Group, and the K Group is 1.9 points and 1.1 points, respectively, and is significant ($t = 2.97$; $p < 0.05$; $t = 2.96$; $p < 0.05$). The difference between the mean values of the HHC Group and the SC Group is not significant ($p < 0.05$). It was also found that in the process of training the level of patience of cadets of all three groups improved, the difference between the indicators of cadets of the 1st and 4th training years in the HHC Group is 6.6 points ($t = 4.32$; $p < 0.001$), in the SC Group – 6.0 points ($t = 5.30$; $p < 0.001$), in the K Group – 4.1 points ($t = 5.79$; $p < 0.001$). The level of cadets' patience across all three groups in the 1st training year was assessed as average, and in the 2nd, 3rd, and 4th training years, as high. However, the most pronounced effect of sports was found in the HHC Group. The results obtained with this methodology indicate a positive impact of HHC training sessions on cadets' patience, which, in the future, will contribute to the effectiveness of performing specific tasks in service and combat activities.

DISCUSSION

According to scientists [3, 18, 21], volitional qualities are relatively stable mental formations that are independent of specific situations and reflect the level of conscious self-regulation of behavior and self-control achieved by an individual. These include purposefulness, determination and courage, independence, perseverance, initiative, endurance, etc. Purposefulness is the ability to focus on a specific goal consciously. This quality includes not only a clear awareness of the goal, but also the choice of the most effective ways to achieve it, and the ability to take systematic action to accomplish it. Determination and courage are individual qualities of willpower associated with the ability and skill to make responsible decisions in a timely and independent manner and to implement them consistently in one's activities. Perseverance is the ability to take sustained, active, energetic action and to be effective in overcoming obstacles. Perseverance is particularly evident when a person is in a problematic situation, facing difficulties and obstacles on the way to achieving a goal. Patience manifests itself in prolonged resistance to adverse factors. In this case, willpower reveals its initiating function, prompting a person to maintain the current state of affairs despite the situation that has arisen. Initiative manifests itself in the ability to take active actions driven by a person's own attitudes, ideas, and beliefs. Initiative manifests itself in creativity, innovation, independence, and resistance to external influences. Self-control (emotional stability) involves maintaining clarity of thought, controlling emotions in challenging situations, and managing one's actions under stress. These qualities manifest differently in specialists' personal potential. Still, together they define the ability to endure and maintain self-control, to withstand physical exertion and mental stress in the course of service and professional activities.

Scientists [6, 8, 10] indicate that the volitional process goes through several basic stages: 1) the emergence of motivation and goal setting; 2) the stage of discussion and struggle of motives; 3) decision making; 4) execution. At the first stage, the emerging need is reflected in consciousness as an urge, the object of which is not yet realized. As the need grows and its object becomes clear, the urge turns into a desire, which becomes the stimulus for action. At this stage, incompatible motives often clash, and a choice must be made between them. In the struggle between motives, willpower is revealed, and the goal of the activity is formulated, which finds expression in the decision-making process. After the decision is made, the tension that accompanied the struggle between motives weakens, and the person feels relief. In a complex volitional act, the decision-making process is followed by planning activities to achieve the set goal and by determining the means to do so. After that, the person proceeds with the planned actions. Achieving a specific goal may involve overcoming external obstacles (objects, people, time,

space) and internal obstacles (fatigue, illness, lack of knowledge, etc.). As previous scientific studies [5, 11, 14] and our results show, existing obstacles often create tension among law enforcement officers, which must be countered by support for the volitional sphere of the personality and movement toward the set goal. Analysis of a complex volitional act shows that it involves various mental processes, skills, abilities, and talents.

As part of our research, the assessment of the volitional qualities of future law enforcement officers was conducted using the following methods: "Willpower Study," "Impulsivity Study," "Volitional Self-Regulation Study," and "Patience Self-Assessment." The assessment of results obtained using the "Willpower Study" methodology shows that in the 1st and 2nd training years, the willpower of cadets in all three groups is at the average level; in the 3rd training year, a high level was observed in the HHC Group. In other groups – average, and in the 4th training year, a high level was found in all groups. This indicates that physical exercise and sports during training at the HEI with SLE contribute to the development of volitional qualities in cadets across all three groups, enabling them to confidently overcome the challenges of training and future service and combat activities. At the same time, hand-to-hand combat training sessions have the most pronounced effect on willpower.

At the same time, it should be noted that the higher the impulsivity, the lower the level of development of volitional qualities. Impulsive people often have vague life plans; they have no interests and are easily distracted [22]. People with low impulsivity, on the contrary, are purposeful, have clear value orientations, show persistence in achieving their goals, and try to bring what they have started to completion. Our analysis of impulsivity levels once again confirmed the effectiveness of physical exercise and sports in general, and HHC in particular, in developing the volitional qualities of future law enforcement officers. According to the normative table, the level of impulsivity among cadets in all three study groups in all training years is assessed as average. The decrease in the level of impulsivity among cadets in all three groups indicates that in the process of training at the HEI with SLE, they improve such qualities as purposefulness (conscious and active focus on a specific result of activity), perseverance and zeal (the ability to overcome external and internal obstacles in achieving the set goal), independence (the ability to act independently, set goals independently, and organize actions independently to achieve these goals), self-control and restraint (the ability and habit of controlling one's behavior, managing oneself, one's movements, speech, refraining from impulsive actions, and ill-considered emotional reactions), clear value orientations are formed (aimed at developing readiness to perform specific tasks of future service and combat activities).

Scientists [12, 17] define volitional self-regulation as the degree of control over one's own behavior across situations, the ability to manage one's actions, states, and impulses consciously. A high level of volitional self-regulation development is characteristic of emotionally mature, active, independent, and self-reliant individuals. They are characterized by calmness, self-confidence, steadfastness of purpose, realistic views, and a developed sense of duty. As a rule, they reflect well on their personal motives, systematically implement their intentions, know how to distribute their efforts, and can control their actions; they have a pronounced socially positive orientation. A low level of volitional self-regulation is observed in people who are sensual, emotionally unstable, vulnerable, and insecure. Their reflectivity is low, and their overall activity level is usually reduced; they tend to be impulsive and unstable in their intentions. This may be due to both immaturity and a pronounced refinement of character, which is not supported by the ability to reflect and exercise self-control [20]. During our research, we found that HHC training sessions have the most pronounced effect on volitional qualities, which determines the expediency of wider introduction of HHC into the physical training of future law enforcement officers. However, it should also be noted that the level of volitional self-regulation of cadets across all three study groups and all training years is assessed as high, indicating the effectiveness of both additional sports activities and the traditional methods of organizing and conducting physical training at the HEI with SLE. Our results confirm the conclusions of many scientists [3, 5, 13, 16, 23] and complement them.

CONCLUSIONS

The study of the level of development of volitional qualities in cadets who were additionally engaged in HHC during their training at the HEI with SLE, compared to cadets who were involved in other sports and cadets who were engaged in the traditional methodology during their SMPEs, shows that according to most of the applied methods, the studied indicators in the HHC Group of cadets in senior training years are significantly better than in the K Group, which indicates a more pronounced effect of HHC training sessions on the level of development of volitional qualities of future law enforcement officers. At the same time, across all methodologies, no significant difference was found between the indicators of cadets from the HHC Group and the SC Group, indicating the effectiveness of additional sports training in developing cadets' volitional qualities. However, such a complex influence on the development of motor skills, service-applied motor skills in HHC, and the education of volitional qualities is not provided

by any other sports. This emphasizes the expediency of wider implementation of HHC in the physical training of cadets at HEIs with SLE to improve the effectiveness of their future service and professional activities.

PROSPECTS FOR FURTHER RESEARCH

It is planned to investigate the impact of various types of motor activity on cadets' physical development and functional state during their training.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Designing and molecular docking analysis of newly acetazolamide derived compounds integrating 4-oxothiazolidine group as possible carbonic anhydrase deactivators

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ABSTRACT

Aim: The study aims to create new acetazolamide products that have a thiazolidin-4-one moiety and test how well they stop the carbonic anhydrase XII enzyme (protein data bank code: (4KP5)) and defeat cancer in silico.

Materials and Methods: We will make four acetazolamide derivatives that have a thiazolidin-4-one group. Acetazolamide will be the starting material. We used the Molecular Operating Environment program 2015.10 to figure out the molecular docking studies. Root Mean Square Deviation (RMSD) and Standard Score (S. score) were used to describe docking how well docked ligands stuck to the target protein carbonic anhydrase XII.

Results: The process of docking showed that the four test ligands (IIIa, IIIb, IIIc, and IIId) bound to the target protein carbonic anhydrase XII more strongly than the reference ligand, which is an acetazolamide.

Conclusions: Of the four chemicals, IIIa had the maximum affinity for binding and a S. score of -7.39. The results propose that the acetazolamide compounds that were designed, especially IIIa, could be carbonic anhydrase inhibitors and anti-cancer drugs that work on the carbonic anhydrase XII enzyme.

KEY WORDS: molecular docking, cancer, acetazolamide derivatives, thiazolidinone moiety, carbonic anhydrase

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INTRODUCTION

Enzymes are very important for many biochemical reactions that happen in living things. Because of how selective and powerful they are at starting certain chemical events, they are very interesting candidates for therapeutic intervention. There has been a rise in the utility of enzymes as promising drug goals in the last few years because they play a role in many diseases, such as cancer, metabolic disorders, and infectious diseases [1-2]. The word "cancer" describes a vast range of disorders that are given rise to the growth of abnormal cells which able to proliferate out of control, infiltrate and damage vital tissue, and destroy it [3]. Cancer is still odd of the most common source of dying around the globe, coming after heart disease [4]. Among the largest problems confronting healthcare agencies is that cancer treatments are failing more often, mostly because tumours are becoming resistant to anticancer drugs [5]. Four new acetazolamide derivatives that have a thiazolidin-4-one moiety are being studied in silico. These derivatives target carbonic anhydrase and are part of new strategies to deal with intrinsic tumour cell heterogeneity, stabilize DNA damage, inactivate drugs, changing targets of drug, and stop cells from dying. These variables all play a big role in drug resistance [6-7]. It seems that tumour protection mechanisms based on the microenvironment have a better chance of being targeted for treatment. The microenvironment is very important in cancer research because it affects how well cancer cells survive and spread [8-9]. The acidity and lack of oxygen in the tumour microenvironment affect how cancer cells work. Acidosis and hypoxia usually make cancer cells more aggressive, move around, and spread, which makes treating tumours much harder. Because of this, therapies that stop these processes are better [10], because of this, there has been a lot of research on the family of carbonic anhydrase enzyme, which affect the pH inside cells [11]. Carbonic anhydrase enzyme (CA) is a group of enzymes which have a metal of zinc ion in the active site and can convert water and carbon dioxide to bicarbonate and a hydrogen ion in a reversible way [12]. There are 15 different types of carbonic anhydrase enzymes in humans. These types have different catalytic activities and locations. CA I, CA III, CA VII, and CA XIII are

examples of cytosolic carbonic anhydrases. CA VA and CA VB are examples of mitochondrial carbonic anhydrases. CA VI is an example of a secretory carbonic anhydrase that can be found in saliva and colostrum. CA IV, CA IX, CA XII, CA XIV, and CA XV are examples of membrane-attached carbonic anhydrases. Three "CA- linked proteins," CA VIII, CA X, and CA XI, are also types that are not active of carbonic anhydrase [13]. There is too much (CA XII) in some solid tumours, but not enough in other healthy tissues. This overexpression helps tumour cells grow, stay alive, and spread in the tumour microenvironment. So, CA XII has been proven as a good objective for cancer examination and management [14]. It changes the acidity, growth, and aggressiveness of tumours, starts the metastatic cascade, and makes tumours less responsive to chemotherapy [15]. When CA XII is overexpressed, it causes cancer to grow. Higher levels of CA XII and CA IX make cancer cells grow faster, start spreading, and make treatments less effective. Hypoxia causes CA XII upregulation in many lines of cells, just like CA IX. However, CA XII expression is found in kidney and breast cells, while CA IX is not. Oestrogen receptors and low oxygen levels also control the expression of CA XII [16]. Targeting CA XII in cancers that show high levels of these markers and stopping their function has been shown to help treat tumours [17]. All catalytic human carbonic anhydrases (hCAs) have a very similar internal binding site that lets zinc ions (Zn2+) bind to them. This is important for carbon dioxide hydration. Because of this, most of the hCA inhibitors found so far have a zinc-attaching part, which is mostly a sulfonamide [18]. Sulfonamides are important because they are used in medicines that have biological activity [19]. Sulfonamides were first widely used as chemotherapeutic drugs and to prevent certain diseases. Since 2005, they have gotten a lot of attention as possible cancer treatments because they can stop carbonic anhydrase [20]. Sulfonamide derivatives can confirm different bindings with the enzyme's unique bipolar structure, based on the groups of substituents on the aromatic moiety, whether they are in the core active site or on the edge [21]. In medicinal chemistry, five-membered heterocyclic chemicals, in specific those with more than one heteroatom, have a wide variety of biological functions [22]. Over time, thiazolidinone has gotten the most attention. It has three distinct atoms: a sulphur atom at stance 1, a nitrogen atom at stance 3, and a carbonyl group at positions 2, 4, or 5 [23]. Researchers are looking into several derivatives of Thiazolidinone with different substituents as possible anticancer agents because thiazolidinone fragments are often used to change lead compounds in anti-tumor drugs [24]. These compounds may have the ability to defeat cancer in a number of methods, like by starting programmed cell death, halting the cell cycle, or making reactive oxygen species. 4-Thiazolidinone-containing chemicals are very good at stopping a number of enzymes, such as tubulin polymerisation, heat shock protein HSP90, carbonic anhydrases, vascular endothelial growth factor receptor 2 (VEGFR2), cell division cycle 25 A (CDC25A), human epidermal growth factor 2 (HER-2), histone deacetyase (HDAC), B-cell leukemia/lymphoma 2 protein (BCl-2), and protein/tyrosine kinases [25-26]. The goal of this study is to make some Inhibitors for Carbonic Anhydrase using derivatives of 4-thiazolidinone. Table (1) shows a list of goal compounds (IIIa to IIId) and the substituents that go with them (R groups). Every R-group stands for a certain chemical part that is linked to the compound's basic structure. This can have a big effect on the physicochemical properties of compound. A molecular docking study was done to see how well the carbonic anhydrase enzyme binds to other molecules. Next, the strongest compounds that have strong enzymatic effects will be chosen for more chemical synthesis.

AIM

The study aims to create new acetazolamide products that have a thiazolidin-4-one moiety and test how well they stop the carbonic anhydrase XII enzyme (protein data bank code: (4KP5)) and defeat cancer in silico.

MATERIALS AND METHODS

SYSTEM AND SOFTWARE CONFIGURATION

The MSI system used in this study had 4.00 GB of random-access memory and Intel Core i3-2330M processors that ran at 2.2 GHz. We set up and installed both ChemDraw 12.0 and MOE 2015.

LIGAND AND RECEPTOR PREPARATION USING MOLECULAR DOCKING TECHNIQUE

The docking process in this work has two parts:

Step 1. Ligand preparation: ChemDraw software (12.0) was utilized to accurately show the molecular structures of the ligand, then, ligand was protonated in 3D, a partial charge was added, energy was lowered, and the findings were restored.

Table 1. Chemicals of Interest, IIIa, IIIb, IIIc, and IIId are target compounds that are OCH_3, Br, OH, and NO_2, respectively

Name	R
IIIa	OCH_3
IIIb	Br
IIIc	OH
IIId	NO_2

Source: compiled by the authors of this study

Table 2. Molecular docking of the test ligands (IIIa-IIId) in comparison to acetazolamide Shows the S score, Rmsd, and main amino acids that are involved in the final ligands' interactions

Name	R group	S score	RMSD	No. of binding sites	binding amino acids
ACZ	****	-5.7	1.5	2	Zn301,Thr198
IIIa	OCH_3	-7.39	1.6	3	Zn301,Thr198,Asn64
IIIb	Br	-7.0	1.9	4	Zn301,Thr198, Asn64,Lys69
IIIc	OH	-6.8	1.6	4	Zn301,Thr198, Asn64,Lys69
IIId	NO_2	-7.0	1.6	4	Zn301,Thr198, Gln89, Trp4

**** - there is no R group

Source: compiled by the authors of this study

Step 2. Protein organization: The crystal framework of Carbonic Anhydrase XII (code: 4kp5) was downloaded to the Molecular Operational Environment (2015) to get the protein ready. The coming steps were taken for preparation of the target protein: only the chains that were involved in the protein action were chosen, small compounds were deleted, water molecules were removed, the active site was built first, the potential of the protein atoms was adjusted, and then hydrogen hide bonds were added. The docking process was done after the MOE had the ligand that had been made earlier from the saved data.

This study is continuation of the article: Ayoub SAZ, Aldabagh NHN, Atiya R (2025) Design and molecular docking study of new acetazolamide derivatives incorporating a 4-Thiazolidinone moiety as potential carbonic anhydrase XII inhibitors. Review of Clinical Pharmacology and Pharmacokinetics – International Edition 39(1): 51-61. https://doi.org/10.61873/RGHB5923.

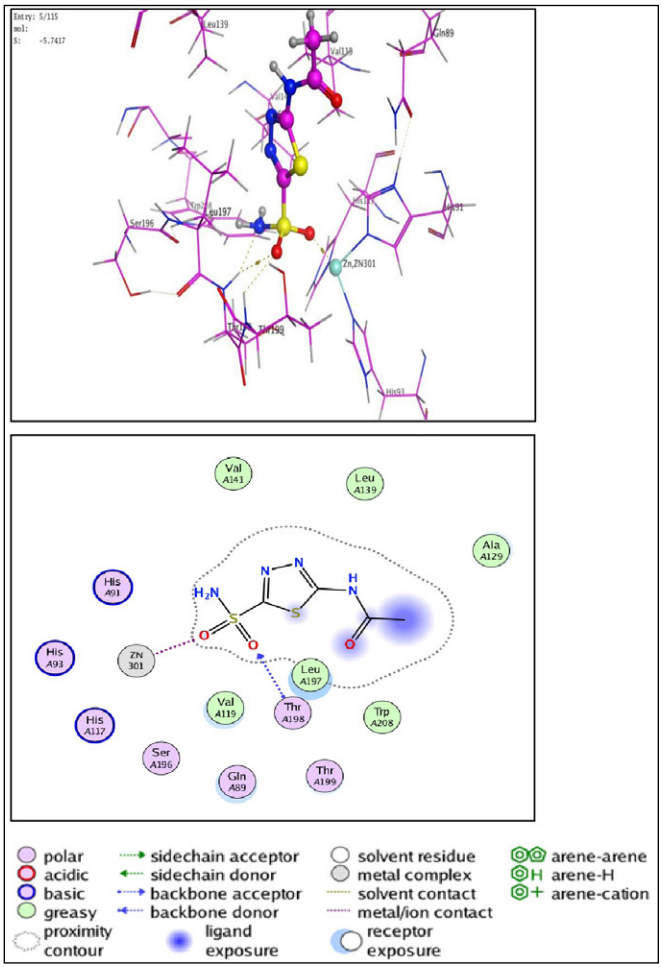

Fig. 1. MOE visualization of reference ligand (acetazolamide) with carbonic anhydrase XII (PDB code: 4KP5), (above) is the 3D structure (below) is the 2D structure

Source: compiled by the authors of this study

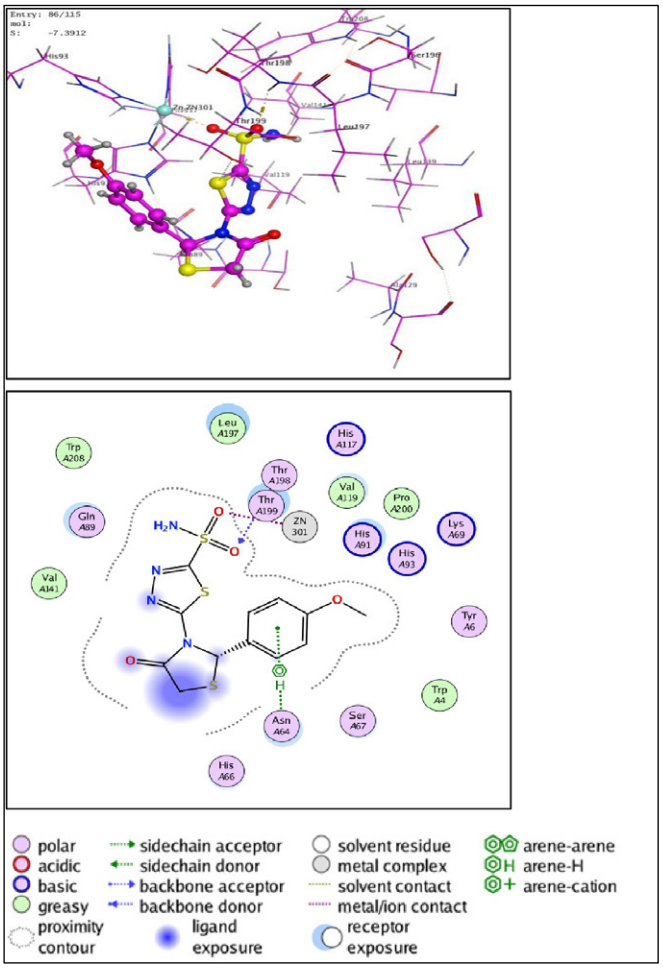

Fig. 2. MOE visualization of compound IIIa with carbonic anhydrase XII (PDB code: 4KP5), (above) is the 3D structure (below) is the 2D structure

Source: compiled by the authors of this study

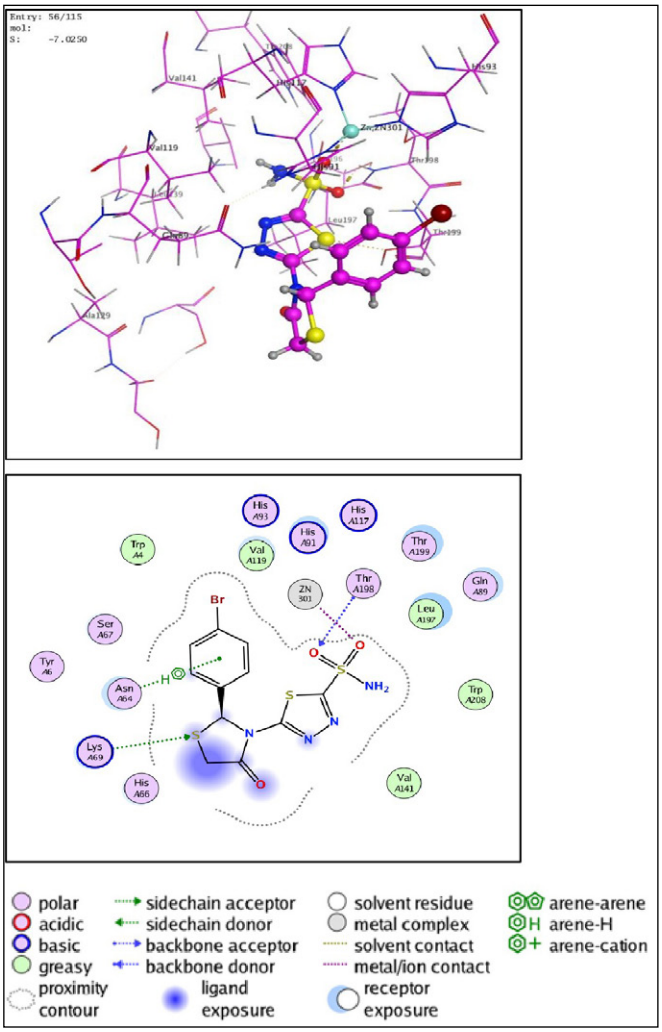

Fig. 3: MOE visualization of compound IIIb with carbonic anhydrase XII (PDB code: 4KP5), (above) is the 3D structure (below) is the 2D structure

Source: compiled by the authors of this study

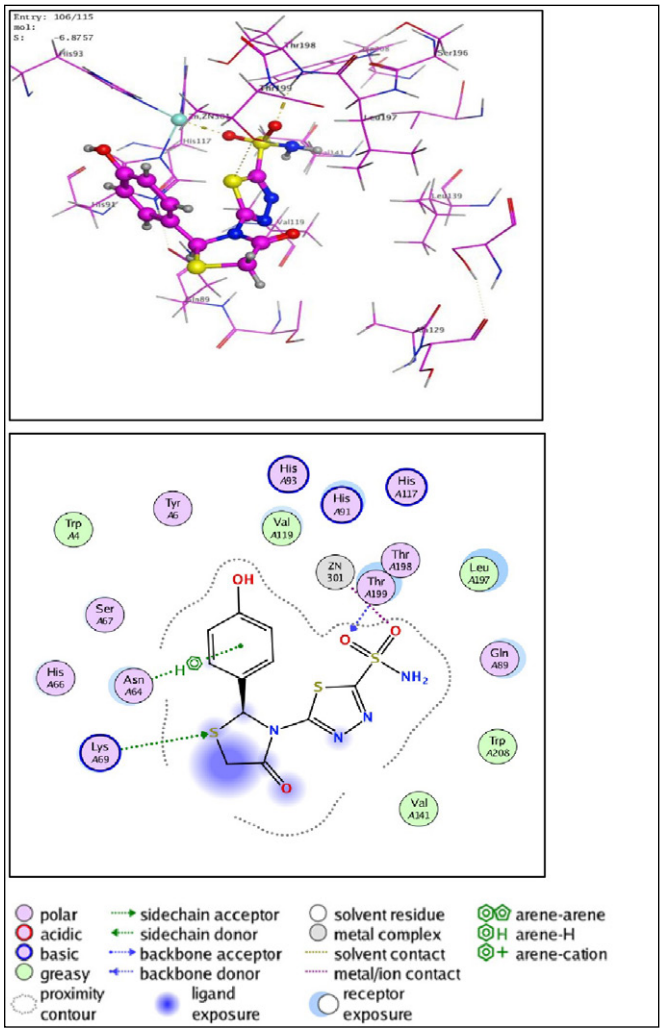

Fig. 4. MOE visualization of compound IIIc with carbonic anhydrase XII (PDB code: 4KP5), (above) is the 3D structure (below) is the 2D structure

Source: compiled by the authors of this study

RESULTS

Molecular docking shows that some ligands interact strongly with an active site in a target. The MOE was created due to it gives a full graphical picture of where ligands are and how they bind to receptor residues. This makes it easy to see, define, and estimate how proteins bind to ligands. The molecular operating environment makes a unique connection between the suspected compounds and the enzyme CA XII, which is in the alike active site as acetazolamide. It is important to quickly figure out how much the compound stops things from happening and how similar the amino acids are to each other and to active sites. Table (1) shows the interaction parameters for the final compounds IIIa, IIIb, IIIc, and IIId.

These parameters include RMSD and S. score values, as well as the identification of important amino acids that are involved in these interactions. These parameters show how well the designed compounds and the target proteins fit together and how much energy it takes to bind them. The S. score and the root mean deviation (RMSD) show how far apart the target point and the ligand atoms are on average for the anti-cancer compound. This shows how this method works. This means that compounds have the best pose when the S. score is high and the RMSD is low. The S. score for all of the target products (IIIa-IIId) is higher than that of the control (acetazolamide). So, they work better than other carbonic anhydrase inhibitors. In addition, their S. scores are -7.39, -7.02, -6.8, and -7.0, and their RMSD values are 1.6, 1.9, 1.6, and 1.6, as shown in Table (2).

The following figure (1) shows the MOE visualization of Acetazolamide with hCA XII (PDB code: 14KP5)

The ligand-protein interaction as 3D structure is shown in the top image, and the 2D structure is shown in the bottom image. In Figure (1), Acetazolamide only binds to zinc and the amino acid threonine 198 in the active site of hCA XII. Using PDB code 4kp5, Figures (2–5) show how compounds IIIa–IIId dock with hCA XII.

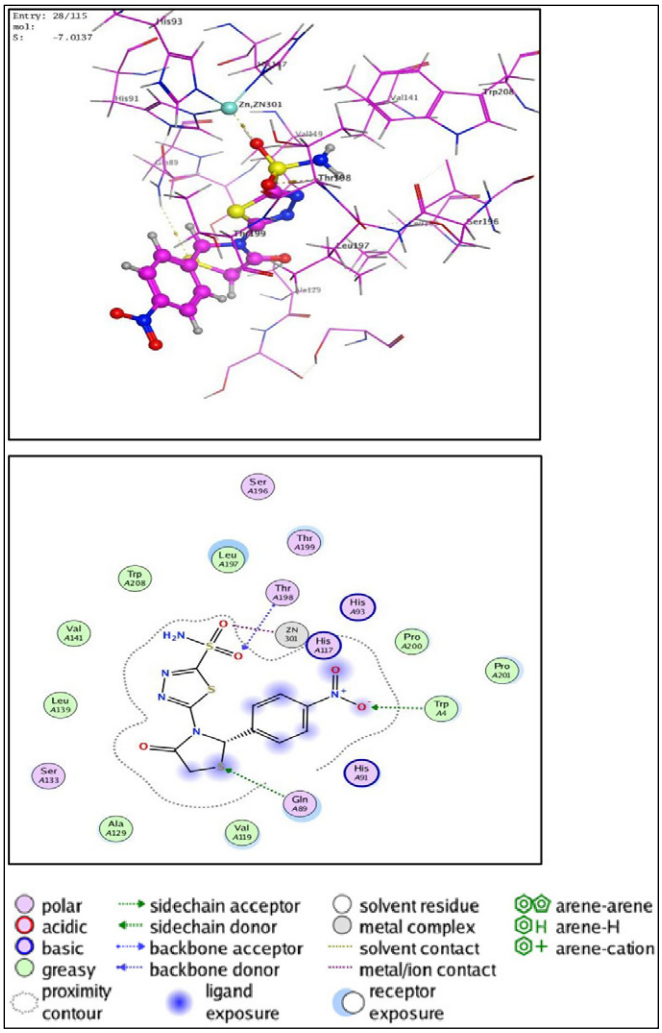

Fig. 5. MOE visualization of compound IIId with carbonic anhydrase XII (PDB code: 4KP5), (above) is the 3D structure (below) is the 2D structure

Source: compiled by the authors of this study

Each figure shows the 3D and 2D structures of the interactions between the ligand and the protein. Every acetazolamide derivative finds zinc and a hydrogen bond with the important amino acid Threonine198. The MOE visualization of compound IIIa with hCA XII is shown in Figure (2). Compound IIIa has the highest S. score of -7.39, which means it has a strong binding affinity. The high S. score could be because of more hydrogen bonding with Asn64, which is important for CAXII's catalytic activity. A methoxy group on this compound may help it fit better inside the receptor pocket. The IIIb binds to important amino acids, especially Lys69 and Asn64, in the active site of carbonic anhydrase XII in ways that are different from the other compounds. Figure (3) shows these interactions in both 3D and 2D forms. In Figure (4), you can see the docking simulation of compound IIIc with hCA XII. It shows that its Thiazolidinone part binds more strongly to the amino acid Lys69, which is vital for substrate interaction at the active site of CAXII. Finally, IIId binds more strongly to the crucial amino acid Trp4 in the active site of CA XII. Figure (5) shows the docking visualization of compound IIId.

DISCUSSION

The active site of human carbonic anhydrase XII has a big conical-figured hole in it that is made up of a hydrophobic part and a hydrophilic part. There is a catalytic cleft inside the hole of this enzyme. Zinc is in this cleft and works as a cofactor. His91, His93, and His117 are three residues of histidine that hold the zinc ion in place and keep its positive charge stable. Threonine 198 is close to the active site and helps keep the enzyme's structure stable. It might also help bind the substrate and keep the transition state stable while catalysis. So, the compounds have to stick to both Thr198 and zinc to stop carbonic anhydrase XII from working. More connecting with other amino acids like Lys67, Gln89, and Asn64 helps the synthesized compounds work better as inhibitors. This is because these amino acids may help the active site cavity stay in the right shape by binding to the substrate and interacting with nearby residues. All of these amino acids are listed in Table (2). Acetazolamide is a very important standard because it is a famous and well-assessed carbonic anhydrase inhibitor. Also, it has a pharmacophore similar to the compounds we looked at.

CONCLUSIONS

The ongoing investigation has shown that four acetazolamide derivatives with thiazolidin-4-one moiety (IIIa-IIId) have both a viable carbonic anhydrase inhibition effect and anti-cancer properties. Most of the compounds had a stronger affinity for the target protein than acetazolamide. The compound that looks the most promising is IIIa, which has an OCH_3 group on the benzene ring and a S. score of -7.39.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Experience of using modern oral iron formulations for the correction of iron deficiency conditions in women of reproductive age

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ABSTRACT

Aim: To scientifically justify the use of the FERRO-EURIKA™ food supplement as a corrective agent for iron deficiency (ID) in women of reproductive age.

Materials and Methods: The study involved 120 women aged 18 to 35 years (median age: 29 years; interquartile range: 24–33 years) with laboratory-confirmed iron deficiency (ID) and iron deficiency anaemia (IDA) related to chronic blood loss (ICD-10 N 92.0 – Excessive and frequent menstruation with regular cycles). In addition to evaluating changes in peripheral blood parameters and iron stores, we analysed the participants' complaints and clinical manifestations of iron deficiency conditions (sideropenic and anaemic syndromes). All subjects were divided into two representative groups of 60 women each. Women of Group 1 (main group, n = 60) received iron bisglycinate 157 mg (equivalent to 30 mg of elemental bivalent iron) in combination with glucosamine salt of (6S)-5-methyltetrahydrofolate – 740 µg (equivalent to 400 µg of folic acid), one capsule twice daily, 30 minutes before meals, for pathogenetically justified correction of ID conditions. Women of Group 2 (comparison group, n = 60) received ferrous sulfate 247.25 mg (equivalent to 80 mg of elemental bivalent iron), one capsule twice daily, 30 minutes before meals. The treatment duration for both groups was 90 days.

Results: We analysed clinical manifestations of iron deficiency conditions and laboratory parameters in women of reproductive age both before the initiation of iron therapy and during correction (on Days 21, 60, and 90 of treatment). Dynamic analysis of clinical symptoms and haematologic indicators in both groups demonstrated a significant decrease in subjective complaints, accompanied by normalization of laboratory parameters, confirming the positive effects of the interventions. Safety and tolerability analyses of the correction of ID conditions were performed to evaluate adherence to long-term iron supplementation among women. An analysis of the causes of treatment discontinuation revealed gastrointestinal adverse effects associated with iron intake: nausea occurred in 5 (8.3%) women of Group 1 and 13 (24.1%) of Group 2; constipation in 2 (3.3%) of Group 1 and 7 (13.0%) of Group 2; and abdominal pain in 2 (3.3%) of Group 1 and 9 (16.7%) of Group 2. These complaints in Group 1 were mild in intensity and resolved by Day 60, allowing all participants in this group to successfully complete the full treatment course. In contrast, gastrointestinal symptoms persisted in Group 2 at Days 60 and 90, resulting in reduced adherence to therapy, with approximately 30% of patients discontinuing further intake of the prescribed iron preparation.

Conclusions: 1. The results of the study indicate that the FERRO-EURIKA™ food supplement may be an effective oral agent for correcting iron deficiency conditions in women of reproductive age. 2. The combination of iron bisglycinate and glucosamine salt of (6S)-5-methyltetrahydrofolate in the composition of FERRO-EURIKA™ contributes to the normalization of haematologic parameters, even at lower doses of iron compared to standard formulations.3. Women who received the FERRO-EURIKA™ food supplement demonstrated better tolerability and a lower incidence of gastrointestinal adverse effects, leading to higher adherence to therapy and successful completion of the treatment course.

KEY WORDS: iron deficiency; iron deficiency anaemia; prevention; treatment; iron bisglycinate; glucosamine salt of (6S)-5-methyltetrahydrofolate

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INTRODUCTION

Iron deficiency (ID) is the most prevalent micronutrient deficiency and a leading cause of iron deficiency anaemia (IDA). This condition remains a significant public health issue, contributing to the global burden of disease [1–3].

Iron plays a fundamental role in oxygen transport, mitochondrial function, DNA synthesis, and aerobic cellular metabolism.

The role of iron in maintaining physiological female hormonal homeostasis is indirect but critically important. Although iron does not directly participate in the

synthesis of oestrogens or progesterone, it indirectly affects hormonal balance, acting as a cofactor for many enzymes required for hydroxylation and transformation of hormonal precursors. Iron is essential for the synthesis of neurotransmitters such as dopamine and serotonin, which influence the secretion of gonadotropin-releasing hormone (GnRH), a key regulator of sex hormone production. As a cofactor, iron is involved in steroidogenesis, the process through which sex hormones (oestrogens, progesterone, testosterone) are synthesized from cholesterol. Consequently, ID and IDA indirectly affect the function of the hypothalamic-pituitary-ovarian axis, leading to clinical manifestations such as ovulatory disorders, abnormal uterine bleeding (AUB), infertility, and tumour-like lesions of the pelvic organs [4,5].

Metabolic disturbances caused by iron deficiency also impact endometrial tissue. In particular, free radical and lipid peroxidation reactions become activated, leading to destabilization of cell membranes and disruption of excitability and functional activity of the endometrium [6].

Among women of reproductive age, AUB is a common cause of ID and IDA, adversely affecting health, quality of life, and healthcare costs. AUB is defined as any deviation from the normal menstrual cycle, manifested by changes in duration, amount of blood loss, or frequency of menstruation. It represents a common gynaecological disorder in women of reproductive age, with a reported prevalence ranging from 10% to 30% in different populations [4–6].

Programs aimed at preventing and treating ID and IDA should focus on eliminating underlying causes, promoting rational nutrition, providing exogenous iron supplementation, and implementing preventive measures to avoid recurrence of the condition. Primary prevention of IDA in women with heavy and prolonged menstruation can be achieved through the monthly administration of 30–40 mg of elemental iron for 7–10 days following menstruation, or through two preventive courses per year, each lasting 6 weeks with daily administrations of 30–40 mg of iron. When selecting an iron compound, it is important to consider the clinical features of ID/IDA, any concomitant diseases, and other individual factors [10,11].

Modern criteria for evaluating the effectiveness of iron compounds include minimal adverse effects, ease of administration, optimal iron content, the presence of components that enhance iron absorption and stimulate haematopoiesis, and a favourable efficacy-to-cost ratio. When prescribing iron therapy, it is essential to consider age, sex, comorbidities, pharmacokinetic properties of the formulation, its pharmacodynamic profile, and potential adverse effects [10,11].

Iron bisglycinate is an amino acid chelate of iron, formed as a result of the reaction between divalent iron and two molecules of the amino acid glycine, covalently bonded during the chelation process. The two glycine molecules bind to iron, protecting it from hydrolysis. As the only form of divalent iron that does not undergo hydrolysis in the stomach, iron bisglycinate is absorbed intact through two types of receptors: DMT-1 (located on duodenal villi) and PEPT-1 (present throughout the gastrointestinal tract). As a result, it demonstrates a bioavailability of 91%. Due to its high bioavailability, a lower dose of iron bisglycinate is sufficient to achieve therapeutic effects compared to other forms of iron, which is particularly beneficial for long-term administration [12–15].

Another important feature of iron bisglycinate is its favourable safety profile. Unfortunately, about half of all outpatients fail to complete their iron therapy due to poor tolerability of conventional iron salts. In contrast, iron bisglycinate is associated with higher patient adherence due to its improved tolerability. Unlike traditional iron salts, iron bisglycinate does not hydrolyse in the stomach, reducing the likelihood of gastrointestinal adverse events [16–19].

There is a relationship between iron absorption and folate levels in the body, both of which are essential for DNA synthesis, the formation of new cells, and normal nervous system function [20,21]. It is recommended that women take daily oral iron and folic acid during preconception preparation and pregnancy to minimize risks of complications for both the mother and the newborn. Experts in gynaecology and nutrition emphasize the importance of exogenous intake of folic acid and iron preparations in women of reproductive age [22].

The glucosamine salt of (6S)-5-methyltetrahydrofolate, a biologically active form of vitamin B9, provides more efficient folate delivery compared to conventional folic acid, particularly for individuals with genetic mutations affecting folate metabolism. The use of glucosamine salt of (6S)-5-methyltetrahydrofolate eliminates this limitation, ensuring effective utilization of folic acid in the body and maintaining adequate red blood cell levels. [20,21].

AIM

To scientifically justify the use of the FERRO-EURIKA™ food supplement as a corrective agent for iron deficiency (ID) in women of reproductive age.

MATERIALS AND METHODS

To achieve the aim of the study, which was to identify differences in the outcomes of correction of ID con-

Table 1. Dynamics of patients' complaints during treatment (absolute number, %)

Parameter	Day of assessment										
	0			21			60			90		
	Group 1 (n=60)	Group 2 (n=60)	p	Group 1 (n=60)	Group 2 (n=54)	p	Group 1 (n=60)	Group 2 (n=47)	p	Group 1 (n=60)	Group 2 (n=42)	p
Fatigue	60 (100.0)	60 (100.0)	>0.999	23 (38.3)	28 (51.9)	0.187	9 (15.0)	15 (31.9)	*0.060*	1 (2.2)	6 (14.3)	**0.019**
Headache	18 (30.0)	19 (31.7)	>0.999	10 (16.7)	13 (24.1)	0.358	5 (8.3)	8 (17.0)	0.235	0 (0)	3 (7.1)	*0.067*
Reduced work capacity	39 (65.0)	38 (63.3)	>0.999	11 (18.3)	21 (38.9)	**0.021**	2 (3.3)	6 (10.6)	0.134	0 (0)	2 (4.8)	0.167
Lack of concentration	33 (55.0)	31 (51.7)	>0.999	2 (3.3)	11 (20.4)	**0.006**	0 (0)	0 (0)	>0.999	0 (0)	0 (0)	>0.999
Memory impairment	30 (50.0)	29 (48.3)	>0.999	9 (15.0)	14 (25.9)	0.167	0 (0)	6 (10.6)	**0.006**	0 (0)	2 (4.8)	0.167
Dizziness	8 (13.3)	9 (15.0)	>0.999	0 (0)	3 (5.6)	0.103	0 (0)	0 (0)	>0.999	0 (0)	0 (0)	>0.999
Tachycardia	6 (10.0)	6 (10.0)	>0.999	1 (1.7)	3 (5.6)	0.344	0 (0)	0 (0)	>0.999	0 (0)	0 (0)	>0.999
Brittle and splitting nails	7 (11.7)	14 (23.3)	0.148	5 (8.3)	7 (13.0)	0.545	0 (0)	5 (10.6)	**0.014**	0 (0)	3 (6.4)	*0.067*
Dry skin	14 (23.3)	13 (21.7)	>0.999	12 (20.0)	11 (20.4)	>0.999	8 (13.3)	9 (19.1)	0.436	0 (0)	3 (4.3)	*0.067*

Note: Fisher's exact test was used

Source: compiled by the authors of this study

Table 2. Dynamics of haematological parameters during treatment (absolute number, %)

Parameter	Day of assessment										
	0			21			60			90		
	Group 1 (n=60)	Group 2 (n=60)	p	Group 1 (n=60)	Group 2 (n=54)	p	Group 1 (n=60)	Group 2 (n=47)	p	Group 1 (n=60)	Group 2 (n=42)	p
RBC count $<3.7\times10^{12}$/L	28 (46.7)	29 (48.3)	>0.999	15 (25.0)	22 (40.7)	0.108	0 (0)	8 (17.0)	**<0.001**	0 (0)	3 (6.7)	*0.067*
Haematocrit < 32%	16 (26.7)	13 (21.7)	>0.999	4 (6.7)	8 (14.8)	0.223	0 (0)	5 (10.6)	**0.014**	0 (0)	2 (4.8)	0.177
MCV < 76 fl	16 (26.7)	15 (25.0)	>0.999	4 (6.7)	10 (18.5)	0.085	0 (0)	6 (12.8)	**0.006**	0 (0)	3 (6.7)	*0.067*
MCH < 27 pg	23 (38.3)	25 (41.7)	>0.999	12 (20.0)	19 (35.2)	0.092	1 (1.7)	7 (14.9)	**0.020**	0 (0)	2 (4.8)	0.177
MCHC < 32 g/dL	15 (25.0)	14 (23.3)	>0.999	5 (8.3)	11 (20.4)	0.103	4 (6.7)	6 (12.8)	0.329	0 (0)	1 (2.4)	0.412
Ferritin < 15 ng/mL	60 (100)	60 (100)	>0.999	27 (45.0)	32 (59.6)	0.138	12 (20)	21 (44.6)	**0.011**	2 (4.3)	5 (11.9)	0.121

Note: Fisher's exact test was used.

Source: compiled by the authors of this study

Table 3. Patients' complaints associated with iron supplementation during treatment (absolute number, %)

ditions between two groups, a minimum sample size calculation was performed. This calculation utilized G*Power v.3.1.9.7 software, with a 5% significance level and 80% power, while assuming a 5% risk of treatment failure and a 20% clinically significant effect. As a result, the minimum required sample size was determined to be 55 patients per group [23].

Thus, the study included 120 women aged 18 to 35 years (median age: 29 years, interquartile range: 24–33 years) who had laboratory-confirmed ID or IDA linked to chronic blood loss (ICD-10 N 92.0 — Excessive and frequent menstruation with regular cycles). In addition to assessing changes in peripheral blood indices and iron storage parameters, the study analysed partici-

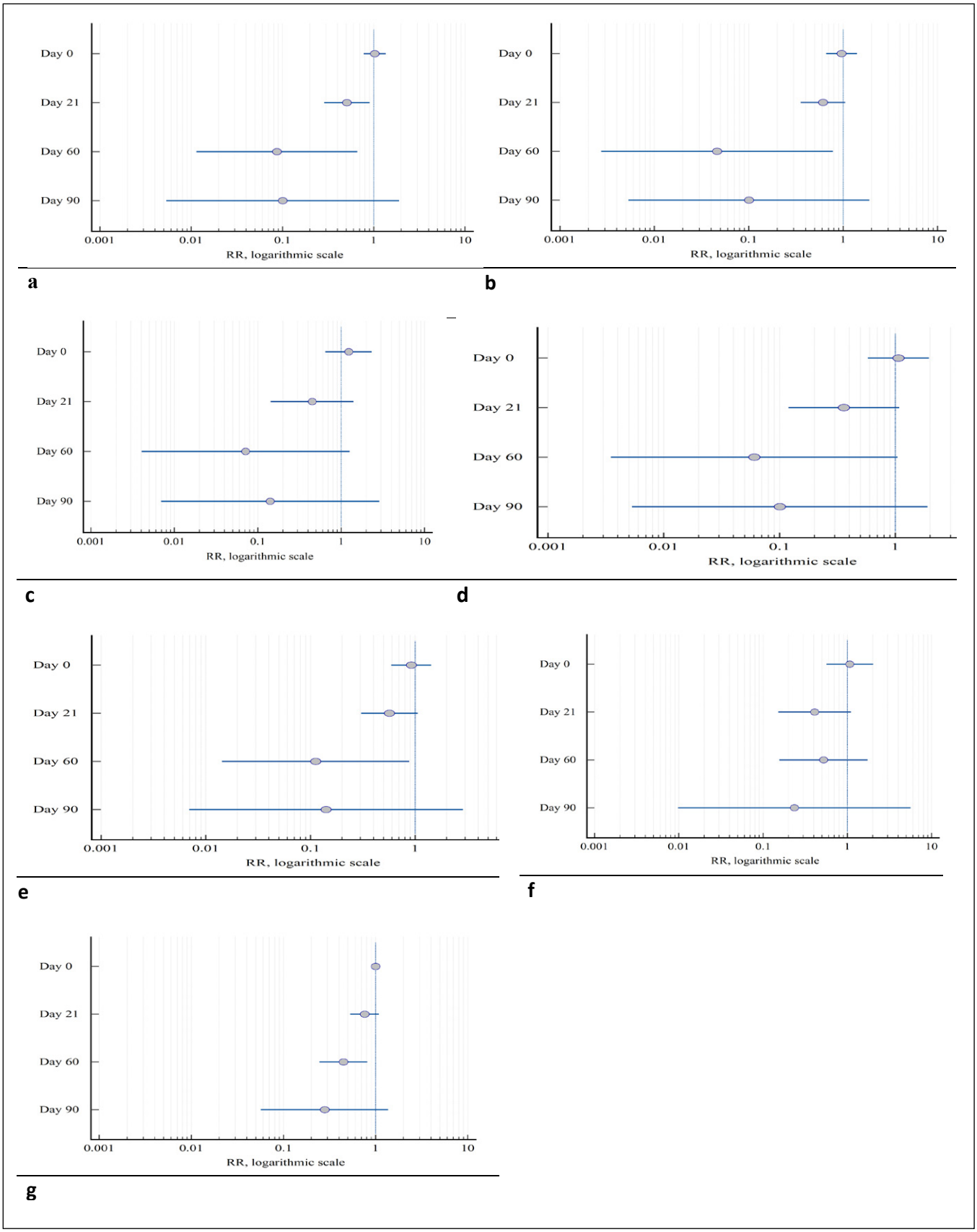

Fig. 1. Dynamics of risk ratio (RR) and 95% confidence intervals (CI) for failure to achieve reference haematological levels between Groups 1 and 2. Legend: (a) Haemoglobin (Hb); (b) Erythrocytes; (c) Haematocrit; (d) Mean corpuscular volume (MCV); (e) Mean corpuscular haemoglobin (MCH); (f) Mean corpuscular haemoglobin concentration (MCHC); (g) Ferritin
Picture taken by the authors

pants' complaints and clinical manifestations associated with iron deficiency (sideropenic and anaemic syndromes).

All subjects were divided into two representative groups of 60 women each. Women of Group 1 (main group, n = 60) received iron bisglycinate 157 mg (equivalent to 30 mg of elemental bivalent iron) in combination with glucosamine salt of (6S)-5-methyltetrahydrofolate – 740 µg (equivalent to 400 µg of folic acid), one capsule twice daily, 30 minutes before meals, for pathogenetically justified correction of ID conditions. Women of Group 2 (comparison group, n = 60) received ferrous sulfate 247.25 mg (equivalent to 80 mg of elemental bivalent iron), one capsule twice daily, 30 minutes before meals. The treatment duration for both groups was 90 days.

Statistical data processing was performed using Microsoft Excel 2016 and Statistica 10.0 (StatSoft Inc.). Additional statistical analysis was carried out using EZR v.1.68 (a graphical interface for R statistical software v.4.3.1, R Foundation for Statistical Computing, Vienna, Austria). Fisher's exact test was applied to determine differences in nominal data. Cochran's Q test assessed significant differences in proportions of binary outcomes among more than two related groups, reflecting treatment dynamics. Risk ratios (RR) and corresponding 95% confidence intervals (95% CI) were calculated to evaluate differences in event risk within the case-control setting. The significance threshold was set at $p < 0.05$ [24].

ETHICS

During the study, the general principles of the Declaration of Helsinki "Recommendations for physicians involved in biomedical research with human subjects" (1964), the Council of Europe Convention on Human Rights and Biomedicine (04.04.1997), and the World Medical Association's ethical principles for medical research involving human subjects (1964–2000) were strictly followed, taking into account the requirements of Directive 2001/20/EC of the European Parliament and of the Council, ICH GCP guidelines, and Order No. 690 of the Ministry of Health of Ukraine dated September 23, 2009. Each participant was informed about the purpose and procedures of the study, and inclusion of the obtained data in the scientific research was carried out only after receiving written informed consent to participate in the clinical study. The informed consent form and the patient examination chart were approved by the Bioethics Committee for Experimental and Clinical Research of the Medical Institute of Sumy State University.

RESULTS

We analysed clinical manifestations of iron deficiency conditions and laboratory parameters in women of reproductive age both before the initiation of iron therapy and during correction (on Days 21, 60, and 90 of treatment).

At baseline, all women in both groups reported fatigue (100.0%). Complaints of decreased work capacity, headache, lack of concentration, and memory impairment were noted by 39 (65.0%), 18 (30.0%), 33 (55.0%), and 30 (50.0%) women in Group 1, and by 39 (63.3%), 19 (31.7%), 31 (51.7%), and 29 (48.3%) women in Group 2, respectively. Periodic dizziness was reported by 8 (13.3%) patients in Group 1 and 9 (15.0%) in Group 2; concurrently, 6 (10.0%) women in each group experienced tachycardia, which could occur even with minor physical exertion. Brittle and splitting nails as well as dry skin were observed in 7 (11.7%) and 14 (33.3%) participants of Group 1, and in 14 (23.3%) and 13 (21.7%) of Group 2, respectively (Table 1).

Laboratory test results confirmed ID in 22 (36.7%), mild IDA in 14 (23.3%), and moderate IDA in 24 (40.0%) patients in Group 1, and correspondingly ID in 23 (38.3%), mild IDA in 13 (21.6%), and moderate IDA in 24 (40.0%) patients in Group 2. According to laboratory evaluation, all women in both groups demonstrated reduced ferritin levels. Both groups also showed decreases in haemoglobin, erythrocyte count, haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC), among other indices (Table 2).

Analysis of the dynamics of clinical manifestations of ID and IDA on Days 21, 60, and 90 of supplementation revealed a downward trend in both groups regarding the frequency of complaints and the proportion of patients presenting any symptom (Table 1).

Throughout the treatment course, normalization of all evaluated hematologic parameters was recorded in both groups. The risk of not achieving therapeutic success on Days 21 and 60 was lower among patients in Group 1 compared to those in Group 2 (Table 2, Fig. 1).

An analysis of reasons for discontinuing iron intake revealed that gastrointestinal complaints were prevalent. During treatment (on Days 21, 60, and 90), nausea was reported by 5 (8.3%) women in Group 1 and 13 (24.1%) in Group 2; constipation by 2 (3.3%) women in Group 1 and 7 (13.0%) in Group 2; and abdominal pain by 2 (3.3%) women in Group 1 and 9 (16.7%) women in Group 2 (Table 3).

All patients linked these adverse events directly to iron intake. However, in Group 1, the symptoms were mild and by Day 60 were absent in all participants,

Parameter	Day of assessment											
	0			21			60			90		
	Group 1 (n=60)	Group 2 (n=60)	p	Group 1 (n=60)	Group 2 (n=54)	p	Group 1 (n=60)	Group 2 (n=47)	p	Group 1 (n=60)	Group 2 (n=42)	p
Nausea	0 (0)	0 (0)	>0.999	5 (8.3)	13 (24.1)	0.545	0 (0)	12 (25.5)	**<0.001**	0 (0)	7 (16.7)	**<0.001**
Constipation	0 (0)	0 (0)	>0.999	2 (3.3)	7 (13.0)	0.082	0 (0)	5 (10.6)	**0.014**	0 (0)	4 (9.5)	**0.026**
Abdominal pain	0 (0)	0 (0)	>0.999	2 (3.3)	9 (16.7)	**0.024**	0 (0)	3 (6.4)	0.082	0 (0)	2 (4.8)	0.177

Note: Fisher's exact test was used.
Source: compiled by the authors of this study

Table 4. Dynamics of patient withdrawal from the study during treatment (absolute number, %)

	Day of assessment			
	0	21	60	90
Group 1 (n=60)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Group 2 (n=60)	0 (0%)	6 (10%)	13 (21.7%)	18 (30%)
p	p>0.999	**0.027**	**<0.001**	**<0.001**

Note: Fisher's exact test was used.
Source: compiled by the authors of this study

allowing all 60 women in this group to complete the entire prescribed course of supplementation (Table 4).

In contrast, the above gastrointestinal symptoms in Group 2 persisted on Days 60 and 90 of the study. Specifically, on Day 60 of therapy, nausea was reported in 12 (25.5%) cases, constipation in 5 (10.6%), and periodic abdominal pain in 3 (6.4%) cases. By Day 90, the incidence of nausea decreased to 7 women (16.7%), constipation to 4 women (9.5%), and periodic abdominal pain to 2 women (4.8%). These adverse events led some patients to discontinue their iron intake (see Table 4).

It is important to note that all women in Group 1 showed good tolerability to long-term iron bisglycinate supplementation, allowing them to successfully complete the recommended treatment course.

DISCUSSION

This study demonstrates that the FERRO-EURIKA™ supplement, containing iron bisglycinate and the glucosamine salt of (6S)-5-methyltetrahydrofolate, is an effective and well-tolerated option for correcting iron deficiency in women of reproductive age. The improvements in hematologic parameters and clinical symptoms are consistent with existing evidence supporting the high bioavailability and favourable safety profile of iron bisglycinate compared with conventional iron salts. Notably, therapeutic efficacy was achieved even with a lower elemental iron dose, highlighting the value of the chelated formulation [13, 14].

A key finding concerns tolerability. Gastrointestinal adverse events were significantly less frequent and milder in the bisglycinate group, resolving by Day 60 and allowing all participants to complete the 90-day course. In contrast, women receiving ferrous sulfate experienced persistent gastrointestinal discomfort that reduced adherence. These results correspond with prior research showing that traditional iron salts are commonly associated with poor tolerability and treatment discontinuation [21, 23].

The inclusion of an active folate form in FERRO-EURIKA™ may have contributed to the hematologic improvements observed, as folate is essential for erythropoiesis and may enhance the response to iron supplementation. Although folate status was not directly assessed, the combination of iron and active folate may offer additional benefit, particularly for individuals with impaired folate metabolism.

Correction of iron deficiency also led to a reduction in sideropenic symptoms, with faster improvement in Group 1, likely related to better absorption and tolerability. This early symptom relief is clinically meaningful for women who frequently experience reduced quality of life due to chronic menstrual blood loss [20, 22].

The study has several limitations, including the 90-day follow-up period, lack of dietary control, and inclusion of only women with iron deficiency related to heavy menstrual bleeding. Thus, results may not be fully generalizable to other etiologies.

Overall, the findings support FERRO-EURIKA™ as a safe, well-tolerated, and effective strategy for managing iron deficiency. Future research should focus on long-term outcomes, broader populations, and the potential preventive use of the supplement in high-risk groups [23].

PROSPECTS FOR FURTHER RESEARCH

The results obtained provide a basis for further study of the effectiveness and safety of the dietary supplement TM FERRO-EURIKA in wider populations of women of reproductive age with various clinical forms of iron deficiency conditions. A promising direction is to conduct long-term prospective observations to assess the stability of the achieved therapeutic effect, prevent recurrence of iron deficiency, and restore reproductive potential.

Further studies may be aimed at comparative evaluation of the effectiveness of FERRO-EURIKA in women of different ages, with different etiologies of iron deficiency (chronic blood loss, malabsorption, vegetarian diets, etc.), as well as in pregnant women, in whom the need for iron increases significantly.

CONCLUSIONS

1. The results of the study indicate that the FERRO-EURIKA™ food supplement may be an effective oral agent for correcting iron deficiency conditions in women of reproductive age.
2. The combination of iron bisglycinate and glucosamine salt of (6S)-5-methyltetrahydrofolate in the composition of FERRO-EURIKA™ contributes to the normalization of haematologic parameters, even at lower doses of iron compared to standard formulations.
3. Women who received the FERRO-EURIKA™ food supplement demonstrated better tolerability and a lower incidence of gastrointestinal adverse effects, leading to higher adherence to therapy and successful completion of the treatment course.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Impact of genetic diversity of PCSK9 polymorphisms on coronary artery disease in a sample of Iraqi individuals

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ABSTRACT

Aim: To assess the association of *PCSK9* SNPs (rs2483205, rs2479394) with CAD susceptibility in an Iraqi population.

Materials and Methods: A case-control study recruited 110 CAD patients, angiographically confirmed, and 110 age-/sex-matched healthy controls. Serum PCSK9 was quantified by ELISA, and lipid profiles were analyzed enzymatically. Genotyping for rs2483205 (C/T) and rs2479394 (G/A) was performed using ARMS-PCR. Associations were evaluated using logistic regression, haplotype analysis, and genotype-phenotype correlations, adjusting for age and sex.

Results: CAD patients exhibited significantly higher PCSK9 levels (54.12 ± 19.04 vs. 20.94 ± 17.72 ng/mL, $p < 0.0001$), LDL-C, TG, and lower HDL-C ($p \leq 0.005$) versus controls. Genotype analysis revealed a significant protective effect of the rs2479394 G/A genotype against CAD (OR = 0.21, 95% CI: 0.04–1.08, $p = 0.024$). The rs2483205 SNP showed no significant association. Haplotype analysis identified four major haplotypes; T-A demonstrated significant protection (OR = 0.45, 95% CI: 0.21–0.88, $p = 0.020$). No significant associations were found between rs2479394 genotypes and phenotypic parameters (blood pressure, lipids, PCSK9) in CAD patients.

Conclusions: The rs2479394 G/A genotype and T-A haplotype are associated with reduced CAD risk in Iraqis, suggesting a protective role for specific *PCSK9* variants. Elevated serum PCSK9 levels and dyslipidemia were confirmed in CAD patients; however, rs2479394 did not correlate with these phenotypic traits.

KEY WORDS: PCSK9, rs2483205, rs2479394, CAD, ARMS-PCR, association

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INTRODUCTION

CAD is characterized by atherosclerotic plaque accumulation in coronary arteries. It is the leading global cause of mortality, responsible for 32% of worldwide deaths [1]. In Iraq, CAD prevalence has surged, affecting 5–10% of adults and manifesting 8–10 years earlier than in Western populations, with hyperlipidemia and smoking as predominant risk factors [2]. The pathophysiology involves endothelial dysfunction, lipid-driven plaque formation, and thrombosis, ultimately leading to myocardial ischemia or infarction [3]. By 2018, CAD caused 18.92% of deaths in Iraq, highlighting an urgent public health crisis [4]. Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9), a serine protease encoded on chromosome 1p32.3, regulates lipid metabolism by degrading hepatic low-density lipoprotein receptors (LDLRs) [5]. This process elevates circulating LDL cholesterol (LDL-C), accelerating atherosclerosis [6]. Beyond lipid modulation, PCSK9 amplifies inflammation via toll-like receptor activation and cytokine release, exacerbating plaque instability [7]. Its dual role positions PCSK9 as a therapeutic target, with inhibitors like evolocumab, reducing LDL-C by 60% and cardiovascular events [6]. Specific PCSK9 single-nucleotide polymorphisms (SNPs) influence CAD susceptibility; rs615563 and rs2479394 associate with subclinical atherosclerosis and hypercholesterolemia [8]. The rs2483205 correlates with elevated LDL-C and CAD risk in multi-ethnic studies [9]. Gain-of function variants increase LDLR degradation, thereby raising LDL-C levels and increasing atherosclerosis risk, while loss-of-function mutations confer protection [10]. Despite evidence from global cohorts, these SNPs remain unstudied in Iraqis, a population with unique genetic and environmental CAD risks [2].

AIM

This study aims to assess the association of *PCSK9* SNPs (rs2483205, rs2479394) with CAD susceptibility in an Iraqi population.

Table 1. Primer Sequence of AS-PCR Genotyping of *PCSK9*

SNP	Type of allele and primer	Sequence (5′ –3′)
rs2483205	Allele C(wild)Fwd	ATGTGGTCCTTGTGTTCGTC
	Allele T(mutant)Fwd	ATGTGGTCCTTGTGTTCGTT
	Reverse primer common	CACTCTGGTTCTCTGGCTCT
rs2479394	Allele G(wild)Rev	GGGAGTCCTCTATGTGACAATG
	Allele A(mutant)Rev	GGGAGTCCTCTATGTGACAATA
	Forward primer common	AGCTTTCTGCCTCCACAAC

Source: compiled by the authors of this study

MATERIALS AND METHODS

STUDY DESIGN AND PARTICIPANT RECRUITMENT

This case-control genetic association study was conducted between August 2024 and March 2025 at the University of Kufa, Iraq. We enrolled 110 patients with coronary artery disease (CAD) (79 males, 31 females) and 110 age-, sex-, and geographically matched healthy controls (81 males, 29 females). CAD diagnosis was confirmed by coronary angiography showing ≥70% obstruction in major coronary arteries or branches at Al-Najaf Center for Cardiac Surgery and Transcatheter Therapy. Patients were recruited from Al-Sadr Teaching Hospital cardiology center following strict inclusion criteria: age >30 years, angiographic confirmation of CAD, and absence of autoimmune disorders or hepatic dysfunction. Controls were asymptomatic individuals randomly selected from the general population with no personal history of CAD, diabetes mellitus, or hypertension, and no family history of CAD. Demographic matching between groups was systematically implemented to minimize confounding effects.

ETHICAL APPROVAL

The study protocol received full approval (MEC-1280) from the Ethical Committee of the Faculty of Medicine, University of Kufa. All participants provided written informed consent before their inclusion. Procedures were conducted in accordance with the principles outlined in the Declaration of Helsinki for human biomedical research.

SAMPLE COLLECTION AND BIOCHEMICAL ANALYSIS

After a standardized 12-hour overnight fast, 5 mL venous blood was collected via peripheral venipuncture. For phenotypic characterization, 3 mL was transferred to plain tubes, incubated at 37°C for 15 minutes for coagulation, centrifuged at 2000 ×g for 10-15 minutes, and serum was stored at -20°C. Biochemical analyses were performed at the Postgraduate Laboratory, Department of Biochemistry. Serum PCSK9 concentrations were quantified using a sandwich ELISA kit (BT LAB, Korea) and measured spectrophotometrically at 450 nm. Serum lipid concentrations were determined by enzymatic methods following the manufacturer's protocols (Randox, UK). They included triglycerides (TG), total cholesterol (TC), and HDL-C. LDL-C and VLDL-C were calculated using the Friedewald equations:

VLDL-C (mg/dL) = TG/5

LDL-C (mg/dL) = TC - (HDL-C + VLDL-C)

GENETIC ANALYSIS

Genomic DNA was extracted from EDTA-treated whole blood using Geneaid purification kits (Taiwan), yielding 20-30 kb fragments. DNA concentration and purity (A260/A280 ratio 1.8-2.0) were verified using a BioDrop spectrophotometer (UK). Two PCSK9 SNPs on chromosome 1, rs2483205 (C>T), and rs2479394 (G>A), were genotyped using Allele-Specific PCR (ARMS-PCR). Primers were designed with Primer3 software based on GenBank sequences, Table 1.

PCR amplification utilized AccuPower® PreMix (Bioneer, Korea) containing Taq DNA polymerase, dNTPs, and optimized buffer components. Reactions contained 6 µL genomic DNA, 2 µL each of allele-specific primers (Macrogen, Korea), and common primers in 20 µL reactions. Thermocycling conditions (Biometra T-professional, Germany) included: initial denaturation at 95°C for 5 minutes; 35 cycles of denaturation (95°C, 20 s), allele-specific annealing (rs2483205: 58.5°C, rs2479394: 59.5°C for 45 s), and extension (72°C, 1 min); with final extension at 72°C for 7 minutes. PCR products were resolved on 2% agarose gels in 1X TBE buffer, stained with Diamond dye (Pioneer, Korea). Electrophoresis was run at 75-80V for 120 minutes alongside 100 bp DNA ladders (Simgen, China). Genotypes were determined by UV visualization of allele-specific amplicons. For the rs2483205 SNP, the amplicons size was 309 bp, and for the rs2479394 SNP, it was 272 bp. homozygous wild-type samples showed bands only in wild-type primer lanes, homozygous mutants only in mutant lanes, and heterozygotes in both lanes, Figure 1 for rs2483205 SNP and Figure 2 for rs2479394 SNP.

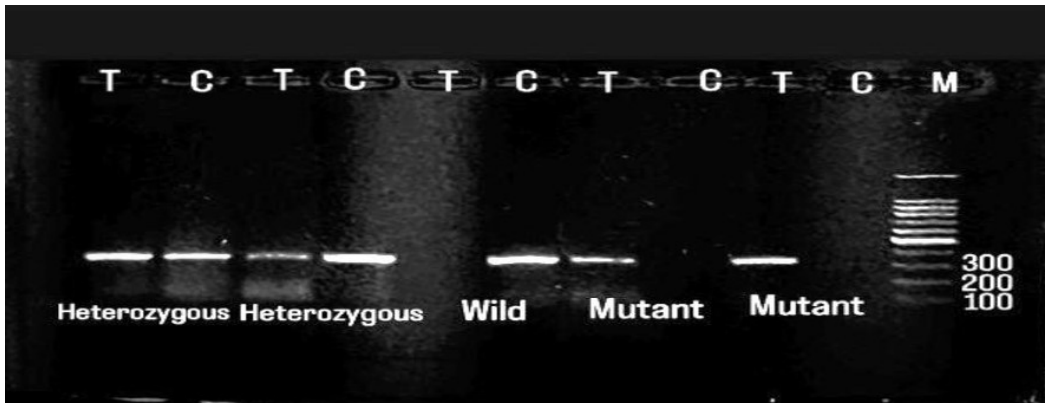

Figure 1: The PCR product of *PCSK9* analyzed for rs2483205 SNP by electrophoresis on an agarose gel. M: Ladder of DNA 100 bp. The PCR product is an amplicons of 309 bp
Source: compiled by the authors of this study

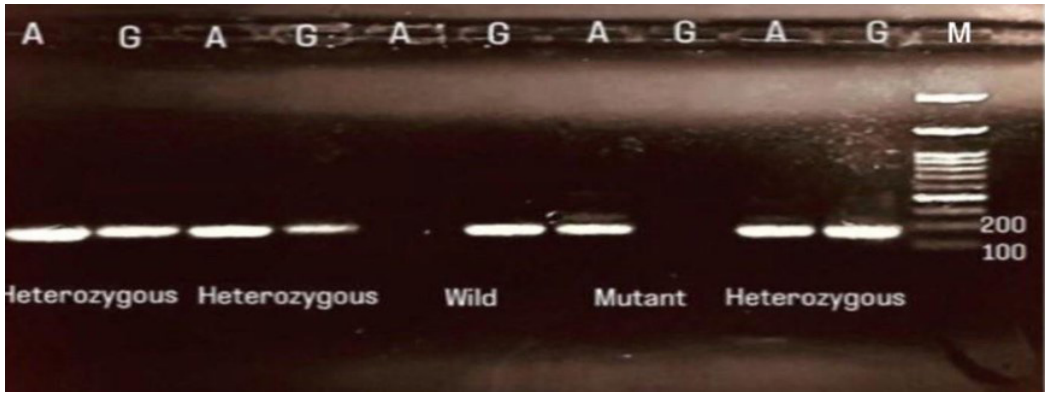

Figure 2: The PCR product of *PCSK9* analyzed for rs2479394 SNP by electrophoresis on an agarose gel. M: Ladder of DNA 100 bp. The PCR product is an amplicons of 272 bp
Source: compiled by the authors of this study

STATISTICAL ANALYSIS

Data are expressed as mean ± standard deviation. Continuous variables were compared using Student's *t*-test. Genotype and allele frequencies were compared using chi-square tests with Yates' correction where appropriate. Odds ratios (ORs) with 95 % confidence intervals (CIs) were estimated from logistic regression models adjusted for age and sex. To ensure consistency between point estimates and hypothesis tests, both *p*-values and CIs were obtained from the same likelihood-based model using profile-likelihood (rather than Wald) methods; this approach reduces the conservatism of CIs in sparse cells. Statistical significance for the prespecified primary analysis (rs615563 under its primary inheritance model in the overall cohort) was set at two-sided $p \leq 0.05$. All other inheritance models, additional SNPs, sex-stratified analyses, and haplotypes were treated as exploratory; for these, the false discovery rate (FDR) was controlled at $q = 0.05$ using the Benjamini–Hochberg procedure. All analyses were performed in SPSS v26 (IBM, USA).

RESULTS

CHARACTERISTICS OF PATIENTS AND CONTROL INDIVIDUALS

The anthropometric and biochemical characteristics of the study groups are summarized in Table 2. There were no significant differences in sex distribution or mean age, however, significant differences were observed in several clinical parameters. Compared to controls, CAD patients had a significantly higher mean BMI, systolic and diastolic blood pressure, FBG levels (195.57 ± 45.41 *vs*. 123.35 ± 11.23 mg/dL, P < 0.0001) and higher serum PCSK9 concentrations (54.12 ± 19.04 *vs*. 20.94 ± 17.72 ng/mL, P < 0.0001). Additionally, total cholesterol (184.70 ± 30.43 *vs*. 155.09 ± 26.76 mg/dL, P < 0.0001), TG (183.89 ± 45.42 vs. 104.25 ± 30.77 mg/dL, P < 0.0001), LDL-C (107.85 ± 24.50 *vs.* 92.63 ± 21.34 mg/dL, P = 0.0035), and VLDL-C (37.12 ± 17.45 *vs.* 21.87 ± 10.23 mg/dL, P < 0.0001) were significantly higher in the CAD patient group, whereas HDL-C was significantly lower in CAD patients (42.84 ± 9.57 *vs.* 46.30 ± 8.50 mg/dL; P = 0.005). These findings indicate significant differences in metabolic and lipid parameters between patients with CAD and healthy controls.

ASSOCIATION OF PCSK9 SNPS WITH CAD

A post hoc power calculation was carried out using the observed genotype frequencies. For rs615563 under the recessive model (G/G *vs*. A/A–A/G; frequency ≈ 0.57 in cases *vs.* 0.33 in controls), at α = 0.05 (two-sided), our study has approximately 85% power to detect an odds ratio of ≥ 2.5. For rare contrasts, such as the A/A genotype in CAD cases (3.6%), power falls below 40% for odds ratios ≤ 2.0.

Table 2. Anthropometric and Biochemical Characteristics of Patients and the Control Group

	Mean ± SD		P-value
	Control	Patients	
Sex (M/F)	81/29	79/31	
Age (y)	62.32±11.62	61.42±8.80	0.517
BMI (Kg/m^2)	26.03±2.44	28.67±4.09	<0.0001
Systolic BP (mmHg)	120.15±9.19	135.31±19.43	<0.0001
Diastolic BP (mmHg)	70.37± 7.09	81.06 ±13.74	<0.0001
Glucose (mg/dL)	123.35±11.23	195.57±45.41	<0.0001
PCSK9 (ng/mL)	20.94±17.72	54.12 ± 19.04	<0.0001
TC (mg/dL)	155.09±26.76	184.70±30.43	<0.0001
TG (mg/dL)	104.25±30.77	183.89± 45.42	<0.0001
HDL (mg/dL)	46.30 ± 8.50	42.84 ± 9.57	0.005
LDL (mg/dL)	92.63 ± 21.34	107.85±24.50	0.0035
VLDL (mg/dL)	21.87 ± 10.23	37.12 ±17.45	<0.0001

Source: compiled by the authors of this study

Table 3. Association of rs2483205 SNP in the PCSK9 with CAD Adjusted by Sex and Age

	Genotype	C	P	OR (95% CI)	P
Codominant	C/C	10 (9.1%)	2 (1.8%)	1.00	
	C/T	94 (85.5%)	104 (94.5%)	3.56 (0.70–18.19)	0.24
	T/T	6 (5.5%)	4 (3.6%)	2.64 (0.31–22.20)	
Recessive	C/C + C/T	104 (94.5%)	106 (96.4%)	1.00	
	T/T	6 (5.5%)	4 (3.6%)	0.78 (0.19–3.25)	0.73
Overdominant	C/C + T/T	16 (14.6%)	6 (5.5%)	1.00	
	C/T	94 (85.5%)	104 (94.5%)	2.13 (0.74–6.14)	0.15

Source: compiled by the authors of this study

Table 4. Association of rs2479394 SNP in the PCSK9 with CAD Adjusted by Sex and Age

	Genotype	C	P	OR (95% CI)	P
Codominant	G/G	2 (1.8%)	8 (7.3%)		
	G/A	104 (94.5%)	102 (92.7%)	0.21 (0.04–1.08)	0.024
	A/A	4 (3.6%)	0 (0%)	NA	
Recessive	G/G + G/A	106 (96.4%)	110 (100%)		
	A/A	4 (3.6%)	0 (0%)	NA	0.075
Overdominant	G/G + A/A	6 (5.5%)	8 (7.3%)		
	G/A	104 (94.5%)	102 (92.7%)	0.50 (0.15–1.68)	0.25

Source: compiled by the authors of this study

The association between the rs2483205 SNP polymorphism in the PCSK9 and CAD, adjusted for sex and age, is shown in Table 3.

The Codominant model exhibited that frequencies of the C/C, C/T, and T/T genotypes were 9.1%, 85.5%, and 5.5% in CAD patients, respectively, compared to 1.8%, 94.5%, and 3.6% in controls. Neither the C/T nor the T/T genotype showed a statistically significant association with CAD. The recessive model illustrated that the T/T genotype also did not differ significantly between cases and controls. Similarly, the over-dominant model demonstrated that the heterozygous C/T genotype showed no significant association with CAD when compared to the combined C/C + T/T genotypes. The rs2479394 polymorphism showed several significant associations with CAD risk in our Iraqi population study Table 4.

Notably, we observed protective effects against CAD in specific genetic models, though some results indicated limited precision due to low genotype counts in certain categories. The Codominant model revealed a 79% reduction in the risk of CAD in the G/A genotype carriers compared to the G/G carriers (OR = 0.21, 95% CI: 0.04-1.08, p = 0.024). No A/A homozygotes were observed in the CAD group, preventing risk estimation for this genotype.

Table 5. Haplotype Frequencies of PCSK9 SNPs and Their Association with CAD in the Iraqi Population

Haplotype	SNP1	SNP2	Frequency	OR (95% CI)	P-value
1	T	G	0.45	1.00	
2	T	A	0.26	0.45 (0.21–0.88)	0.020
3	C	G	0.15	0.83 (0.34–1.67)	0.073
4	C	A	0.14	0.32 (0.07–1.31)	0.094

SNP1: rs2483205 C/T, SNP2: rs2479394 G/A
Source: compiled by the authors of this study

Table 6. Phenotype-rs2479394 SNP Genotype Relationship in CAD Patients under the codominant Model

	Mean ± SD		P-value
Genotype	G/A (102)	G/G (8)	
Systolic BP (mmHg)	131.21±19.23	133.14±19.21	0.231
Diastolic BP (mmHg)	79.11 ±13.62	79.92± 13.45	0.112
Glucose (mg/dL)	195.61±44.90	194.15±44.31	0.456
PCSK9 (ng/mL)	54.12 ± 19.5	48.93±18.5	0.426
TC (mg/dL)	181.65±29.63	184.10±25.49	0.222
TG (mg/dL)	178.77± 44.86	181.32±29.56	0.415
HDL (mg/dL)	41.67 ± 9.45	43.05 ± 9.42	0.141
LDL (mg/dL)	105.46±23.42	108.78 ± 22.91	0.423
VLDL (mg/dL)	36.11 ±17.32	38.34 ± 16.94	0.323

Source: compiled by the authors of this study

HAPLOTYPE ANALYSIS

Haplotype-based association analysis of the PCSK9 SNPs rs2483205 (C/T) and rs2479394 (G/A) highlighted significant differences between the CAD patients and healthy controls. Four major haplotypes were identified Table 5, i.e., T-G, T-A, C-G, and C-A. The T-G haplotype (frequency 0.45) served as the reference. Notably, the T-A haplotype (frequency 0.26) demonstrated a significant protective association against CAD (OR = 0.45, 95% CI: 0.21–0.88, p = 0.020), while the C-G haplotype (frequency 0.15; OR = 0.83, 95% CI: 0.34–1.67, p = 0.073) and the C-A haplotype (frequency 0.14; OR = 0.32, 95% CI: 0.07–1.31, p = 0.094) also exhibited odds ratios below 1.0, suggestive of potential protective effects, these associations did not reach statistical significance at the $p<0.05$ level

GENOTYPE-PHENOTYPE ANALYSIS

The relationship between the rs2479394 genotype and various phenotypic parameters in CAD patients under the Codominant model is shown in Table 6. No statistically significant differences were observed between the two genotypic groups across all measured clinical and biochemical parameters. Specifically, systolic blood pressure, diastolic blood pressure, glucose, and serum PCSK9 levels were comparable between the G/A, G/G groups. Similarly, no significant differences were noted in lipid profile parameters, including TC, TG, HDL-C, LDL-C, and VLDL-C. These findings suggest that the rs2479394 polymorphism does not significantly influence the phenotypic characteristics evaluated in CAD patients.

DISCUSSION

This investigation critically examines the clinical, biochemical, and genetic implications of *PCSK9* in patients with CAD. The results revealed significantly higher BMI, blood pressure, fasting glucose, and dyslipidemia, along with elevated PCSK9 concentrations in CAD patients, highlighting the multifaceted role of PCSK9 in lipid regulation, inflammation, and atherosclerotic progression. Additionally, the significant association of the rs615563 variant with CAD risk is a key finding that enhances our understanding of the disease. The protective effect of the A–T haplotype, a promising discovery, underscores the potential for genetic interventions in CAD management.

METABOLIC AND PHENOTYPIC CHARACTERISTICS

Iraqi CAD patients exhibited a 2.6-fold elevation in serum PCSK9 levels compared to controls ($p < 0.0001$), consistent with global studies linking heightened PCSK9 levels to advanced atherosclerosis [1]. This enzyme drives CAD pathogenesis through two pathways: the hepatic degradation of LDL receptors elevates systemic cholesterol levels, while vascular expression

in endothelial cells and macrophages directly promotes inflammation, foam cell formation, and endothelial dysfunction, independent of lipid metabolism [2-3]. These synergistic mechanisms establish PCSK9 as a master regulator of plaque vulnerability, particularly through its amplification of matrix metalloproteinase activity and reduced collagen stability within fibrous caps. Significant dyslipidemia characterized CAD cases, with elevated total cholesterol, triglycerides, LDL-C, and VLDL-C alongside reduced HDL-C (all $p \leq 0.005$), directly reflecting PCSK9's catalytic enhancement of LDL receptor catabolism [4]. Genetic epidemiology solidifies this relationship: gain-of-function variants, such as E670G, elevate CAD risk through LDL-C accumulation [5]. In contrast, loss-of-function polymorphisms confer a risk reduction of up to 88% by stabilising hepatic LDL receptor expression [6]. Notably, elevated fasting glucose, BMI, and blood pressure indicate insulin resistance. This condition upregulates PCSK9 transcription through SREBP-2 activation, creating a pathological feedback loop in which hyperglycemia accelerates LDL receptor degradation and disrupts the endothelial glycocalyx [7]. The metabolic-inflammatory crosstalk amplifies plaque vulnerability through epigenetic reprogramming: hypermethylation of the PCSK9 promoter in individuals with diabetes perpetuates expression even during statin therapy. At the same time, histone acetylation enhances NLRP3 inflammasome assembly within plaque macrophages [8]. This positions PCSK9 at the intersection of lipid dysregulation, glucotoxicity, and vascular inflammation. This triad explains the 2.6-fold elevation of PCSK9 observed in Iraqi patients, which is consistent with that seen in cohorts from China and Korea [10]. Critically, these findings validate PCSK9 inhibitors as universal therapeutic tools, with studies showing that alirocumab reduces cardiovascular events by 15% in patients with high baseline PCSK9 levels, regardless of ethnicity [11].

POPULATION - SPECIFIC GENETIC ARCHITECTURE OF PCSK9

Our analysis of 220 Iraqis revealed profound ethnic divergence in the effects of PCSK9 variants: rs2483205 exhibited complete neutrality, whereas rs2479394 G/A conferred a striking 79% reduction in CAD risk (OR = 0.21, $p = 0.024$). This contrasts sharply with global patterns, where rs2483205-T/T reduces risk in Han Chinese [12] but increases severity in Egyptians [13] divergence attributable to Iraqi-specific linkage disequilibrium with functional variants, epigenetic modulation via war-related stress exposures, and gene-environment interactions like high saturated fat diets amplifying LDL-C effects [14]. Crucially, rs2479394's protection aligns with PCSK9 loss-of-function principles but extends beyond lipid modulation, as experimental models confirm that such variants suppress caspase-1 activation and NLRP3 inflammasome assembly in macrophages, reducing interleukin-1β release by 40%(3) Mechanistically, rs2479394 disrupts the lipid-inflammation-metabolism triad: enhanced LDL receptor recycling reduces apoB100-containing particles by 22% [15], downregulates IL-6/CRP signaling, mitigates endothelial ICAM-1 expression [16] and improved insulin sensitivity blunts SREBP-2-mediated PCSK9 upregulation in metabolic syndrome [17]. The absence of A/A homozygotes in CAD patients suggests a dose-dependent threshold effect, mirroring near-complete CAD resistance in North Indian rs505151-GG carriers, where homozygosity reduces LDL-C by 38% [18]. This protective gradient suggests that homozygous rs2479394 A/A may confer absolute CAD protection, a hypothesis that requires validation in larger Iraqi cohorts. The exceptional effect size (OR=0.21) exceeds most reported variants (e.g., rs505151 OR=1.5 in Indians [19], suggesting unique haplotype structures or synergistic interactions with Mesopotamian genetic backgrounds. This variant's clinical utility is transformative: G/A carriers may be able to forego intensive interventions in resource-limited settings while genotyping predicts a superior response to PCSK9 inhibitors, similar to rs662145-T carriers, who experience 30% fewer cardiovascular events post-therapy [20].

HAPLOTYPE CONTEXT AND ANCESTRAL DIVERGENCE

The absence of single-SNP effects underscores how ancestry-specific haplotype structures modulate CAD risk, as identical variants exhibit opposing effects across populations due to divergent linkage disequilibrium patterns. While rs2483205-T was neutral in Iraqis, it anchors the protective H4 haplotype (T-G) in Han Chinese, reducing CAD risk by 50% through coordinated LDL-C reduction and improvements in glucose metabolism (12). Conversely, the E670G-encompassing haplotype elevates LDL-C by 3.5% and coronary lesion complexity in Caucasians by disrupting the PCSK9 autocatalytic domain [21]. This divergence highlights how Iraqi-specific LD blocks may decouple SNPs from causal variants, necessitating haplotype-level analysis to resolve actual genetic effects, a principle validated in African ancestry cohorts where distinct LD enables fine-mapping of causal variants at 1p13 loci [22]. The protective T-A haplotype identified here (OR=0.45, $p = 0.020$) operates through integrated biological pathways mirroring global PCSK9 loss-of-function haplotypes, like the Italian R46L block (15.4% LDL-C reduction, 33% lower MI risk;(23)It en-

hances LDL receptor recycling, while similar to Chinese H4, it suppresses endothelial TRAIL-mediated apoptosis and macrophage NLRP3 activation [23]. These haplotypes transcend lipid modulation by disrupting metabolic-inflammatory cascades, particularly insulin resistance-driven PCSK9 upregulation, which in turn amplifies foam cell formation via CD36 overexpression. The dose-dependent protection gradient (heterozygote benefit vs. homozygote resilience) mirrors Cohen's landmark observation of near-complete CAD resistance in PCSK9 nullizygotes [24]. Clinically, PCSK9 haplotypes enhance CAD management: the Chinese H4 haplotype improves polygenic risk score accuracy beyond single SNPs (AUC 0.74 vs. 0.68). At the same time, rs562556-T predicts 40% fewer cardiovascular events post-inhibitor therapy due to an exaggerated reduction in LDL-C [25]. In Iraq, implementing T-A haplotype screening could optimize prevention by stratifying patients into three tiers: haplotype-protected individuals (standard care), moderate-risk heterozygotes (aggressive risk factor control), and high-risk non-carriers (early PCSK9 inhibition). This approach disrupts the lipid-inflammation-metabolism triad driving Iraq's CAD epidemic, potentially reducing mortality in a nation where CAD claims 45% of lives annually.

METABOLIC NEUTRALITY OF RS2479394 SNP

The absence of associations with lipid profiles, PCSK9 levels, or glycemic parameters for rs2479394 in Iraqi CAD patients ($p > 0.05$) starkly contrasts with functional variants like E670G, which exhibits dose-dependent LDL-C elevation (GG > AG > AA), explaining 3.5% of LDL variability [21] and rs562556, which drives carotid plaque progression via PCSK9-mediated LDLR degradation [26]. This metabolic neutrality stems from three mechanistic layers: first, rs2479394's intronic location likely lacks splicing regulatory function versus coding variants in the prodomain (e.g., R46L); second, population-specific LD patterns decouple it from causal variants in Iraqis, as ancestrally distinct haplotype blocks alter variant correlations [22]. Third, disease-stage heterogeneity dilutes genetic effects, with PCSK9 influences being most pronounced in pre-disease cohorts under the age of 45 [23]. Clinically, the neutrality of rs2479394 refines risk stratification; however, rs2479394 lacks utility for metabolic prediction, paralleling Pereira's 31-variant GRS, which excluded PCSK9 due to non-significant haplotypic effects in Portuguese CAD [27]. Regarding disease progression, the metabolic disconnection associated with rs2479394 implies a minimal impact on atherosclerosis pathogenesis. PCSK9 haplotypes predominantly elevate CAD risk through LDL-C-mediated pathways, as evidenced by Mendelian randomization, which shows that LDL-C reduction mediates 89% of PCSK9's protective effect [28]. Nevertheless, its strong CAD protection suggests that it operates through non-lipid pathways, such as inflammation, potentially via modulation of JAK-STAT signaling in vascular smooth muscle cells. This pathway can be measured via IL-6 trans-signaling assays in future studies. The rs2479394 locus heterogeneity within PCSK9, where functional variants (R46L, E670G) consistently modulate lipids versus non-coding SNPs with population-specific effects, is echoed across West Asia: Namordizadeh observed no haplotype effects for Iranian MI risk despite individual SNP associations [29] while Li identified PCSK9 rs662145 as a triglyceride-elevating locus in Han Chinese through enhancer-promoter interactions [30]. Such context dependency underscores that intronic variants may gain metabolic relevance only within specific haplotypic or environmental contexts, necessitating the functional validation of rs2479394 via CRISPR-edited hepatocyte models to quantify PCSK9-LDLR binding kinetics.

LIMITATIONS

This study has several limitations that should be considered when interpreting the findings. First, although we adjusted for age and sex, residual confounding by other cardiovascular risk factors (such as smoking, hypertension, diabetes, and BMI) could not be entirely ruled out because detailed adjustments were beyond the available dataset. This may influence the observed associations and should be addressed in future analyses with more comprehensive covariate data.

Second, our case–control design, although useful for initial discovery, cannot establish causality and is prone to selection bias. Replication in an independent Iraqi or regional cohort will be necessary to confirm these results and enhance generalizability. Third, the genetic analysis was restricted to two common PCSK9 SNPs (rs615563 and rs2483205). While these were selected based on prior evidence, this limited scope does not capture the full range of PCSK9 variation, especially rare or regulatory variants that may influence CAD risk. Broader genotyping or sequencing studies could provide a more complete picture of PCSK9's role in Iraqi populations. Finally, functional assays to directly test the biological effects of the identified variants were beyond the scope of this study but would strengthen causal inference. Despite these constraints, our rigorous genotyping, detailed biochemical profiling, and haplotype and sex-stratified analyses offer valuable initial insights and provide a foundation for larger, multi-centre studies to validate and extend these findings.

CONCLUSIONS

This study shows that the PCSK9 rs2479394 G/A genotype and the T-A haplotype significantly reduce CAD risk in Iraqis, while rs2483205 shows no association. CAD patients exhibited elevated PCSK9 and marked dyslipidemia, though rs2479394 did not affect metabolic traits. These findings highlight population-specific genetic influences on CAD and support further large-scale and functional investigations.

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CONFLICT OF INTEREST

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ORIGINAL ARTICLE

CONTENTS

Features of metabolism and peculiarities of organs' structural reorganization by sodium nitrite with underlying tobacco intoxication

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ABSTRACT

Aim: To determine the characteristics of metabolism in rats of different ages exposed by sodium nitrite with underlying tobacco intoxication.

Materials and Methods: The experiments were conducted on male white rats kept on a standard vivarium diet. The rats were divided into three age groups: sexually immature with a body weight of 60–80 g (3 months old), sexually mature with a body weight of 180–200 g (12 months old), and elderly with a body weight of 300–350 g (18 months old).

The animals received a single intragastric dose of sodium nitrite via a probe in the form of an aqueous solution at a dose of 45 mg/kg body weight, which is 1/4 of the LD50. The study was conducted 24 and 72 hours after exposure to this toxicant.

Results: It has been established, that during the whole experiment, after the affection of the rats, the hyperproduction of active forms of oxygen is present. Simultaneous exposure of rats to sodium nitrite and tobacco intoxication leads to increased generation of active oxygen species by neutrophil granulocytes in the blood. By the end of the experiment, sexually immature rats showed a maximum increase in the content of TBK-active products in all organs (in the liver – 3.3 times, in the kidneys – 3.6 times, in the lungs – 4.3 times, myocardium – 6.0 times). At the end of the experiment, the content of methemoglobin in the blood of young animals after exposure increased significantly (2.6 times). The simultaneous effect of sodium nitrite and tobacco smoke causes disturbances in the antioxidant system. Suppression of superoxide dismutase activity in the blood serum and liver of rats of all age groups was detected. Suppression of superoxide dismutase activity was detected in the blood serum and liver of rats of all age groups. By the end of the experiment, ceruloplasmin content had increased in the group of sexually immature rats, exceeding the level in control animals by 2.4 times at the end of the experiment. Glutathione levels decreased in the liver, lungs, kidneys, and myocardium of rats of all age groups, most significantly in sexually immature rats. At the same time, nitrooxidative stress developed, as evidenced by an increase in nitrite ion levels in all organs during the experiment. After exposure to sodium nitrite, rats of different ages that had been poisoned with tobacco smoke for 45 days showed a significant increase in enzyme activity in their blood serum (alanine aminotransferase activity in blood serum increased 7.7 times, aspartate aminotransferase 5.0 times, lactate dehydrogenase by 1.8 times, gamma-glutamyl transpeptidase by 4.1 times, and alkaline phosphatase by 3.6 times. Secondary endogenous toxins accumulated in the affected organism – medium-weight molecules, the content of which in blood serum was highest at the end of the experiment in sexually immature rats. The myocardium of elderly rats was most sensitive to the action of toxicants. In the lungs of sexually immature and aged rats, cytochrome oxidase activity decreased 1.4 times at the end of the experiment.

Microscopic examination of organs revealed that structural reorganization of lungs, liver, kidneys, myocardium of the animals of all age groups was characterized by changes in vascular bed, main morpho functional components (acini, hepatocytes, nephrons and muscle fibers of the myocardium).

Conclusions: Exposure of rats of all age groups to sodium nitrite and tobacco smoke causes intensification of free radical oxidation processes in the rats' bodies, manifested by the occurrence of oxidative and nitrooxidative stress, activation of lipoperoxidation and oxidative modification of proteins, suppression of the antioxidant system, energy supply processes, formation of endogenous intoxication, and increased intensity of inflammatory processes in the body. The most pronounced metabolic changes with underlying nitrite-tobacco intoxication were evidenced in the immature rats that were proved by the experimental data. The identified violations lead to severe intoxication of the body, which requires the additional introduction of corrective factors.

KEY WORDS: metabolic disorders, endogenous intoxication, heart, lungs, liver, blood vessels, sodium nitrite, tobacco smoke

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INTRODUCTION

In everyday life, people are exposed to a number of toxic factors, which leads to general poisoning of the body [1, 2, 3]. Harmful habits such as smoking, alcohol, and medication abuse play a significant role in the development of pathology. Active and passive smoking can cause the formation of active forms of oxygen, which activate free radical oxidation processes in the body [4]. At the same time, the components of cigarette smoke cause mitochondrial oxidative stress in various types of

cells. Reactive oxygen species cause a complex pro-inflammatory response in the body, resulting in increased production of pro-inflammatory cytokines [3, 5].

At the same time, a significant environmental and medical-biological problem in the agricultural and industrial regions of Ukraine is the combined effect of inorganic nitro compounds on the human and animal organism, accompanied by nitrate-nitrite intoxication [6, 7]. The intake of nitrates and nitrites into the body leads to the formation of excessive amounts of nitrogen oxide, which can initiate chain free radical reactions [8]. This creates the conditions for the formation of other active forms of nitrogen, which can cause hypoxic and free radical necrobiosis.

The increase in the number of diseases and pathological conditions, in which disturbances in oxidative processes, immune and inflammatory responses play a significant role, leads to a deepening of the manifestations of endogenous intoxication.

Thus, given the prevalence of smoking and the active use of nitrites in the national economy, it is important to study the mechanisms of the simultaneous effect of toxicants on the body, taking into account the age aspect.

AIM

To determine the characteristics of metabolism in rats of different ages exposed by sodium nitrite with underlying tobacco intoxication.

MATERIALS AND METHODS

The experiments were conducted on male white rats kept on a standard vivarium diet. The rats were divided into three age groups: sexually immature with a body weight of 60–80 g (3 months old), sexually mature with a body weight of 180–200 g (12 months old), and elderly with a body weight of 300–350 g (18 months old).

The animals received a single intragastric dose of sodium nitrite via a probe in the form of an aqueous solution at a dose of 45 mg/kg body weight, which is 1/4 of the LD50. The study was conducted 24 and 72 hours after exposure to this toxicant. A model of chronic tobacco smoke exposure was created using a sealed chamber with a volume of 30 liters, exposing the animals to the toxicant daily through appropriate openings. Six animals were placed in the chamber simultaneously for 6 minutes. After 15, 30, and 45 days from the start of exposure to tobacco smoke, the animals were removed from the experiment. With simultaneous exposure to both toxicants on the 30th day of tobacco intoxication (24 and 72 hours before the specified date), rats were administered sodium nitrite intragastrically. Similarly, sodium nitrite exposure was modeled on the 45th day of tobacco smoke poisoning in rats.

The study involved 792 rats. For biochemical studies, 720 rats were used, divided into groups depending on the duration of the study, with 36 animals in each group. For morphological studies, 72 rats were used.

The study material consisted of homogenates of the liver, heart, lungs, kidneys, whole blood, and blood serum.

Two series of studies were conducted. In the first series of experiments, the following indicators were determined: the content of active oxygen species (AOS), TBK-active products, oxidative modification of proteins (OMP), carboxyhemoglobin (HbCO), superoxide dismutase activity (SOD), catalase activity (CAT), and the erythrocyte intoxication index (EII). In the second series of experiments, the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), γ-gamma-glutamyltranspeptidases (GGTP), alkaline phosphatase (ALP), succinate dehydrogenase activity (SDH), cytochrome oxidase activity (CO), C-reactive protein (CRP) content, interleukin-4 (IL-4) content, and interleukin-6 (IL-6) content.

RESULTS

Tobacco smoke intoxication caused the generation of ROS (O_2, OH-, H_2O_2), which led to oxidative stress in the body, accompanied by the activation of lipid peroxidation (LPO) and oxidative modification of proteins. The intensification of free radical oxidation processes under the action of AFO led to the destruction of nucleic acids and carbohydrates, causing structural and metabolic disorders in cells.

The content of TBK-active products in the blood serum of rats affected by both toxicants simultaneously significantly exceeded the content recorded after the separate action of each factor.

The highest content of TBK-active products was found in the liver of young and mature rats after poisoning, which gradually increased and reached its maximum value at the end of the study. Similar activation of LPO processes after sodium nitrite exposure against the background of tobacco intoxication was observed in the kidneys of rats of different ages. By the end of the experiment, the content of TBK-active products had increased significantly in the kidneys of sexually immature rats by 3.6 times, in old rats by 3.5 times, and in sexually mature rats by 2.4 times ($p \leq 0.05$).

The lungs and myocardium are the targets of the toxic effects of tobacco smoke: the lungs of sexually immature rats, in which the content of TBK-active products was highest ($p \leq 0.05$) on both the 15th day

of intoxication and the 45th day of TS damage (24 and 72 hours after exposure to sodium nitrite against this background).

A study of the content of TBK-active products in the myocardium of rats after exposure to toxicants showed an increase in all age groups during the experiment ($p \leq 0.05$). The most pronounced changes in the content of TBK-active products were recorded in the myocardium of sexually immature animals. The content of TBK-active products in the myocardium of young rats increased after exposure depending on the duration of the study and at the end of the experiment was 12.3 times higher than that of control animals. This may be due to the increased sensitivity to stress of this particular group of animals. One of the reasons for this condition is the increased release of catecholamines into the blood in response to a stress factor (smoke), in particular adrenaline, which, when increased, has a toxic effect on the myocardium, further activating the processes of lipoperoxidation and increasing the content of its intermediate products in the heart.

During the study of OMP in rats, an increase in the content of 2,4-dinitrophenylhydrazones (2,4-DPH) was observed as both basic (λ=430 nm) and neutral (λ=370 nm) in the blood serum and organs of animals of all age groups after exposure to sodium nitrite against the background of tobacco smoke intoxication.

When studying the content of neutral OMP products in the lungs of rats, it was found that the most sensitive to the action of toxicants were sexually immature animals, in which this indicator increased by 2.4 times ($p \leq 0.05$) by the end of the experiment compared to the group of control animals.

The kidneys of sexually immature rats were the most sensitive to the effects of toxicants, in which 72 hours after the administration of sodium nitrite against the background of 45-day TS intoxication, the content of neutral OMP products increased 2.9 times ($p \leq 0.05$) compared to the control animals.

A similar increase in basic 2,4-DHFG was observed in the blood serum and organs of rats after exposure to toxicants.

Another indicator that characterizes pathological changes in the body of animals after exposure to tobacco smoke and indicates the deepening of hypoxia is carboxyhemoglobin. By the end of the experiment, the HbCO content in the blood of sexually immature rats (under the action of both toxicants) increased the most – 3.0 times higher than the control level, in sexually mature rats – 1.5 times higher, and in elderly rats – 2.3 times higher than in the control group.

Under conditions of sodium nitrite poisoning against a background of tobacco intoxication, superoxide dismutase activity was studied in the blood serum and liver of rats of all age groups. The latter in the blood serum of sexually immature rats decreased 2.5 times by the end of the experiment when poisoned with two toxicants, in sexually mature rats – 1.6 times, and in elderly rats – 1.9 times ($p \leq 0.05$) compared to the control group. The study of SOD activity in the liver confirmed its decrease at all stages of the study in all age groups. The enzyme activity decreased most significantly during the experiment in the liver of sexually immature animals.

Catalase activity in the blood serum and organs of rats of all age groups after exposure to toxicants was studied. It was found that CAT in the blood serum of rats of all age groups decreased throughout the experiment, which could lead to hydrogen peroxide intoxication of the body. The lowest CAT was recorded in the blood serum of sexually immature rats, which at the end of the experiment was 1.9 times lower than the control level, in sexually mature rats – 1.6 times lower, and in elderly animals – 1.4 times lower ($p \leq 0.05$).

A decrease in this indicator was observed in the liver of rats affected by toxicants, and it was lowest at the end of the study. The greatest decrease in CAT was observed in the liver of elderly rats.

CAT in the lungs of rats of different ages decreased depending on the duration of the experiment, and by the end of the study, it was 2.4 times lower than the control in young animals, 2.7 times lower in mature animals, and 2 times lower in elderly rats.

A pronounced decrease in CAT was recorded in the kidneys of rats of all age groups after sodium nitrite exposure against the background of tobacco intoxication. After 15 days of mildronate administration, catalase activity significantly increased in sexually mature and elderly animals.

The myocardium of elderly rats was most sensitive to the effects of both toxicants, with CAT decreasing 2.2 times compared to the control animals at the end of the study.

To assess the functional state of erythrocyte cytoplasmic membranes, it is important to determine their permeability percentage. Fifteen days after tobacco smoke poisoning and 72 hours after sodium nitrite poisoning, EII increased by 38.7% in the blood of sexually immature rats, after 30 days of tobacco intoxication and 72 hours of sodium nitrite poisoning, it increased by 31.6%, and at the end of the experiment, it exceeded the level of control animals by 55.6%. In sexually mature rats, this indicator exceeded the control values by 38.0% at the end of the experiment, and in elderly animals – by 54.1%.

When studying the functional state of the liver in rats affected by sodium nitrite against the background of

tobacco intoxication, cytolytic syndrome was diagnosed based on ALT and AST activity indicators, and cholestatic syndrome was diagnosed based on γ-glutamyltranspeptidase and alkaline phosphatase activity.

Sodium nitrite poisoning in rats against the background of tobacco intoxication led to an increase in serum ALT (a marker enzyme of the liver) activity in all age groups. By the end of the experiment, serum ALT activity in sexually immature rats increased 7.7 times compared to the control level, and in the other two age groups — 5.3–5.4 times ($p \leq 0.05$).

When determining ALT activity in the liver, the changes were reversible. Enzyme activity decreased in all groups of rats at all study periods, which was progressive in nature.

By the end of the experiment, ALT activity in the liver of sexually immature rats decreased by 73%, i.e., it was only 27% of the level in control animals.

A decrease in ALT activity in the lungs of affected rats was observed at all study periods. A more pronounced decrease was observed in the lungs of elderly rats, in which enzyme activity was 3.5 times lower than the control level at the end of the study. In sexually immature and elderly rats, this indicator decreased in the lungs by 2.4 and 2.3 times, respectively.

The myocardium of elderly rats was most sensitive to the effects of toxicants. After 72 hours of sodium nitrite poisoning against a background of 45-day intoxication, ALT activity in this group of animals decreased 4.5 times. During the same period, ALT activity was 4.3 times lower than the control in the myocardium of sexually immature rats and 3.7 times lower in sexually mature rats.

A study of enzyme activity in the kidneys of rats of different ages showed that the most sensitive to the simultaneous action of sodium nitrite and tobacco intoxication were elderly rats, in which the studied indicator decreased by 5.7 times, in sexually immature rats it decreased 3.0 times, and in sexually mature rats it decreased 2.3 times.

The highest AST activity was recorded in the blood serum of elderly rats after exposure to sodium nitrite against the background of tobacco intoxication. The experimental indicator increased during the experiment and reached its highest level at the end of the study (8.2 times higher than in control animals). In sexually immature rats during this period, it increased 5.0 times, and in sexually mature rats, 6.9 times compared to the control.

The most pronounced changes in AST activity after exposure to toxicants were observed in the liver of sexually immature rats, in which this indicator decreased by 4.8 times compared to the level of control animals at the end of the study. During the same period, enzyme activity decreased by 2.7 and 3.7 times, respectively, in the livers of sexually mature and elderly rats.

Considering that increased AST activity in the blood serum of rats of all age groups is a test for the degree of myocardial damage, this indicator was studied specifically in the myocardium. After poisoning rats with sodium nitrite against the background of 45-day tobacco intoxication, AST activity in the myocardium of rats of different ages decreased. In the myocardium of sexually immature and elderly rats, the indicator decreased depending on the duration of the study. By the end of the experiment, enzyme activity had decreased by 3.8 and 3.9 times, respectively, compared to the level in the control group. A study of AST activity in the kidneys of rats of different ages after exposure to sodium nitrite showed a similar decrease ($p \leq 0.05$) during the experiment.

To confirm the development of cytolytic syndrome in the affected organism, the activity of another organ-specific enzyme, LDH, was studied. Its activity in the blood serum of rats of all age groups increased and reached its highest value at the end of the experiment in sexually immature animals. After 45 days of tobacco smoke poisoning and 72 hours from the moment of sodium nitrite exposure, the enzyme activity in this group of animals was 1.8 times higher than in the control group, and 1.7 times higher in sexually mature and elderly rats.

The study of LDH activity in the myocardium showed its decrease in rats of all experimental groups. The lowest enzyme activity after exposure of rats to both toxicants was recorded in the myocardium of sexually immature and elderly rats at the end of the experiment – 1.5 times lower. The lowest LDH activity was recorded in the liver of sexually immature and elderly rats as a result of exposure to toxicants. In the lungs of affected sexually immature rats, LDH activity was lowest (1.6 times lower than the control level) at the end of the study.

Exposure of rats of all age groups to sodium nitrite against the background of 45-day tobacco smoke intoxication led to a decrease in LDH activity in their kidneys. The most pronounced changes were observed in the kidneys of young and elderly rats, in which the indicator was 1.7 times lower than the control level.

GGTP activity in the blood serum of rats increased after exposure to both toxicants simultaneously. In the blood serum of sexually immature rats, GGTP activity increased after exposure in the last term the most – 4.1 times compared to control animals. During this period, the indicator increased 1.6 times in the blood serum of sexually mature rats and 2.3 times in elderly animals.

A study of GGT activity in the liver showed a decrease

after injury in all experimental groups of animals. The greatest decrease in the activity of this enzyme was observed in the liver of sexually immature rats. In these animals, the experimental indicator decreased continuously depending on the duration of the experiment and at the end of the study reached 48% of the control.

Damage to the membrane structures of hepatocytes is evidenced by the results of studies of the organ-specific enzyme (a marker of cholestasis) – alkaline phosphatase in blood serum. The LP study showed a statistically significant increase in serum during the experiment in all experimental groups of animals. The enzyme activity in serum ($p \leq 0.05$) increased linearly and reached its highest value at the end of the study. ALP activity in the blood serum of sexually immature rats at the end of the study increased 3.6 times, in sexually mature rats – 1.6 times, and in elderly rats – 3.4 times after their damage to the NN against the background of tobacco intoxication.

In the kidneys of sexually immature rats, this indicator decreased by 2.7 times, in mature rats by 1.5 times, and in elderly rats by 2.5 times.

Similar results were obtained when studying the activity of LF in the liver of intoxicated rats. The activity of LF decreased the most after poisoning with NN against the background of tobacco intoxication in the liver of sexually immature animals (by 76% relative to the control) at the end of the experiment.

Under conditions of simulated pathology in the tissues of the liver, lungs, and myocardium, the indicators of the mitochondrial electron transport system decreased at the beginning of the study, with maximum energy deficiency in cells at the end of the experiment.

In the liver of rats of all age groups, SDG activity decreased sharply depending on the duration of the experiment. The greatest decrease in enzyme activity was achieved in the liver of sexually immature rats at the end of the study (45 days of TD and 72 hours from the moment of NN poisoning) – 2.2 times ($p \leq 0.05$) below the level of control rats.

After exposure to toxicants, a decrease in SDG activity was observed in the lungs of rats of all age groups throughout the experiment. The decrease was significantly more pronounced than when poisoned with each of the toxicants separately. By the end of the study, SDG activity in the lungs of sexually immature rats decreased by 2.1 times, in sexually mature rats by 1.9 times, and in elderly rats by 1.7 times ($p \leq 0.05$) compared to the control group.

The study of SDG activity in the organs of rats poisoned with sodium nitrite against the background of tobacco intoxication and the detected decrease in its activity created the prerequisites for studying CO activity in animals of different ages under such pathological conditions. The greatest decrease in CO activity was observed in the liver of sexually immature rats – by the end of the experiment, it had decreased 2.7 times compared to the control.

A similar decrease in CO activity after damage was observed in the myocardium of rats of different ages. The myocardium of elderly rats was the most sensitive to the action of toxicants: enzyme activity decreased throughout the study period. By the end of the experiment, it was 2.2 times lower than that of the control group.

Damage by both toxicants led to a profound disruption of CO activity in the lungs of rats. In sexually immature and elderly rats, this indicator decreased by 60.0% at the end of the experiment, and in sexually mature rats, by 50.0%.

A decrease in CO activity in the mitochondria of various organs when exposed to toxicants can be explained by a restriction in the flow of electrons from the substrate link of the respiratory chain through cytochromes b-c. Inhibition of CO activity may also occur due to the binding of free oxygen radicals to metal atoms present in the enzyme under study.

The study of C-reactive protein content is one of the most acceptable markers for early diagnosis and monitoring of inflammatory diseases. The highest content of this indicator was recorded in the blood serum of elderly rats poisoned with both toxicants, in which it increased 3.3 times by the end of the experiment. Similar changes in CRP content were observed in the blood serum of sexually immature and sexually mature rats after injury and the application of corrective factors. Sexually mature rats were more resistant to such changes, with the smallest increase in this indicator after exposure. It is known that CRP synthesis and secretion occurs in the liver and is regulated by pro-inflammatory cytokines, primarily IL-6, but it can also be produced by macrophages and lymphocytes.

Exposure of rats to sodium nitrite against the background of 45-day tobacco smoke intoxication led to an increase in the content of pro-inflammatory cytokine IL-6 in the blood serum of rats in all experimental groups. The highest IL-6 levels were observed in the serum of sexually immature rats, which had already increased significantly at the beginning of the study. By the end of the experiment, after exposure to both toxicants, the content of pro-inflammatory cytokine in the blood serum of sexually mature rats increased 3.5 times, and in elderly animals – 2.6 times compared to the control group.

The basis for the development of the inflammatory process is the launch of a cytokine cascade, which

includes pro-inflammatory cytokines on the one hand and anti-inflammatory mediators on the other. The balance between the two oppositely acting groups of cytokines largely determines the course and outcome of the disease. It is known that IL-4 has a powerful anti-inflammatory effect and plays a key role in the onset of the inflammatory response.

The experimental indicator progressively decreased in all age groups of animals depending on the duration of intoxication. By the end of the study, the content of anti-inflammatory cytokine decreased most significantly in the blood serum of sexually immature rats – by 2.2 times, while in mature and elderly rats – by 1.7 and 1.8 times ($p \leq 0.05$), respectively (compared to the control).

Oxidative stress and the accumulation of toxic products of exogenous and endogenous origin in the bodies of affected rats led to the development of inflammatory processes, which intensified depending on the duration of the experiment and the age of the animals. This was confirmed by an imbalance of pro- and anti-inflammatory cytokines and an increase in acute phase protein in the blood serum.

To confirm the results obtained, morphological studies of the organs of rats of different age groups after exposure to sodium nitrite were conducted.

Microscopic studies of the internal organs of white rats affected by sodium nitrite against the background of tobacco intoxication showed that the structural organization of the lungs and heart of animals of all age groups is characterized by changes in the vascular bed and the main morphofunctional components.

The most pronounced changes occur in the lungs of sexually immature and elderly animals. In the respiratory section of the lungs, the alveolar area is significantly increased compared to the control group of animals. There is a thickening of the interalveolar septa, blood filling of the hemocapillaries, and lymphoid infiltration under conditions of toxicant exposure.

The results of the studies allow us to conclude that when rats of different ages are exposed to sodium nitrite and tobacco smoke, oxidative and nitrooxidative stress develops in the body, which is exacerbated by the simultaneous use of toxicants. Sexually immature rats were the most sensitive to the effects of the xenobiotics we used.

DISCUSSION

Poisoning rats with sodium nitrite against a background of chronic tobacco intoxication is accompanied by hyperproduction of aggressive ROS in the affected organism, leading to the development of oxidative stress. This is indicated by the intensification of lipoperoxidation and oxidative modification of proteins after damage and a decrease in the activity of the antioxidant system components [9]. Sodium nitrite causes increased methemoglobin formation through a free radical mechanism. The primary reaction of tobacco smoke on the body is the formation of carboxyhemoglobin. Both hemoglobin derivatives are unable to transport oxygen to organs and tissues. It is known that the entry of sodium nitrite into the body is accompanied by the development of hemic hypoxia, as indicated by the increased content of methemoglobin in the blood of poisoned rats. After exposure to both toxicants, sexually immature rats were found to be the most sensitive, with this indicator progressing and reaching its highest level by the end of the experiment – 3.3 times higher than in control animals.

Thus, nitrite-tobacco toxicosis leads to tissue hypoxia, which is confirmed by a decrease in the activity of mitochondrial enzymes, resulting in the suppression of energy production processes [5, 10]. The above factors cause the destruction of plasma and cytoplasmic membranes and changes in their permeability. At the same time, the activity of organ-specific enzymes in the blood serum increases and decreases in the organs of animals. The identified disorders are accompanied by a syndrome of endogenous intoxication caused by the accumulation of degradation products of lipid and protein molecules, in particular MSM, in the body. A significant amount of endogenous toxins, as well as exogenous toxins that enter the body with tobacco smoke, leads to the activation of inflammatory processes. Under conditions of nitrite-tobacco toxicosis, an imbalance occurs in the content of pro- and anti-inflammatory cytokines. Prolonged hypoxia caused by sodium nitrite and tobacco smoke leads to increased formation of nitric oxide under the action of inducible NO synthase, which is activated in pathological conditions. Nitrooxidative stress occurs in the affected organism, confirmed by the suppression of endothelial NO synthase activity and the accumulation of nitrite ions in the organs of animals.

The proposed scheme of development of nitrite-tobacco toxicosis suggests that the primary response to the entry of these toxicants into the body is the activation of free radical oxidation processes, resulting in the development of oxidative and nitrooxidative stress, deepening endogenous intoxication, and activation of inflammatory processes, disruption of energy supply processes, and changes in the body's defense systems [11].

It has been found that after rats are exposed to toxicants, there is hyperproduction of active forms of oxygen, which leads to the activation of free radical oxidation processes (lipoperoxidation and oxidative modification of proteins) in the animals' bodies [12]. Under these conditions, hypoxia occurs due to the significant formation of methemoglobin and carboxyhemoglobin. The toxic products formed provoke the destruction of biomembranes and the release

of intracellular components into the blood. Secondary endogenous toxins (medium-weight molecules) accumulate in the body, and their content increases in the blood serum of rats of all age groups. Increased endogenous intoxication leads to changes in the antioxidant system (inhibition of enzymatic and non-enzymatic links).

The inhibition of mitochondrial oxidation system enzymes is more pronounced when both toxicants are present simultaneously. The development of inflammatory processes has been observed, as evidenced by an imbalance of pro- and anti-inflammatory cytokines and an increase in serum C-reactive protein levels. There are disturbances in the functioning of the NO system and nitrooxidative stress occurs, manifested by an increase in the activity of inducible NO synthase and a decrease in the activity of its endothelial isoform.

Given that LF is an organ-specific liver enzyme, an increase in its activity is a typical sign of cholestasis, and the results obtained should be considered as confirmation of hepatocyte damage with manifestations of inflammatory processes, cytolysis, and bile stasis in the bile capillaries and ducts. All this together contributes to the overall endogenous toxicosis, which manifests itself in an increase in MSM – markers of toxic syndrome.

Poisoning rats with sodium nitrite against the background of tobacco intoxication leads to oxidative stress in the body with the formation of a significant amount of AFO, accompanied by the development of destructive processes and cytolytic syndrome, as well as a change in the permeability of cell membranes with subsequent disruption of the functions of intracellular organelles. Irreversible disturbances in the structure and functioning of mitochondria caused by the action of excessive amounts of ROS lead to a shift in energy metabolism towards increased glycolysis and inhibition of oxidative phosphorylation [13, 14].

Sodium nitrite poisoning causes nitrooxidative stress in rats due to the formation of significant amounts of nitric oxide. Tobacco smoke contains nitrogen dioxide and nitrogen oxide, which, when ingested, are toxic due to the formation of peroxynitrite, nitrite, and nitrate ions that initiate LPO reactions. Thus, nitrooxidative stress developed in the body along with oxidative disorders.

Research results have shown that simultaneous exposure to sodium nitrite and tobacco smoke leads to significant activation of nitrite ion formation processes in the bodies of rats of different ages. In the blood serum of sexually immature rats after exposure to both toxicants, the nitrite ion content increased 2.3 times at the end of the study, which can cause significant formation of endogenous nitric oxide and the development of nitrooxidative stress. At the same time, the indicator exceeded the control level by two times in the blood serum of sexually mature and elderly animals. The maximum formation of nitrite ions occurs at the end of the study.

After exposure to toxicants, the nitrite ion content increased in the liver, myocardium, and kidneys of rats of all age groups. Sodium nitrite poisoning of animals against the background of 45-day tobacco smoke intoxication caused an increase in the nitrite ion content in the lungs of rats in all experimental groups. In the lungs of sexually immature animals, this indicator increased 3.7 times by the end of the experiment, in sexually mature animals – 3.2 times, and in elderly rats – 2.7 times compared to the control group, which confirms the tropism of tobacco smoke to this organ.

Excessive accumulation of nitrite ions in the organs of rats after injury can cause increased formation of nitric oxide.

It has been proven that after rats were injured with sodium nitrite against a background of tobacco intoxication, iNOS activity progressively increased in the blood serum in all age groups. iNOS was most active in the blood serum of sexually immature rats, increasing 3.3 times ($p \leq 0.05$) by the end of the experiment after exposure to toxicants.

A study of iNOS activity in the liver of rats of different ages after exposure to toxicants showed that it was most active in sexually immature animals. At the beginning of the experiment, the enzyme activity in the liver of this group of rats increased 3.0 times ($p \leq 0.05$), and by the end of the experiment, it exceeded the level of control animals by 4.3 times ($p \leq 0.05$).

The activity of eNOS in the blood serum and liver of rats of different age groups after exposure to toxicants was studied. The enzyme activity in the blood serum of sexually immature rats decreased almost equally at all stages of the study and at the end of the experiment was 56.0% lower than the control. In aged rats, this indicator decreased by 56.0%, and in sexually mature rats, by 50.0% ($p \leq 0.05$).

Exposure to both toxicants simultaneously led to a decrease in eNOS activity in the liver of animals of all age groups. The lowest eNOS activity was recorded in the liver of sexually immature rats at the end of the experiment; it decreased 5.1 times after exposure compared to the intact control group of the same age. In sexually mature rats, this indicator decreased by 3.0 times, and in elderly animals, by 3.7 times compared to the control group at the end of the study.

Sodium nitrite poisoning against the background of tobacco intoxication caused oxidative and nitrooxidative stress in the animals, which determined the severity of the pathological process.

The results obtained allow us to conclude that NO hyperproduction, which is associated with an increase in nitrite ion content after injury and iNOS activity, along with the activation of oxygen free radical reactions, is

one of the key links in toxic damage to the body, particularly in cases of sodium nitrite poisoning against the background of tobacco intoxication.

CONCLUSIONS

Exposure of rats of all age groups to sodium nitrite and tobacco smoke causes intensification of free radical oxidation processes in the rats' bodies, manifested by the occurrence of oxidative and nitrooxidative stress, activation of lipoperoxidation and oxidative modification of proteins, suppression of the antioxidant system, energy supply processes, formation of endogenous intoxication, and increased intensity of inflammatory processes in the body. The most pronounced metabolic changes with underlying nitrite-tobacco intoxication were evidenced in the immature rats that were proved by the experimental data. The identified violations lead to severe intoxication of the body, which requires the additional introduction of corrective factors.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Assessment of surgical antimicrobial prophylaxis practices in a Tertiary Hospital in Iraq

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ABSTRACT

Aim: Surgical Antimicrobial Prophylaxis (SAP) is critical in reducing surgical site infections (SSIs). However, inappropriate antibiotic use remains common concern in many healthcare settings. Therefore, the aims was to assess the use of surgical antibiotic prophylaxis.

Materials and Methods: A retrospective cross-sectional study was conducted among 419 discharged surgical and gynecological patients at Al-Salam Teaching Hospital, Mosul, Iraq, during August 2023. Patient records were analyzed for SAP indication, antibiotic selection, timing, and duration according to international guidelines. This study aimed to assess both the prescribing patterns and the appropriateness of surgical antimicrobial prophylaxis practices.

Results: SAP was indicated for most patients; however, appropriate antibiotic selection was documented in only 35.1%. Ceftriaxone was predominantly prescribed (73.0%), followed by amoxicillin (8.6%), and cefotaxime (6.2%). Furthermore, most patients received antibiotics after surgical incision, and the duration exceeded 7 days in a significant proportion of cases, contrary to standard recommendations. Of 419 patients, 117 (27.9%) received appropriate prophylaxis and 302 (72.1%) inappropriate prophylaxis. The mean antibiotic cost per patient was 2.16 USD in the appropriate group compared with 4.85 USD in the inappropriate group. Inappropriate prophylaxis accounted for 85.3% of the total antibiotic expenditure.

Conclusions: The study highlights substantial gaps in adherence to SAP guidelines, emphasizing inappropriate antibiotic selection, timing, and excessive duration. These findings underscore the urgent need for implementing antimicrobial stewardship interventions to optimize SAP practices and reduce potential antibiotic resistance and SSIs.

KEY WORDS: surgical site infections, prescribing patterns, surgical antibiotic prophylaxis, inappropriate antibiotic use

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INTRODUCTION

Effective Surgical Antimicrobial Prophylaxis (SAP) significantly reduces surgical site infections (SSI) incidence, which are infections occurring at or near surgical incisions within 30 days or up to one year post-operation [1, 2]. SSI rank third among all nosocomial infections in hospitals; therefore, optimal prophylaxis should be used, involving correct indication based on microbiological evidence, precise timing (within 60 minutes prior to surgical incision), selection of antibiotics covering likely pathogens, and maintaining adequate bactericidal concentrations throughout the surgical procedure [3, 4].

Multiple factors contribute to the development of SSIs, including type and duration of the surgical procedure, appropriate antimicrobial prophylaxis, and patient-related risk factors such as advanced age, diabetes mellitus, cigarette smoking, and immunocompromised states [5, 6]. Surgical wound classifications, ranging from clean and clean-contaminated to contaminated and dirty, directly influence infection risk [7, 8].

Irrational antibiotic prescribing practices is a major contributor to the high burden of surgical site infections (SSIs) in low- and middle-income countries, where the World Health Organization (WHO) estimates SSI rates to range from 1.2% to 23.6% [9-11]. Inappropriate prophylactic antibiotic use, characterized by incorrect drug choice, delayed timing, or excessive duration, exacerbates medical costs, prolongs hospital stays, increases superinfection risk, promotes antibiotic resistance, and raises adverse drug reactions [12, 13].

Data regarding SAP utilization in Iraq, particularly in Al-Salam Teaching Hospital, Mosul, remain scarce. Hence, this retrospective study aimed to assess SAP practices, including antibiotic selection, timing, duration, and adherence to international guidelines among patients discharged after surgical operations. Investigating these parameters at the hospital level will provide essential

insights into current prescribing patterns, identify gaps in antibiotic stewardship, and inform targeted interventions to enhance patient care outcomes [14-16]. The results from this study will serve as a baseline for future interventions, aiding policymakers and healthcare professionals in optimizing SAP protocols.

AIM

The primary aim of this study was to evaluate the utilization and appropriateness of surgical antimicrobial prophylaxis (SAP) among patients admitted to the surgical and gynecological wards at Al-Salam Teaching Hospital in Mosul, Iraq. Specifically, the study sought to:

1. Assess the indications for SAP in relation to the type and class of surgical procedures performed.
2. Evaluate the selection of antibiotics used for prophylaxis against established clinical guidelines.
3. Examine the timing and duration of antimicrobial administration in the perioperative period.
4. Identify patterns of inappropriate SAP use, including overuse of broad-spectrum antibiotics.

MATERIALS AND METHODS

STUDY DESIGN AND SETTING

A retrospective cross-sectional study was conducted at Al-Salam Teaching Hospital, located in Mosul, Iraq. The study included patients admitted to the surgical and gynecological wards who underwent surgical procedures and were subsequently discharged. The data collection covered a period of one month, specifically August 2023.

STUDY POPULATION

All patients who underwent surgical intervention and received antimicrobial therapy during hospitalization were considered eligible. Patients with incomplete medical records or non-surgical admissions were excluded. A total of 419 patient files were reviewed and included in the final analysis.

DATA COLLECTION

Data were extracted from physical patient records using a structured data collection form. The following variables were recorded:

- **Demographics**: age, sex, and residence
- **Surgical data**: diagnosis, type of surgery (elective/emergency), wound classification (according to CDC), duration of surgery
- **Antibiotic prophylaxis details**: name, timing (relative to incision), duration, and combination use
- **Clinical data**: comorbidities, smoking history, and penicillin allergy status

ASSESSMENT OF SAP APPROPRIATENESS

The appropriateness of Surgical Antimicrobial Prophylaxis (SAP) was evaluated based on ASHP 2013 and WHO 2015 guidelines. Four components were assessed:

1. **Indication**: SAP should be given for clean-contaminated, contaminated, or selected clean procedures
2. **Antibiotic selection**: Use of narrow-spectrum antibiotics like cefazolin was preferred; broad-spectrum agents (e.g., ceftriaxone, meropenem) were considered inappropriate when not indicated
3. **Timing**: Antibiotics were considered timely if administered within **60 minutes before surgical incision**
4. **Duration**: SAP was deemed appropriate if limited to a single preoperative dose or discontinued within 24 hours postoperatively.

ETHICAL CONSIDERATIONS

The study protocol was approved by the ethical committee of Al-Salam Teaching Hospital. All data were handled with strict confidentiality.

DATA ANALYSIS

Data were entered and analyzed using Microsoft Excel. Descriptive statistics such as mean standard deviation, frequencies and percentages were used to summarize demographic data, surgical characteristics, and SAP compliance indicators. A cost analysis was conducted from the hospital perspective. Only direct acquisition costs of antibiotics were considered. Unit prices were obtained from the official pharmacy procurement list and converted to U.S. dollars at the official exchange rate. For each patient, the total cost of antibiotic prophylaxis was calculated as the number of units administered multiplied by the unit price. Mean cost per patient and total cost were calculated separately for the appropriate and inappropriate prophylaxis groups. Indirect costs, such as staff time, infusion materials, and costs related to complications, were not included.

RESULTS

In this study (Table 1), a total of 419 patients were included in the study. The mean age was 39 ± 19 years, and the average duration of hospital stay was 2.1 ± 2.5 days. The majority of patients were male

Table 1. Socio-demographic and clinical characteristics of patients (N = 419)

Characteristic	Number of cases	Percent (%)
Number of Patients	419	
Male / Female	204 / 115	73 /17
Age (Mean±SD)	39 ± 19	
Duration of hospital stay (Mean SD)	2.1 ± 2.5	
Residence (Urban/Rural)	252 / 167	60 / 40
Fever	23	5
Smoking	17	4
Penicillin Allergy	14	3
Chronic disease		
Cardiovascular disease	41	10
Diabetes Mellitus	24	6
Past surgical history	39	9
Past caesarean section	16	4

Source: compiled by the authors of this study

Table 2. Classification of surgical procedures by type: elective *vs* emergency among hospitalized patients (N = 419)

Surgery Type	Number of Cases	Percent (%)
Elective	140	33.4 %
Emergency	279	66.6 %
Total	**419**	**100.0%**

Source: compiled by the authors of this study

Table 3. Classification of surgical procedures by duration

Surgery Duration	Number of Cases	Percent (%)
< 1 hour	184	43.9 %
> 1 hour	235	56.1 %

Source: compiled by the authors of this study

Table 4. Frequency and percentage of surgical diagnoses among hospitalized patients (N = 419)

Diagnosis	Number of Cases	Percent [%]
C-section	141	33.7%
Appendicitis	51	12.2%
Cholecystitis	24	5.7%
Renal stone	23	5.5%
Abscess	10	2.4%
Acute abdomen	10	2.4%
Fracture	9	2.1%
Circulage	9	2.1%
Cystoscopy	7	1.7%
Hernia	7	1.7%
Thyroidectomy	7	1.7%
Abortion	7	1.7%
Diabetic foot	6	1.4%
Ureteroscopy	6	1.4%
Tonsillitis	6	1.4%
Hydatid cyst	5	1.2%
Road Traffic Accident (RTA)	5	1.2%
Ectopic pregnancy	5	1.2%
Others	81	19.3%
Total	**419**	**100%**

Source: compiled by the authors of this study

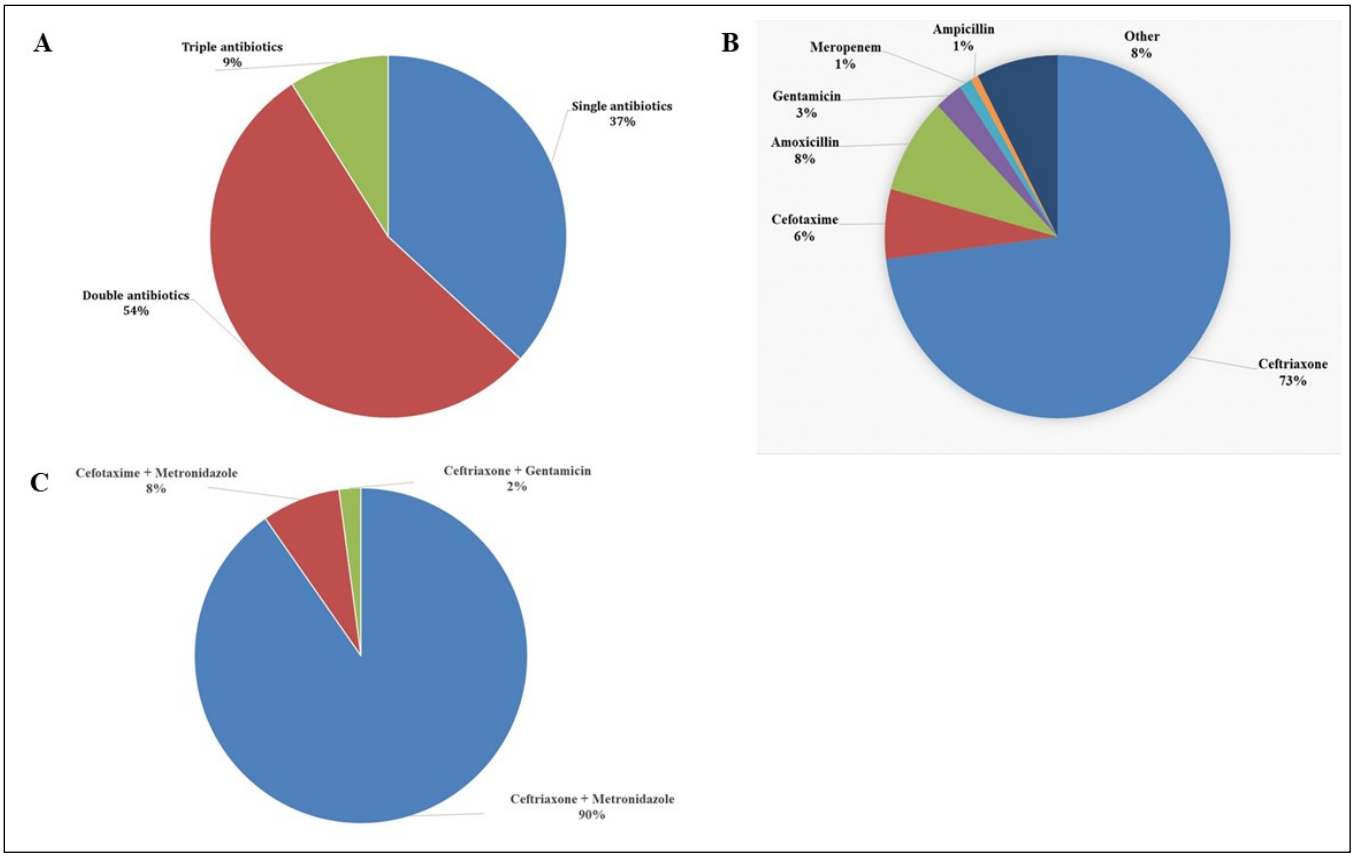

Fig. 1. (A) Distribution of Antibiotic Regimens Among Hospitalized Patients. (B) Distribution of Commonly Prescribed Antibiotics Among Hospitalized Patients. (C) Distribution of Common Dual Antibiotic Combinations Used in Hospitalized Patients
Source: compiled by the authors of this study

(204 patients, 73%) and residents of urban areas (252 patients, 60%).

Regarding the Clinical characteristics of patients, revealed that 5% had fever, 4% were smokers, and 3% reported a penicillin allergy. Regarding comorbidities, 10% had cardiovascular disease and 6% had diabetes mellitus. Additionally, 9% of patients had a history of previous surgery, while 4% had a past caesarean section.

Out of 419 surgical procedures analyzed (Table 2), 279 (66.6%) were classified as emergency surgeries, while 140 procedures (33.4%) were categorized as elective surgeries. This indicates that the majority of surgical interventions were performed under urgent or unplanned clinical circumstances. The high proportion of emergency surgeries may reflect the nature of hospital admissions, with acute conditions such as trauma, obstetric emergencies, or infections being common triggers for surgical intervention.

The distribution of surgical duration (Table 3) revealed that 235 procedures (56.1%) lasted more than one hour, whereas 184 procedures (43.9 %) were completed within one hour. These findings suggest that a substantial proportion of surgeries performed in this hospital setting involve complex or time-intensive procedures. Duration of surgery is a critical factor associated with the risk of postoperative complications, wound classification, and resource utilization in the operating room.

The most frequently recorded diagnosis (Table 4) among hospitalized surgical patients was cesarean section (C-section), accounting for 141 cases (33.7%). This was followed by appendicitis (12.2%), cholecystitis (5.7%), and renal stones (5.5%). Other less common diagnoses included abscesses, fractures, and various elective surgical conditions such as hernias, thyroidectomy, and cystoscopy. Collectively, these findings highlight the diversity of surgical conditions managed, with a predominance of emergency and obstetric-related cases.

Based on the CDC surgical wound classification system (Table 5), 259 procedures (61.8%) were categorized as Class II (Clean-Contaminated), indicating controlled entry into sterile body cavities such as the gastrointestinal, genitourinary, or respiratory tracts. Class I (Clean) wounds accounted for 50 cases (11.9%), while Class III (Contaminated) and Class IV (Dirty-Infected) comprised 78 (18.6%) and 31 (7.6%) cases, respectively. This distribution reflects the high prevalence of intra-abdominal and obstetric surgeries, which inherently involve entry

Table 5. Surgical wound classification of procedures performed in hospitalized patients according to CDC criteria (N = 419)

Wound Class	Class Description	Total Cases	Percent [%]
Class I	Clean (no entry into GI, GU, or respiratory tracts)	50	11.9%
Class II	Clean-Contaminated (controlled entry into GI, GU, respiratory, or genital tracts)	259	61.8 %
Class III	Contaminated (open wounds, spillage, or major sterile break)	78	18.6%
Class IV	Dirty-Infected (existing infection, pus, or perforation)	31	7.6%
Total	—	**419**	**100.0%**

Source: compiled by the authors of this study

Table 6. Appropriateness and mean cost of antibiotic selection for SAP

Antibiotic Use	No. of Patients	Percent [%]	Mean Cost/ patient	Total Cost USA [$]	Percent [%)]	Justification
Appropriate	117	27.9 %	2.16$	253	14.7%	Narrow-spectrum (e.g., cefazolin-equivalent, gentamicin, appropriate metronidazole use)
Inappropriate	302	72.1 %	4.85$	1,464.9	85.3%	Broad-spectrum agents (e.g., ceftriaxone, cefotaxime, meropenem) used without indication
Total	**419**	**100%**		**1,717.9**	**100%**	—

Source: compiled by the authors of this study

into body tracts, thereby increasing the risk of contamination and postoperative infection.

Antibiotic prescribing patterns among hospitalized patients showed a predominant use of combination therapy. As illustrated in Figure 1 A, dual antibiotic therapy was the most frequently prescribed regimen, accounting for 67% of patients, followed by single antibiotic use in 31%, while triple therapy was used in only 2%. This reflects a strong reliance on empirical broad-spectrum coverage.

The patient included in the current study was given SAP with one, two or even triple antibiotics for the prevention of SSI. In terms of individual antibiotic use (Figure 1 B), Ceftriaxone was the most frequently prescribed antibiotic, administered in 73.0% of patients, followed by Amoxicillin at 8.6%, and Cefotaxime at 6.2%. Gentamicin and Meropenem were prescribed in 2.6% and 1.2% of cases, respectively. Ampicillin was used in 0.7%, while other antibiotics collectively accounted for 7.6% of prescriptions.

Among the dual antibiotic combinations (Fig. 1 C), the most commonly used regimen was Ceftriaxone plus Metronidazole, representing 90% of dual therapy cases. This was followed by Cefotaxime plus Metronidazole (8%), and Ceftriaxone plus Gentamicin (2%). These findings highlight a practice of using broad-spectrum agents targeting both Gram-negative organisms and anaerobes, particularly in abdominal and surgical infections.

Although SAP was indicated in most patients (Table 6), the antibiotic selection was appropriate in only 27.9% of cases. The widespread use of ceftriaxone (73%) and cefotaxime (6.2%), both broad-spectrum antibiotics not recommended for standard prophylaxis, significantly contributed to inappropriate use. In addition to poor antibiotic choice, other critical deviations were observed. The timing of administration was inappropriate in the majority of cases, with antibiotics often given after surgical incision, reducing their preventive effectiveness. Moreover, the duration of prophylaxis frequently exceeded 7 days, far beyond the recommended single-dose or ≤ 24-hour regimen advised by international guidelines such as WHO and ASHP. These combined issues - broad-spectrum overuse, delayed administration, and prolonged duration - reflect major gaps in antimicrobial stewardship and highlight the urgent need for protocol adherence to reduce antimicrobial resistance and surgical site infections.

The cost analysis revealed substantial differences in expenditure between the two groups (Table 6). The mean antibiotic cost per patient in the appropriate prophylaxis group was 2.16 USD, whereas patients in the inappropriate group incurred a mean cost of 4.85 USD. The total expenditure on surgical antimicrobial prophylaxis amounted to 1,717.9 USD, of which 1,464.9 USD (85.3%) was attributable to inappropriate use. Thus, although inappropriate prophylaxis was administered to 72.1% of patients, it consumed more than four-fifths of the total antibiotic budget.

DISCUSSION

This retrospective evaluation identified significant deviations from recommended SAP practices. While SAP was indicated for most patients, appropriate antibiotic selection occurred in only 27.9% of cases. The frequent use of broad-spectrum antibiotics such as ceftriaxone (73%) and cefotaxime (6.2%) illustrates a common yet

inappropriate practice due to their unnecessary broad coverage and potential for resistance development. The inappropriate use of antibiotics observed in this study is consistent with findings from other studies conducted in Asian and neighboring countries, where inappropriateness rates range from 40% to 100%, and is notably higher compared to rates reported in other developing countries, which range from 20% to 50% [17-20]. According to guidelines from ASHP and WHO, cefazolin or equivalent narrow-spectrum antibiotics are recommended as first-line choices for most surgical procedures, reflecting a clear gap in local practice adherence [21-23].

In addition to antibiotic selection issues, timing was identified as significantly suboptimal, with most antibiotics administered after surgical incision, substantially diminishing their prophylactic efficacy. Evidence strongly supports administering antibiotics within 60 minutes prior to incision to achieve optimal tissue concentrations during surgery [24]. Furthermore, antibiotic prophylaxis duration commonly exceeded seven days in this study, contrary to recommended guidelines, which emphasize prophylaxis limited to a single dose or less than 24 hours postoperatively Prolonged prophylaxis increases the risk of adverse events, antibiotic resistance, and healthcare costs without offering additional protection against SSIs [25].

These findings are consistent with other international reports highlighting poor adherence to SAP guidelines in resource-limited settings [17, 18, 20, 25]. Contributing factors may include inadequate training, lack of standardized hospital protocols, and limited awareness of antimicrobial stewardship principles among healthcare providers. Therefore, targeted educational interventions, implementation of hospital-specific guidelines, and ongoing audit-feedback mechanisms are strongly recommended to enhance compliance and improve clinical outcomes [26]. One limitation of this study is that it was conducted in a single tertiary hospital, which may limit the generalizability of the findings to other healthcare settings. Future prospective studies assessing the impact of stewardship interventions are warranted. Additionally, strategies to strengthen institutional oversight and continuous professional training on evidence-based SAP practices could significantly mitigate these issues, optimizing patient outcomes and preserving antibiotic efficacy.

Beyond clinical implications, our findings demonstrate the considerable financial impact of inappropriate prophylaxis. The mean cost per patient receiving inappropriate prophylaxis was more than double that of patients who received guideline-concordant regimens. Inappropriate use accounted for 85% of total antibiotic expenditure despite being administered to fewer than three-quarters of patients. Extrapolating the observed excess cost to an annual scale suggests that a large and preventable financial burden is imposed on the institution.

These results underscore the importance of implementing regular audits and reinforcing adherence to evidence-based guidelines. Restricting the use of broad-spectrum and costly agents, such as ceftriaxone and meropenem, to appropriate indications would not only improve clinical outcomes but also result in substantial cost savings.

CONCLUSIONS

This study revealed a high rate of inappropriate SAP practices at Al-Salam Teaching Hospital, particularly concerning the misuse of broad-spectrum antibiotics. In addition, most patients did not receive prophylactic antibiotics within the recommended preoperative window, and the duration of antibiotic administration frequently exceeded the guideline-recommended limit of 24 hours. These deviations from standard SAP protocols contribute heightened antimicrobial resistance and unnecessary healthcare expenditures.

The findings underscore the urgent need to develop and implement standardized, evidence-based local guidelines for SAP. Moreover, the establishment of an effective Antimicrobial Stewardship Program (ASP) is essential to monitor and improve antibiotic prescribing practices. Such measures will help optimize antibiotic use, reduce SSIs, and mitigate the growing threat of antimicrobial resistance. Future studies with larger, multicenter data are recommended to better assess SAP practices across various surgical disciplines and settings in Iraq. In addition to its clinical risks, inappropriate surgical antimicrobial prophylaxis imposes a significant and avoidable economic burden. Adherence to guideline-recommended regimens would reduce both patient harm and unnecessary institutional costs.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Characteristics of the development of motor and volitional qualities of future law enforcement officers in the process of their specialized training

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ABSTRACT

Aim: To investigate the impact of specialized training (hand-to-hand combat training) on the development of motor and volitional qualities indicators in cadets during their studies at higher educational institutions with a specific learning environment.

Materials and Methods: The research, conducted in the 2024-2025 year, involved 298 male cadets, who were divided into three groups: HHC Group (n = 47), whose cadets were involved in hand-to-hand combat; SC Group (n = 82), whose cadets trained in other sports clubs (games, strength, athletics); C Group (n = 169), whose cadets did not engage in additional sports activities. Research methods: analysis and generalization of literary sources, testing, psychodiagnostic methods, and statistical methods.

Results: It has been found that, according to most of the tests and methods used, the cadets of the HHC Group showed significantly better results in terms of motor and volitional qualities in their senior training years than those in the C Group. At the same time, no significant difference was found between the indicators of cadets in the HHC and SC groups, which indicates the effectiveness of additional sports activities in developing cadets' motor and volitional qualities.

Conclusions: The comprehensive impact of hand-to-hand combat training on indicators of motor and volitional qualities in future law enforcement officers has been demonstrated. This emphasizes the advisability of a wider introduction of hand-to-hand combat techniques into the specialized training of future law enforcement officers during their studies, to improve their professional training and increase the effectiveness of their future service activities.

KEY WORDS: motor qualities, volitional qualities, specialized training, hand-to-hand combat, cadet

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INTRODUCTION

The profession of a law enforcement officer is among the most responsible and dangerous in the world. In carrying out their service duties, law enforcement officers perform several essential tasks, which determine the high level of requirements for their professional training [1, 2]. An analysis of the specifics of law enforcement activities, especially in conditions of martial law, gives reason to conclude that modern law enforcement officers must possess not only knowledge of the legislative framework, but also developed motor and volitional qualities, as well as established skills and abilities to apply physical force and coercion [3, 4]. Therefore, improving the professional readiness of future law enforcement officers is a key area of activity for higher educational institutions with a specific learning environment (HEIs with SLE), which require specialists to search for effective means to ensure the maximum efficiency of the educational process [5].

Experts argue that the basis for the professional training of future law enforcement officers in the current conditions of training in HEIs with SLE should be specialized physical training (SPT), which is as close as possible to the real conditions of service activities and which is capable of ensuring a high level of physical and psychological readiness of future law enforcement officers for effective actions in extreme conditions [6, 7]. According to many scientists [8, 9], one of the most effective SPT methods for future law enforcement officers is hand-to-hand combat (HHC). HHC has a large number of tools for developing motor and volitional qualities that determine the physical and psychological readiness of law enforcement officers to act effectively in extreme situations during their service activities [10].

Scientists [11] argue that during HHC training, law enforcement officers effectively develop motor qualities such as speed, coordination, agility, explosive power, speed and strength, and endurance. Among the volitional qualities that are developed during HHC training are: determination, courage, initiative, perseverance, durability, self-control, and confidence in one's own abilities [12].

AIM

The aim is to investigate the impact of specialized training (hand-to-hand combat training) on the development of motor and volitional qualities indicators in cadets during their studies at higher educational institutions with a specific learning environment.

MATERIALS AND METHODS

PARTICIPANTS

The research, conducted in the 2024-2025 academic year, involved 298 male cadets from Lviv State University of Internal Affairs (LSUIA, Lviv, Ukraine). To achieve the research aim, based on the results of the survey, we formed three groups of cadets: HHC Group (n = 47) included cadets who, while studying at the HEI with SLE, in addition to attending compulsory training sessions in SPT, also systematically participated in a hand-to-hand combat sports club during their sporting and mass participation activities (SMPAs) (1st training year – 12 people, 2nd training year – 13 people, 3rd training year – 11 people, 4th training year – 11 people); SC Group (n = 82) included cadets who also additionally participated in sports in various sports clubs of the HEI with SLE during their SMPAs hours, including sports games, strength sports, and athletics (1st training year – 21 people, 2nd training year – 24 people, 3rd training year – 20 people, 4th training year – 17 people); C Group (n = 169) included cadets who, during their studies, attended only compulsory training sessions in SPT and did not additionally engage in sports within their SMPAs hours being conducted according to the standard program (1st training year – 43 people, 2nd training year – 38 people, 3rd training year – 42 people, 4th training year – 46 people). The amount of physical activity per week (in hours) did not differ between the study groups. The primary variable was the content of the training sessions across the groups. Inclusion criteria: male cadets, willingness to engage in a particular sport during training (determined by a survey at the beginning of the academic year), no health contraindications to sports; exclusion criterion – the cadet's desire to withdraw from the research at any convenient time.

RESEARCH METHODS

Analysis and generalization of literary sources, testing of motor qualities, psychodiagnostic methods, and statistical methods. Analysis and generalization of literary sources were used to conduct an analytical review of scientific sources on the outlined range of issues (24 sources from PubMed, Scopus, Web of Science, and Index Copernicus were analyzed).

Testing was used to assess the development of cadets' motor skills during their HHC training sessions. The following tests were used: 100 m run (cadets' speed qualities were assessed); exercises on the horizontal bar (1st training year – pull-ups, 2nd training year – hip-swing-ups, 3rd and 4th training years – breast-ups) (strength qualities were assessed); 3 km run (overall endurance was assessed); 6x100 m shuttle run (agility and applied motor skills in accelerated movement were evaluated). Psychodiagnostic methods were used to determine the development of cadets' volitional qualities during their HHC training sessions. Two methodologies were used: "Are you decisive?" (according to E. Berne) and "Study of the Locus of Subjective Control" (according to J. Rotter) [13, 14].

STATISTICAL METHODS

Statistical methods were used to process the data obtained. The compliance of the sample data distribution with the Gauss' law was assessed using the Shapiro-Wilk W test. The reliability of the difference between the indicators was determined using the Student's t-test. The reliability of the difference was set at $p<0.05$. All statistical analyses were performed using SPSS software, version 10.0, adapted for medical and biological research.

ETHICS

The procedure for organizing the study and the topic of the article were previously agreed with the Committee on compliance with Academic Integrity and Ethics of the LSUIA. Also this study followed the regulations of the World Medical Association Declaration of Helsinki. Informed consent was received from all cadets who took part in this study.

FRAMEWORK

This scientific article was carried out according to the plan of the research work of the Lviv State University of Internal Affairs for 2020-2026 "Psychological, pedagogical and sociological support of law enforcement officers» (state registration number 0113U008196).

Table 1. Level of development of cadets' motor qualities in the HHC Group (n = 47), the SC Group (n = 82), and the C Group (n = 169), M ± m

Training year	Number of people	HHC Group	Number of people	SC Group	Number of people	C Group	Level of significance, p		
							HHC-SC	HHC-C	SC-C
100 m run, s									
1st	12	14.2±0.17	21	14.1±0.15	43	14.4±0.13	>0.05	>0.05	>0.05
2nd	13	14.0±0.15	24	13.9±0.13	38	14.2±0.12	>0.05	>0.05	>0.05
3rd	11	13.8±0.16	20	13.7±0.13	42	13.9±0.11	>0.05	>0.05	>0.05
4th	11	13.5±0.18	17	13.3±0.14	46	13.7±0.11	>0.05	>0.05	<0.05
Strength exercises on the horizontal bar, times									
1st	12	14.2±0.58	21	14.5±0.71	43	13.9±0.38	>0.05	>0.05	>0.05
2nd	13	10.9±0.85	24	11.3±0.92	38	8.8±0.54	>0.05	<0.05	<0.05
3rd	11	8.9±0.79	20	9.1±0.63	42	7.5±0.49	>0.05	>0.05	<0.05
4th	11	10.2±0.82	17	10.3±0.68	46	8.2±0.43	>0.05	<0.05	<0.05
3 km run, s									
1st	12	767.8±9.06	21	761.9±7.85	43	774.1±5.97	>0.05	>0.05	>0.05
2nd	13	742.9±8.81	24	737.2±7.42	38	756.5±6.13	>0.05	>0.05	<0.05
3rd	11	725.2±9.23	20	718.6±7.61	42	743.2±6.04	>0.05	>0.05	<0.05
4th	11	707.4±9.85	17	696.7±7.79	46	733.8±5.87	>0.05	<0.05	<0.01
6x100 m shuttle run, s									
1st	12	135.4±3.17	21	136.7±2.59	43	132.9±1.88	>0.05	>0.05	>0.05
2nd	13	146.7±3.02	24	148.3±2.67	38	141.2±1.92	>0.05	>0.05	<0.05
3rd	11	158.1±2.87	20	162.1±2.32	42	153.5±1.79	>0.05	>0.05	<0.01
4th	11	171.3±3.15	17	177.2±2.18	46	162.1±1.66	>0.05	<0.05	<0.001

Notes: M – arithmetic mean; m – error of the arithmetic mean; p – reliability of the difference between the indicators of the cadets of the studied groups, which was determined using Student's t-test

Source: compiled by the authors of this study

RESULTS

The analysis of the level of development of cadets' speed qualities based on the results of the 100 m run showed that in the 1st training year, cadets' results across all three study groups were not significantly different ($p > 0.05$). In the 2nd and 3rd training years, the best results were observed in cadets of the SC Group (13.9 and 13.7 s, respectively); however, they did not differ significantly from those of cadets of the HHC and C groups ($p > 0.05$). In the 4th training year, the results of the 100 m run in cadets of the SC Group were significantly better than those of cadets of the C Group by 0.4 s ($t = 2.25$; $p < 0.05$). However, no significant difference was found between the results of the HHC and SC groups, as well as the HHC and C groups ($p > 0.05$) (Table 1).

A significant improvement in the results of the 100 m run was also observed among cadets from all three groups during their training at the HEI with SLE ($p < 0.01$; $p < 0.001$). At the same time, the level of motor skill development in this test among cadets from all three groups studied is rated as excellent. The analysis showed that both the traditional SPT program at the HEI with SLE and, in particular, additional training sessions in various sports have a positive effect on the development of speed qualities in cadets – future law enforcement officers.

The analysis of cadets' results in strength exercises on the horizontal bar showed that in the 1st training year, there was no significant difference in indicators across all three groups ($p > 0.05$). However, in the HHC and SC groups, the indicators are better than in the C Group by 0.3 and 0.6 times. In the hip-swing-up exercise in the 2nd training year, the results ratio between the groups studied did not change: the best results were found in the SC Group (11.3 times), the worst in the C Group (8.8 times). At the same time, the results of cadets in the HHC Group and the SC Group are significantly ($t = 2.06$; $t = 2.34$; $p < 0.05$) better than those of cadets

Table 2. Level of development of cadets' volitional qualities in the HHC Group (n = 47), the SC Group (n = 82), and the C Group (n = 169) by the "Are You Decisive?" methodology, M ± m

Training year	Number of people	Possible amount of points	Answer options					
			A	B	C	D	F	J
HHC Group								
1st	12	120	9.3	35.0	7.9	12.9	17.8	17.1
2nd	13	130	8.7	36.3	5.6	10.0	15.0	24.4
3rd	11	110	12.3	35.4	6.2	10.7	16.2	19.2
4th	11	110	9.6	35.8	3.6	4.5	18.2	28.3
Total	47	470	10.4	35.1	5.9	9.8	16.7	22.1
SC Group								
1st	21	210	8.8	32.1	9.6	14.6	17.9	17.0
2nd	24	240	9.6	32.7	7.3	11.2	18.1	21.1
3rd	20	200	9.1	36.1	6.5	6.9	16.5	24.9
4th	17	170	9.3	40.1	5.6	6.1	11.7	27.2
Total	82	820	9.9	34.4	7.4	10.0	16.4	22.0
C Group								
1st	43	430	15.7	16.4	15.8	16.4	20.2	15.5
2nd	38	380	15.8	17.6	14.0	15.3	22.7	14.7
3rd	42	420	17.9	17.6	13.0	13.9	21.3	16.3
4th	46	460	18.5	18.0	12.3	12.9	21.6	16.7
Total	169	1690	17.1	17.4	13.7	14.6	21.4	15.8

Note: A, B, C, D, F, J - the answer options offered in the methodology questionnaire
Source: compiled by the authors of this study

in the C Group, by 1.1 and 1.5 times, respectively; the difference between the HHC Group and the SC Group is insignificant ($p > 0.05$). In the 3rd and 4th training years, the results of the breast-ups in cadets of the HHC Group and the SC Group were also significantly ($t = 2.07$-3.61; $p < 0.05$–$p < 0.01$) better than those of the C Group, which indicates a positive effect of additional systematic sports activities (HHC or other) on the development of motor skills of future law enforcement officers. A comparison of the average results of cadets in pull-ups on the horizontal bar in the senior training years confirmed the previous conclusions: the indicators for cadets in the HHC and the SC groups are significantly the same ($p > 0.05$), while cadets in the C Group had significantly worse strength indicators than those in the HHC and the SC groups by 1.7 ($t = 2.51$; $p < 0.05$) and 1.8 ($t = 4.33$; $p < 0.001$) times. However, the assessment of cadets' strength qualities showed that cadets from all three groups received excellent results in strength exercises in all training years.

The study of endurance development indicators in cadets shows that in the 1st training year, the results of the 3 km run did not differ significantly across all studied groups ($p > 0.05$). However, in the HHC and the SC groups, endurance development was rated as good, whereas in the C Group it was rated as satisfactory. In the 2nd training year, the 3 km run results of cadets in the SC Group (12 min 17.2 s) were significantly ($t = 2.01$; $p < 0.05$) than those of cadets in the C Group (12 min 36.5 s), by 19.3 s, and were significantly the same as the results of cadets in the HHC Group (12 min 22.9 s) ($p > 0.05$). In the 2nd training year, the endurance level of cadets in the HHC Group and the C Group was rated as good, and that of the SC Group as excellent. In the 3rd training year, both the SC Group (11 min 58.6 s) and the HHC Group (12 min 05.2 s) showed better results than the C Group (12 min 23.2 s), by 24.6 s and 18 s, respectively. In the 4th training year, a significant difference was already found between the HHC Group and the C Group (26.4 s; $t = 2.30$; $p < 0.05$), and between the SC Group and the C Group (37.1 s; $t = 3.80$; $p < 0.01$), while no significant difference was found between the HHC Group and the SC Group ($p > 0.05$). In the senior training years, the level of endurance development of cadets in the HHC Group and the SC Group is rated as excellent, and in the C Group as good, which allows us to conclude that additional HHC training sessions and other sports activities have a greater impact on the development of endurance in future law enforcement officers than SPT sessions. Studying the indicators of cadets in the 4th and 1st training years of all the groups studied, we found that the results improved significantly in all groups: in

Table 3. Level of development of cadets' volitional qualities in the HHC Group (n = 47), the SC Group (n = 82), and the C Group (n = 169) by the "Study of the Locus of Subjective Control" methodology, M ± m

Training year	Number of people	HHC Group	Number of people	SC Group	Number of people	C Group	Level of significance, p		
							HHC-SC	HHC-C	SC-C
1st	12	28.8±2.46	21	27.5±1.67	43	27.8±1.38	>0.05	>0.05	>0.05
2nd	13	31.4±1.35	24	30.8±1.42	38	30.2±1.09	>0.05	>0.05	>0.05
3rd	11	34.6±1.22	20	33.7±1.16	42	32.9±0.95	>0.05	>0.05	>0.05
4th	11	38.5±1.15	17	37.9±1.02	46	35.6±0.78	>0.05	<0.05	>0.05

Notes: M – arithmetic mean; m – error of the arithmetic mean; p – reliability of the difference between the indicators of the cadets of the studied groups, which was determined using Student's t-test

Source: compiled by the authors of this study

the HHC Group – by 1 min 04.0 s ($t = 4.51$; $p < 0.001$); in the SC Group – by 1 min 5.2 s ($t = 5.30$; $p < 0.001$); in the C Group – by 40.3 s ($t = 4.81$; $p < 0.001$).

The evaluation of cadets' results in the 6x100 m shuttle run shows that, as in the analysis of other motor skills, no significant difference was found between the results of all three groups in the 1st training year ($p > 0.05$). In the 2nd training year, an important difference was found between the results of cadets in the SC Group (2 min 05.1 s) and the C Group (2 min 09.1 s) – 4 s ($t = 2.22$; $p < 0.05$). In the 3rd training year, the ratio of the results of the 6x100 m shuttle run between the studied groups did not change – the best results were found in cadets of the SC Group (2 min 00.9 s); they are significantly better than those of cadets in the C Group (2 min 06.3 s) by 5.4 s ($t = 2.22$; $p < 0.05$) and significantly the same as the results of the HHC Group (2 min 02.3 s). In the 4th training year, the results of cadets in the C Group (2 min 03.9 s) were significantly worse than those of the SC Group and the HHC Group by 7.5 s ($t = 4.48$; $p < 0.001$) and 4.6 s ($t = 2.11$; $p < 0.05$), respectively. The difference between the results of cadets in the SC Group and the HHC Group in the 4th training year is insignificant ($p > 0.05$), which indicates the effectiveness of additional sports activities (HHC and other types) on the formation and improvement of applied motor skills of future law enforcement officers than in the C Group, by 4.9 s ($t = 4.27$; $p < 0.001$) and 3.3 s ($t = 2.31$; $p < 0.05$), respectively. It should be noted that the results of cadets in the 6x100 m shuttle run in all three study groups in all training years are rated as excellent. The analysis of the dynamics of the results of the 6x100 m shuttle run shows that in all groups they significantly improve during the training process at the HEI with SLE ($p < 0.001$), however, the difference between the results of cadets in the 1st and 4th training years in the HHC Group is 11 s ($t = 4.34$), in the SC Group – 12.3 s ($t = 6.44$), and in the C Group – 7.5 s ($t = 4.86$).

The analysis showed that cadets who additionally participated in the HHC sports club during their training at the HEI with SLE had a significantly ($p < 0.05$-0.001) better level of development of all qualities than cadets who used the traditional SPT program and did not participate in additional sports. Compared with cadets who also practiced other sports, all indicators studied for cadets in the HHC Group did not show a significant difference from those in the SC Group ($p > 0.05$). This indicates that additional sports activities during training at the HEI with SLE are more effective than the traditional SPT methodology in promoting the development of cadets' motor skills.

The impact of HHC training sessions on the volitional qualities of cadets was assessed according to such qualities as determination and locus of subjective control. Determination, as a volitional quality of personality, enables a person to carry out a decision without unnecessary hesitation. Law enforcement officers who lack this quality often cannot bring a case to a successful conclusion and doubt the correctness of their chosen execution method. An essential condition for determination is courage, i. e., the willingness to take justified risks. Of course, determination must be based on deep prudence and only come into play when a person finally decides what they need. There is a whole chain here: consideration of goals, struggle of motives, doubts, etc. The presence of doubts during this period does not indicate indecisiveness, but rather the opposite, because an intense battle of motives is characteristic of a thinking person. But decisive people quickly struggle with their motives; they know how to act quickly, calmly, and professionally, despite possible danger, while indecisive people take a long time to

make a decision. The level of decisiveness among cadets was studied using the "Are You Decisive?" methodology (by Eric Berne). Cadets had to choose one answer from the options provided (A, B, C, D, F, J) for each of the 10 questions. If the majority of the cadet's answers (more than 50 %) corresponded to letters B and J, his level of decisiveness was assessed as high; if the majority of the cadet's answers (more than 50 %) corresponded to letters A and F and B and J, his level of decisiveness was assessed as average; if the majority of the cadet's answers (more than 50 %) corresponded to the letters C and D, his level of determination was assessed as low. The study of cadets' determination shows that during their training, the level of determination improves: the number of people in each group who chose answers B and J increased (in the HHC Group from 35.0 and 17.1 % to 35.8 and 28.3 %, respectively; in the SC Group from 32.1 and 17.0 % to 40.1 and 27.2 %, respectively); those who chose options A and F also increased slightly (in the HHC Group from 9.3 and 17.8 % to 9.6 and 18.2 %, respectively; in the SC Group from 8.8 to 9.3, respectively); and those who chose options C and D decreased (in the HHC Group from 7.9 and 12.9 % to 3.6 and 4.5 %, respectively; in the SC Group from 9.6 and 14.6 % to 5.6 and 6.1 %, respectively) (Table 2). In the HHC and the SC groups, the distribution of answers across all training years shows a similar trend: the majority (over 50 %) of cadets chose options B and J, and less than 10 % chose options D and C. In the C Group, there is a practically even distribution of answer options in all training years: options B and J were chosen by 31.9-34.7 % of cadets; options A and F – by 35.9-40.1 % of cadets; options C and D – by 32.2-25.2 % of cadets.

The analysis of the overall ratio of cadets' responses confirmed the above trend—a high level of determination in the HHC and the SC groups, and an average level in the C Group. The analysis showed that sports, and HHC in particular, have a positive effect on the development of a volitional quality among future law enforcement officers, namely determination. It is essential to add that the most significant number of cadets who chose answer options characterizing a high level of determination was found in the HHC Group, further confirming the effectiveness of HHC training sessions in developing the volitional qualities of future law enforcement officers.

Using J. Rotter's methodology, we determined the locus of subjective control of cadets, which reflects their level of development of volitional qualities. The locus of control is a characteristic of a person's volitional sphere that demonstrates their tendency to attribute responsibility for the outcomes of their activities to external forces or to their own abilities and efforts. Attributing responsibility for the results of one's actions to external forces is called an external locus of control, while attributing responsibility to one's own abilities and efforts is called an internal locus of control. Every person has a specific position on the continuum from external to internal. With a low level of internal locus of control, people see little connection between their actions and the events in their lives that are important to them. They do not consider themselves capable of controlling the development of such events and believe that most of them are the result of chance or the actions of other people. Therefore, "externalists" are emotionally unstable, prone to informal communication and behavior, uncommunicative, have poor self-control, and are highly strung. A high level of internality corresponds to a high level of subjective control over any significant events. People with this locus of control believe that most important events in their lives are the result of their own actions, that they can control these events, and that they feel responsible for these events and for how their lives unfold in general. "Internals" with high levels of subjective control are emotionally stable, stubborn, decisive, sociable, and characterized by significant self-control, restraint, and tolerance. The level of subjective control among cadets was assessed using the internality indicator, which is considered low at 11 points or fewer and high at 33-44 points. Thus, the analysis of the obtained indicators shows that in the 1st, 2nd, and 3rd training years, the level of subjective control among cadets in all three groups is reliably the same ($p > 0.05$) (Table 3).

In the 4th training year, the level of subjective control in the HHC Group is the best among the groups (38.5 points), significantly better than in the C Group (35.6 points), by 2.9 points ($t = 2.02$; $p < 0.05$), and significantly the same as in the SC Group (37.9 points) ($p > 0.05$). The analysis of the mean values shows that, although the indicators in the HHC and the SC groups are better than those in the C Group, no significant difference was found between them ($p > 0.05$). The study of changes in cadets' indicators of subjective control shows that across all three groups, there was a significant improvement, with the greatest changes observed in the HHC and the SC groups. Thus, the difference between the indicators of the 1st and 4th training years in the HHC Group is 9.7 points ($t = 3.57$; $p < 0.001$), in the SC Group – 10.4 points ($t = 5.23$; $p < 0.001$), and in the C Group – 7.8 points ($t = 4.92$; $p < 0.001$). Assessment of cadets' subjective control in the study groups shows that in the 1st and 2nd training years, the indicators of all three groups were at the average level, and in the 3rd and 4th training years, at the high level. It was also found that, during senior

training, more than 70 % of cadets in each group had an internal locus of subjective control.

The results show that the vast majority of cadets in all groups, especially in the senior training years, have a conscious motivation to achieve success, both in their service activities and in their personal lives, demonstrate independence in decision-making, responsibility for the results of their activities, and resilience in overcoming difficulties, linking their successes in academic training to achievements in their future professional careers. All these traits and qualities of cadets are most effectively revealed during sports activities, including HHC, which confirms the need for their widespread introduction into the SPT process for future law enforcement officers.

DISCUSSION

Scientists [3, 15, 16] note that a modern law enforcement officer must be able to ensure their own safety and that of those around them, using sufficient force and methods if necessary; distinguish between dangerous situations; and act without prejudice in all circumstances, guided by ethical standards. The analysis of law enforcement practices shows that the functional duties of a law enforcement officer can only be performed by a person who has a high level of responsibility for the results of their own professional activities, possesses a complex of knowledge, practical skills, and professionally important motor and volitional qualities [10, 17]. In view of this, it should be noted that these components ensure that law enforcement officers are professionally ready to perform complex, sometimes atypical, and dangerous tasks effectively.

As noted by scientists [6, 7], the basis of professional training for future law enforcement officers during their studies at HEIs with SLE is SPT, one of the most effective types of which is hand-to-hand combat. Experts [8, 9] believe that hand-to-hand combat, as a type of martial arts, belongs to physical exercises of variable intensity, of an acyclic nature, with continuous responses to the opponent's actions. HHC is associated with significant motor activity and, depending on its intensity and the opponent's actions, can involve moderate, high, or submaximal physical exertion at certain moments. Scientists [12] note that in HHC, work is performed in a mixed aerobic-anaerobic zone, and oxygen demand and oxygen debt are determined by the difficulty of the work and by the high nervous and mental stress associated with the risk to life and the possibility of injury. The authors, studying the results of the Rufier test, the Kerd index, the level of physical working capacity, and maximum oxygen consumption, found that cadets who systematically engage in HHC have a high level of functional capacity of the cardiovascular and respiratory systems [18]. This is confirmed by the results we obtained.

Repeated performance of various actions with weapons and means of physical influence and without weapons (hand and foot strikes, pain techniques, throws, chokeholds, etc.) during HHC training sessions primarily contributes to the development of motor skills, where the leading role belongs to psychomotorics as a means of motor response to external stimuli. At the same time, a complex spatial-temporal conditioned reflex plays a special role – law enforcement officers develop a reaction to a moving object [11]. The need to constantly keep your distance during HHC, to compare the moment of applying force with the opponent's movement, requires a high level of analysis (visual, motor, and vestibular), as well as the ability to assess the opponent's distance, speed of movement, and location. The use of painful techniques also has a beneficial effect on the pain analyzer, increasing pain sensitivity thresholds [6]. Instantaneous switching of attention, its stability, distribution, and concentration, rapid processing of information, independence and correctness of decision-making, the ability to develop action plans when making decisions and make adjustments to them taking into account the opponent's countermeasures, the ability to maintain performance and an optimal emotional state in extreme situations, the ability to interact with partners (brothers-in-arms, comrades) – these are the components of successful law enforcement actions as a result of HHC training [19]. Our research also showed that cadets who additionally participated in HHC training sessions during their studies at the HEI with SLE demonstrated a higher level of development in all the above-mentioned qualities than those who did not participate in HHC training.

As experts point out [11], systematic HHC training effectively affects the central nervous system: constant stimulation of multiple nerve centers during hand-to-hand combat promotes the development of skills for performing movements that require considerable speed, strength, and endurance.

According to scientists [12], in leading countries worldwide, HHC is considered a specialized training tool and an indispensable means of psychological hardening for law enforcement officers, instilling self-confidence, courage, determination, and resilience in the face of stressful conditions. Studying the impact of HHC training on cadets' psychological readiness for future professional combat activities, scientists [5] found that mastering HHC techniques is inevitably associated with a specific fear: fear of pain from missed enemy blows, unsuccessful falls after throws, etc. Overcoming this fear requires the manifestation of qualities such as

courage and determination. Improving combat techniques and actions involves their repeated duplication, which also requires cadets to demonstrate discipline and independence. Constantly changing situations in which HHC techniques are perfected foster ingenuity and quick-wittedness in cadets [10]. Training fights – conditioned, semi-conditioned, and especially unconditioned (free) – are particularly valuable for improving the psychological preparedness of cadets, since their conduct requires the manifestation, and therefore the cultivation, of the entire spectrum of volitional qualities that make up their psychological preparedness [2, 5].

According to scientists [13, 20, 21], the volitional qualities cultivated in combat training sessions as a result of the phenomenon of transfer will also be manifested in the course of performing service duties. The specificity of HHC lies in its ability to activate internal motivation, which, in turn, leads to self-improvement and self-education. We also noticed this during our research. After all, the vast majority of cadets across all groups have a conscious motivation to achieve success in both their service activities and their personal lives, and demonstrate independence in decision-making. They gain experience in overcoming psychological difficulties and improve their assessment of absolute risk, which is quite important and valuable in dangerous situations in their professional activities.

Scientists [22] identify the following main characteristics of HHC: direct contact with the enemy; the aggressiveness of the enemy and their active opposition; a lack of time and information, and the need to make quick and responsible decisions on which the safety and lives of both the soldier and their brothers-in-arms depend; experiencing negative emotional states, primarily fear, etc. According to scientists [23], well-trained personnel in HHC techniques are characterized by self-control, a sense of physical and psychological superiority over the enemy, and a constant, functional, proactive attitude. Our results confirm the conclusions of many scientists [8, 9, 11, 17, 22, 24] and complement them. It has been established that HHC training sessions have a positive effect on the development of cadets' working capacity and endurance, as well as their motor and volitional qualities (in particular, determination, courage, responsibility, etc.), which will generally contribute to improving the effectiveness of performing specific tasks in their future service activities.

CONCLUSIONS

It has been found that hand-to-hand combat is an effective means of specialized training for cadets as future law enforcement officers in interaction with offenders, as well as an effective means of developing and improving their physical and psychological preparedness, forming various motor skills, fostering courage, determination, confidence in their own abilities, etc.

It has been established that during hand-to-hand combat training, cadets effectively develop both motor skills (speed, coordination, agility, strength, endurance) and volitional qualities (determination, courage, responsibility, etc.). In the HHC Group, most indicators studied in the senior training years are significantly better than in the C Group, indicating a more pronounced effect of HHC training sessions on the development of motor and volitional qualities of future law enforcement officers compared with the traditional SPT methodology. At the same time, no significant difference was found between the indicators of cadets in the HHC Group and the SC Group, which allows us to conclude that additional training in any sport is effective in developing the motor and volitional qualities of cadets. However, no other sports activities have such a comprehensive impact on the development of motor skills and applied motor skills, as well as on the cultivation of volitional qualities, as HHC training sessions. This emphasizes the advisability of wider implementation of HHC into the physical training programs of cadets during their studies at HEIs with SLE to improve the effectiveness of their future service activities.

PROSPECTS FOR FURTHER RESEARCH

It is planned to investigate the impact of strength sports on the development of motor and volitional qualities of cadets during their studies at HEIs with SLE.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Effect of boiled and macerated cinnamon sticks and powder on diabetic and biochemical profile in patients with type 2 diabetes mellitus

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ABSTRACT

Aim: We examined possible impact of cinnamon on blood glucose and some biochemical parameters among type 2 diabetic patients treated with cinnamon in Najaf.

Materials and Methods: Patients with type 2 diabetes were received cinnamon (boiled branches, soaked branches, boiled powder and soaked powder) twice a day after meals. Fasting and random blood glucose in addition to urine biochemical markers; glucose, ketones, specific gravity, total protein, were measured.

Results: Our study support previous ones regarding the beneficial effect of cinnamon in lowering blood glucose levels in diabetic patients. However, there are several points that our study highlighted them. Firstly, it more effective in lowering fasting more than random blood glucose. Secondly, soaked parts are more effective than boiled parts. In addition, the only urine biochemical parameters that were affected by cinnamon (reduced urine concentration) were urine glucose and urine ketones with no effect on urine pH, specific gravity or total proteins. Further studies are required to extend the duration of treatment to a longer period, scheduling concentration series of cinnamon and using double blind study including placebo.

Conclusions: Fasting blood glucose, urine glucose and urine ketones were shown to be reduced by the treatment with cinnamon, with soaking more than boiling and branches more than powder. In conclusion, soaked cinnamon has potentially more effective than boiled on lowering fasting blood level.

KEY WORDS: type 2 diabetes, biochemical urine parameter, cinnamon

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INTRODUCTION

Diabetes is an epidemic of pandemic proportions, with over 100 million cases occurring annually worldwide and the estimated prevalence continuing to rise from 171 million in 2000 to 366 million in 2030 [1-2] and 642 million by 2040 [3] as the diabetic population is expected to increase significantly. Diabetes Mellitus is a metabolic disease that affects how fat, protein, and carbohydrates are metabolized. It is caused by abnormalities in the action or production of insulin, or both, which raise blood glucose levels [4]. Particularly in low-income nations, diabetes mellitus (DM) and its consequences have spread like an epidemic, posing a serious threat to world health and the economy [5]. Diabetes mellitus is classified into three main kinds based on clinical presentation: type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM), and gestational diabetes mellitus (GDM) [6], T2DM makes up about 90% of DM cases [7]. Cinnamon is a spice that consists of the dried bark of Cinnamomum. The most common Cinnamomum species is *Cinnamomum verum* which belongs to *Cinnamomum Lauraceae* family. Approximately 250 species of cinnamon have been found in the world [8]. Nowadays cinnamon trees are cultivated in China, Indonesia, Vietnam, India, Lao, Seychelles, and Madagascar, especially in Sri Lanka. Numerous species are referred to as cinnamon, but true cinnamon is from the bark of Cinnamomum [9]. Because of its content (mainly polyphenols) cinnamon is suggested to clearly affect glucose homeostasis, though investigations on glucose fluctuations have generated contrasting findings [10-13]. It is not clear if

Table 1. Study design and the stages of study

Stage	Day	Supplement	Measurement
One	Day one	Boiled cinnamon sticks after dinner	Fasting blood sugar Components of urine Random blood glucose one hour after dinner
	Day two	Same above	Same above
	Day three	Same above	Same above
	Day four		Break
Two	Day one	Soaked cinnamon sticks after dinner	Fasting blood sugar Components of urine Random blood glucose one hour after dinner
	Day two	Same above	Same above
	Day three	Same above	Same above
	Day four		Break
Three	Day one	Boiled cinnamon powder after dinner	Fasting blood sugar Components of urine Random blood glucose one hour after dinner
	Day two	Same above	Same above
	Day three	Same above	Same above
	Day four	Break	
Four	Day one	Soaked cinnamon powder after dinner	Fasting blood sugar Components of urine Random blood glucose one hour after dinner
	Day two	Same above	Same above
	Day three	Same above	Same above
	Day four	Break	

Source: compiled by the authors of this study

Table 2. Gender-related distribution of patients

Gender	n (%)
Male	67 (60%)
Female	43 (40%)

Source: compiled by the authors of this study

Table 3. Age-related distribution of patients

Age group (years)	n (%)
40–50	27 (24%)
51–60	62 (56%)
>60	23 (20%)

Source: compiled by the authors of this study

Table 4. Diabetic complications among patients

Complications	n (%)
None	78 (70%)
Cardiovascular disease	26 (23%)
Neuropathy	8 (7%)

Source: compiled by the authors of this study

cinnamon has any effect on glucose control. However, prior investigations have demonstrated that cinnamon reduced glucose and lipid levels in individuals with type 2 diabetes [14-17] Prediabetes [18-19] and healthy adults [20-21]. Other investigations have shown that cinnamon improved the glycemic profile but not HbA_{1c} [10] Vanschoonbeek and colleagues, in other hand, reported no significant difference between placebo and cinnamon group. Their negative results may be because the participants involved in the study are postmenopausal diabetic patients [11]. Negative findings were also reported by Tang and co-workers, however, they used cinnamon powder as capsules, an administration method that is different from ours, boiling and maceration [13]. Cinnamon doses used in studies were of a wide range, from 0.5 g/d to 6 g/d, and the durations of the study differed from 4 to 12 weeks. Reappraisal of evidence shows that supplementation with cinnamon or cinnamon extracts might reduce blood glucose levels [22-29], but the inconsistent results regarding the effect of cinnamon on glycemic control, which were from studies with small effect sizes or very heterogeneous effects, reveal the need for further investigations.

AIM

We examined possible impact of cinnamon on blood glucose and some biochemical parameters among type 2 diabetic patients treated with cinnamon in Najaf. We performed a 4-week randomized, controlled crossover trial to determine the impact of cinnamon intake on glucose

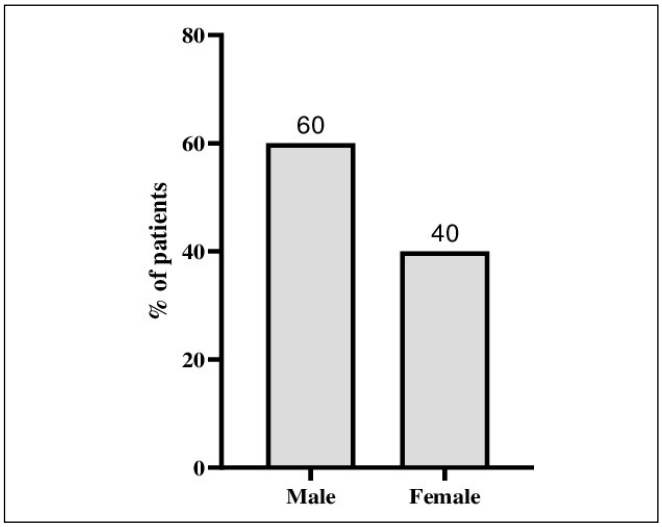

Fig. 1. Characterization of patients according to their age
Source: compiled by the authors of this study

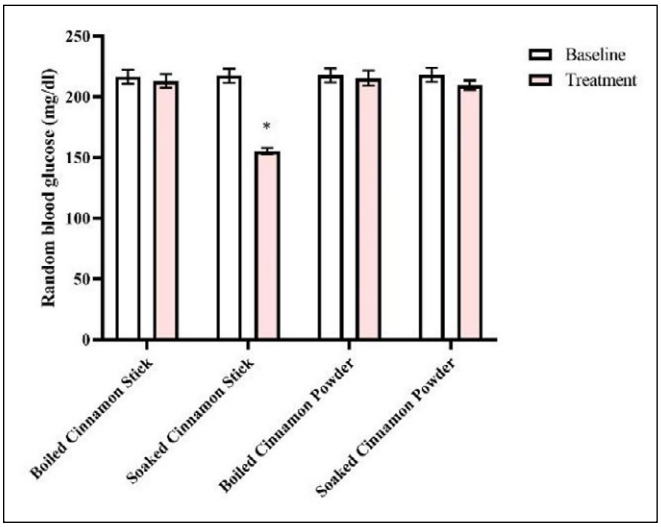

Fig. 2. Effect of cinnamon on fasting glucose level. Data presented as mean ± SEM, n=112, *: significant p<0.05 when compared to the baseline (before treatment), t-test
Source: compiled by the authors of this study

amplitude throughout the day, determined by fasting and random glucose monitoring in patients with type2 diabetes. We also evaluated urine biochemical markers.

MATERIALS AND METHODS

STUDY DESIGN, DATA COLLECTION AND PARTICIPANTS

Table 1 shows the study design and the stages of our study

A total of 112 participants diagnosed with T2DM were involved in this study visiting outpatient clinics from three cities (Najaf, Diwaniya and Babylon). Participants were selected based on established diagnostic criteria for T2DM and were between the ages of more than 40 years. Informed consent was obtained from all individuals prior to their inclusion in the study. Exclusion criteria included patients with type 1 diabetes, those with end stage liver or kidney disease, pregnant or lactating women, individuals taking insulin or other herbal supplements, and those with a history of allergy to cinnamon. Participants with poor medication adherence or those involved in other clinical trials were also excluded to ensure the accuracy and consistency of the results. Patients for whom antidiabetic management program was modified during the study period were also excluded.

PREPARATION AND ADMINISTRATION OF CINNAMON

Preparation of cinnamon: 3 grams of cinnamon sticks or powder were either boiled for 30 minutes until a total

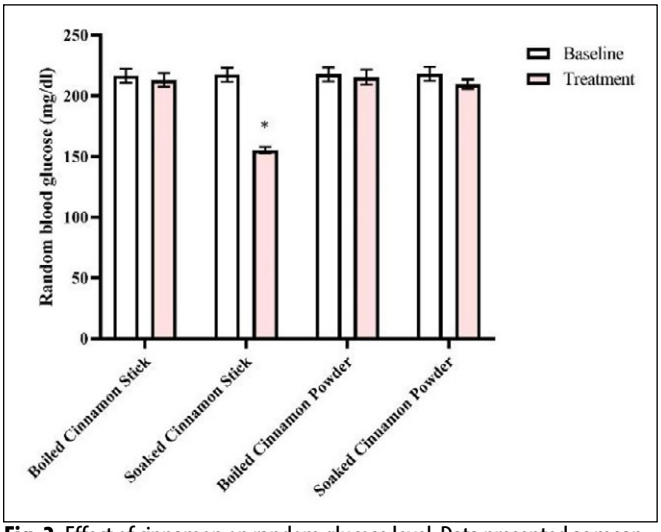

Fig. 3. Effect of cinnamon on random glucose level. Data presented as mean ± SEM, n=112, *: significant p<0.05 when compared to the baseline, t-test
Source: compiled by the authors of this study

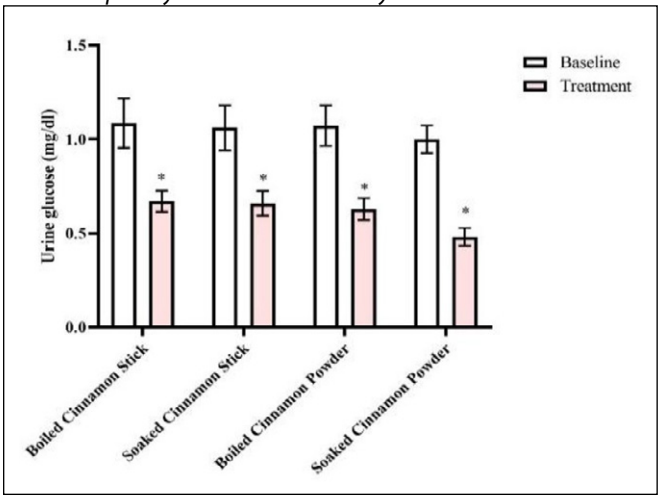

Fig. 4: Effect of cinnamon on urine glucose level. Data presented as mean ± SEM, n=112, *: significant p<0.05 when compared to the baseline, t-test
Source: compiled by the authors of this study

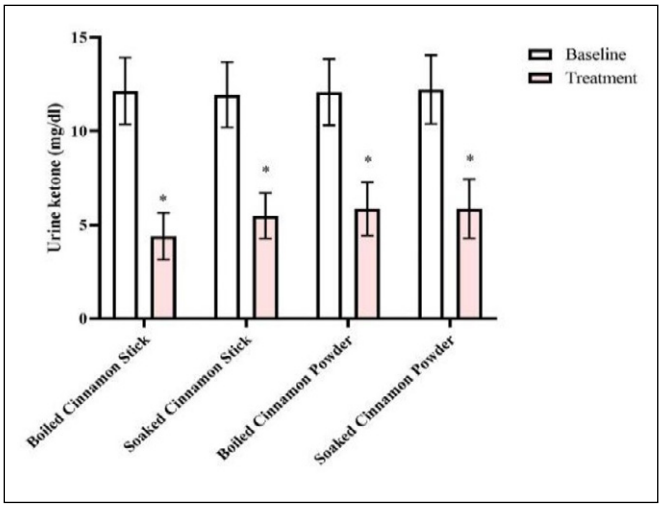

Fig. 5. Effect of cinnamon on urine ketone level. Data presented as mean ± SEM, n=112, *: significant $p<0.05$ when compared to the baseline, t-test
Source: compiled by the authors of this study

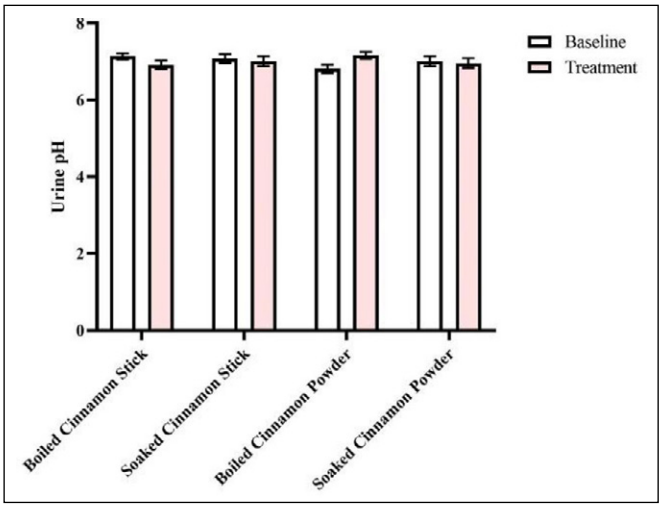

Fig. 6. Effect of cinnamon on urine pH, data presented as mean ± SEM, n=112
Source: compiled by the authors of this study

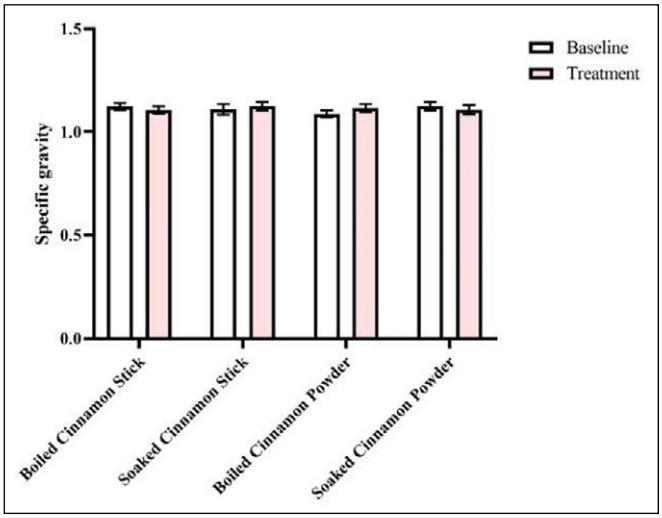

Fig. 7. Effect of cinnamon on urine specific gravity. Data presented as mean ± SEM, n=112
Source: compiled by the authors of this study

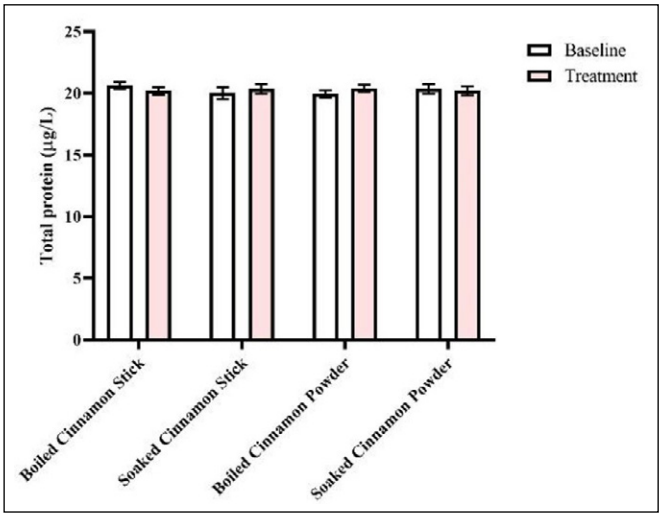

Fig. 8. Effect of cinnamon on urine total protein. Data presented as mean ± SEM, n=112
Source: compiled by the authors of this study

volume of 20 ml or soaked overnight in 20 ml of water and given 10 ml twice a day. Conventional methods were used to mimic the usual consumption of cinnamon as an herbal product not a pharmaceutical product.

MEASUREMENT OF STUDY PARAMETERS

Blood glucose was measured using On Call plus Glucometer (ACON/USA) while urine biochemical markers were measured using a commercial kit (urine glucose; 120916-0018, urine protein; 4015630019069, urine pH; 120916-0019, urine specific gravity and urine ketones; URISIGN/China).

PLACE AND TIME OF DATA COLLECTION

The study was conducted and data were collected during the period (25th of August to 25th of December 2024) for patients with T2DM visiting outpatient clinic in the center of endocrinology in Najaf.

ETHICAL APPROVAL

Data were collected officially after permission achieved from the health directorate as a part of coordination between the University of Alkafeel and the health directorate of Najaf. Written consents from participants were obtained and an ethical approval was obtained from the College of Pharmacy at the University of Alkafeel.

DATA ANALYSIS AND PRESENTATION

Both data analysis and presentation were performed using the latest GraphPad Prism software (version is 9.3.1), USA. Data are presented as percent or mean ±

SEM and t-test was used to test any significant difference between groups. Those data with p value <0.05 was considered significantly different.

CHARACTERIZATION OF PATIENTS ACCORDING TO THEIR GENDER

The gender-related distribution of demographic details in Table 2 and Fig. 1. is explained here in this study. Of the total sample (n = 112) males comprised 60%, and females 40%. This male preponderance may be due not only to higher prevalence of diabetes among men in this cohort but also because presentation with complications was chosen in some uncommon instances.

CHARACTERIZATION OF PATIENTS ACCORDING TO THEIR AGE

According to age distribution, most of the patients were in the 51–60 years age group (56%). Next were patients between 40 and 50 years (24%) and those above 60 years accounted for 20% (Table 3). The higher prevalence in middle-aged and older adults is consistent with the established increase in prevalence of type 2 diabetes with age, with risk rising steeply after the age of 40 and reaching a peak in the sixth decade of life.

CHARACTERIZATION OF PATIENTS ACCORDING TO DIABETIC COMPLICATIONS

On assessing diabetic related complications, 70% of patients had no complications at time of study. Of those who did develop complications, cardiovascular disease was the most common, affecting 23% of patients, followed by diabetic neuropathy in 7% of patients (Table 4). The greater extent of cardiovascular involvement is also in line with the well-established position of diabetes as a leading risk factor for cardiovascular morbidity and mortality.

RESULTS

EFFECT OF CINNAMON ON FASTING BLOOD GLUCOSE LEVEL

A differential effect on fasting glucose levels to cinnamon preparations was identified in the intervention. Fasting blood glucose level was also statistically significantly lower than baseline blood glucose levels on both soaked branches and soaked powder of cinnamon ($p<0.05$). However, both boiled sticks and boiled powder did not show remarkable hypoglycemic effect (Fig. 2). These data suggest that preparation method might be very important in attenuating/altering the bioactivity of cinnamon against fasting hyperglycemia.

EFFECT OF CINNAMON ON RANDOM GLUCOSE LEVEL

Regarding random blood glucose, there was a significant difference relative to baseline only in the soaked branches of cinnamon ($p<0.05$). However, no significant effect was observed for the both soaked powder and boiled preparation (sticks or powder) (Fig. 2). This selective action implies that the bioactive components of postprandial glucose regulation might be relatively more maintained or extracted in infusions of the soaked branches rather than in other preparations.

EFFECT OF CINNAMON ON URINE GLUCOSE LEVEL

There was a significant effect of cinnamon treatment on urine glucose level. All four types of cinnamon preparation (boiled-branches, soaked-branches, boiled-powder, soaked-powder) lowered urinary glucose (glucosuria) versus the pretreatment (baseline) level ($p<0.05$) (Fig. 4). This uniform effects among the preparations stressed cinnamon's capacity to modify renal glucose handling or reduce glucosuria through alterations in systemic glucose regulation.

EFFECT OF CINNAMON ON URINE KETONE LEVEL

There were also significant decreases in urinary levels of ketones after treatment with all groups of cinnamon (boiled at branch part, soaked branch part, boiled powder or soaked powder) ($p<0.05$) (Fig. 5). This implies enhanced metabolic control, since the lower ketonuria indicates a lesser dependence upon fat oxidation and ketogenesis, possibly as a result of better glucose utilization post cinnamon in supplementation.

EFFECT OF CINNAMON ON URINE PH

With respect to the urine pH that was investigated in this work, no significant difference in all treated groups (boiled branch, soaked branch, boiled powder, and soaked powder) compared to the baseline value was found (Fig. 6). This suggests that cinnamon supplementation has no effect on systemic acid-base balance and renal hydrogen ion excretion.

EFFECT OF CINNAMON ON URINE SPECIFIC GRAVITY

Regarding the urine specific gravity measured in this study, the absence of negative urine specific gravity effects was demonstrated in any of the cinnamon groups

(Fig. 7). This indicates that supplement with cinnamon does not modify the renal concentration's capacity of the urine and general fluid-electrolyte homeostasis of these patients.

EFFECT OF CINNAMON ON URINE TOTAL PROTEIN

No significant difference in 24-h urinary total protein compared to the baseline was observed after the administration of either form of cinnamon supplement (Fig. 8). Perhaps, it is because – as the data indicate- that cinnamon does not affect renal glomerular permeability and/or the status of proteinuria in this cohort.

DISCUSSION

In order to compare the effect on blood glucose urine biochemical markers in diabetic person, different preparations of cinnamon were used. The glucose data on day zero (before treatment) represent the baseline data in diabetic subjects prior to initiation of supplementation. On the first day of experiment (day 0), mean fasting blood glucose in diabetic rats of all four groups, which were weighed to boiled and soaked branches/ powder from cinnamon was estimated. When diabetic subjects in these groups 3g/cinnamon/day received for a period of three days, the mean fasting blood glucose from patients at (day 3) was significantly lower compared to the mean fasting baseline value.

The result showed that soaked cinnamon dose could reduce mean fasting blood glucose in both sets of patients, while there seems to have been a decrease the drop in blood glucose level is not statistically significant in relation to the mean fasting blood sugar values of patients who received boiled cinnamon. This fact might be explained by the wide range of people's blood glucose levels at the outset, which means that boiled power was not effective. Also, boiling itself may alter both components [30-31]. Furthermore, the boiling time may influence the quality and/or quantity of compositions [32-34]. Hence, there may be a need for time-scale to ensure the effective period for boiling in order to give the highest yield of components. Even the mean fasting blood glucose levels further plummeted which might owe to cumulative action on the cinnamon powder. The same group members taking part in lengthy tests were rewarded with a further drop in fasting blood sugar, and they used a lot more cinnamon than the previous two doses put together. This means that, the longer the use of cinnamon – the better. However, such cumulative effect was not shown with cinnamon powder which may attributed to the reasons mentioned above. Furthermore, it was found that even boiled water with powder can still packed quite a wallop in lowering blood glucose levels. In summary, 3 g/d cinnamon (3 g in powder form) can simply be conveniently incorporated into the regular diet as part of food and its use may be convenient, especially if we recall that 3 g/d cinnamon are within an effective and safe range [35-36]. Another advantage of cinnamon is its ability to reduce blood glucose, which is especially significant for those with type 2 diabetes. It is advised that diabetics regularly incorporate cinnamon into their dietary preparations in light of this findings. To prevent exceeding the suggested quantity, its consumption must be controlled. Otherwise, improper usage of cinnamon is still linked to health problems [37]. Numerous theories have been suggested to explain how cinnamon affects diabetes. Sangal suggests that cinnamon could mimic the effects of insulin [38]. Another process that is thought to be responsible for cinnamon's action is "inhibiting glycogen synthase" [39]. It may also have the effect of decreasing the absorption of glucose in the small intestine by inhibiting intestinal ATPase and boosting glycosidase enzymes [40-42]. Additional hypothesized mechanisms could include up-regulating insulin receptor gene expression [43], activating PPAR γ and AMP kinase [44], activating IGF1 signaling in fibroblasts [45], modifying mitochondrial physiology and elevating cellular metabolism [46], Cyclic-AMP signaling, and autophagy [47], in addition to inhibiting alpha-amylase, up-regulating GLUT4 translocation in muscle and adipose tissues [39], phosphorylating AMPK and acetyl-CoA carboxylase, and inhibiting AMPK to reduce glucose uptake by adipose tissues and anti-AGEs formation [48]. The participants who were not taking cinnamon dosages during the break days showed a steady rise in their mean fasting blood glucose levels, suggesting that cinnamon had a long-lasting hypoglycemic impact on diabetics. It's possible that the cinnamon dosage caused certain cellular metabolic changes, which is why the mean fasting blood glucose level did not increase to the level observed on day 0 of the experiment. Although we are unsure, it appears that cinnamon may have caused some biochemical or physiological alterations in insulin resistance sites, glucose transport across cell membranes, the enzyme system involved in the metabolism of carbohydrates, and receptor sites [49]. Using a rat model, Jayaweera and colleagues used a range of high doses of cinnamon (1500, 2250, 3000 mg/kg) to evaluate the diuretic effect of cinnamon [50]. Beside its diuretic effect, they found that cinnamon increase the renal excretion of several minerals. Consistent with our findings, Jayaweera and co-workers reported no significant difference in specific gravity. The urine pH, however, reported by the same team was slightly different when compare treatment to

control. This might be due to the high dose of cinnamon. In addition, it is more likely to be due to changes in the change in sodium bicarbonate nor hydrogen ion as they reported no changes in the concentration of hydrogen ion. It is noteworthy to mention that cinnamon supplementation may be associated with health problem. For example, it could increase the secretion of oxalate in the urine which may increase the incidence of renal oxalate stone [13, 51], however, the World Health Organization (WHO) suggested Acceptable Daily Intake level of cinnamon to be 0.7 mg / kg body weight [51], and a dose of 3 g per a day I very low compared to the Acceptable Daily Intake. This means it that can be considered safe if we consider that the average body weight is 70 kg.

CONCLUSIONS

Our study support previous ones regarding the beneficial effect of cinnamon in lowering blood glucose levels in diabetic patients. However, there are several points that our study highlighted them. Firstly, it more effective in lowering fasting more than random blood glucose. Secondly, soaked parts are more effective than boiled parts. In addition, the only urine biochemical parameters that were affected by cinnamon (reduced urine concentration) were urine glucose and urine ketones with no effect on urine pH, specific gravity or total proteins. Further studies are required to extend the duration of treatment to a longer period, scheduling concentration series of cinnamon and using double blind study including placebo.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Physical literacy in social work: current status and prospects implementation of foreign experience in Ukraine

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ABSTRACT

Aim: To initiate a scholarly dialogue on exploring the potential integration of physical literacy into social work practice and the possibilities for its implementation in helping to support diverse client groups, taking into account the experience of Ukrainian-German cooperation.

Materials and Methods. A review of English-language literature on physical literacy was conducted using three scientometric databases to assess the current research landscape and practical applications of physical literacy within the social domain. Factual information about the target population was gathered via a structured questionnaire. The empirical data were analysed using mathematical and statistical techniques, including descriptive statistics and correlation analysis. All statistical analyses were performed with SPSS Statistics software, version 19.0.0.

Results: A review revealed a wide range of interpretations of physical literacy in contemporary scientific literature, and common standard characteristics were identified across various cultural and scientific perspectives. In social work, physical literacy is predominantly equated with engagement in physical activity. Consequently, its primary perceived benefit is physical and mental health improvement. The influence of physical literacy on cognitive and social dimensions of well-being is acknowledged to a lesser degree.

Conclusions: Social workers are generally prepared to integrate the concept of physical literacy into their professional practice or are already applying some aspects of it, often without explicitly recognizing them as such. Systemic changes are required to ensure the effective implementation of physical literacy in social work and to achieve a meaningful impact on all dimensions of health. These include improvements to the legislative framework—particularly a revision of workload standards for social work professionals—appropriate training for social workers, increased funding, enhanced material and technical resources, and the adoption of an individualized approach to working with clients.

KEY WORDS: physical literacy, social work, social worker, social welfare, health, components of health

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INTRODUCTION

The concept of physical literacy (PL) and its rationale, initially articulated by Australian researcher and nurse practitioner Margaret Whitehead in 1987 and publicly introduced in 1993, is regarded as a fundamental mode of interaction without which we would not be able to realize our human potential [1]. PL is characterized as an "embodiment of human nature and personal potential, serving as a means to enhance individual health" [2]. Scholars from various countries and regions have contributed to the evolution of the definition and conceptualization of PL [3-12]. Today, the concept is widely recognized in North America, Australia, New Zealand, the United Kingdom, and several Western European countries, including Germany, and is increasingly being adopted in Ukraine [13-14]. PL involves continuous learning that allows people to achieve their goals, develop their knowledge and potential, and participate fully in the life of their community and society [2; 15]. More modern interpretations of PL describe it as «motivation, confidence, physical competence, knowledge and understanding, appreciation, and responsibility for participating in physical activity throughout life» [16].

Lviv Polytechnic National University (Lviv, Ukraine), in cooperation with Julius Maximilian University of Würzburg (Germany), has accumulated extensive experience in applying the concept of physical literacy within the field of social work. This collaboration includes joint projects (2015–2019), a series of scientific and practical seminars and conferences (2017–2025), elective courses delivered by Prof. Dr. Olaf Huz for undergraduate and graduate students in social work (online, 2022–2024), as well as thematic lectures for practicing social workers as part of professional development programs organized by the Department of Sociology and Social Work (online, 2023–2025). This practical experience, combined with a thorough analysis of theoretical literature and empirical research findings, as well as the current socio-political context of Ukraine during wartime and

the anticipated post-war recovery, has contributed to a deeper understanding of the potential role of PL in social work. Consequently, the focus of this study was to explore how the concept of physical literacy is represented in contemporary academic discourse in relation to public health and well-being. Additionally, the research aims to identify the areas of social work where PL is already being integrated into professional practice, and to examine how social workers perceive the feasibility of implementing PL in their daily work under martial law and in the context of Ukraine's post-war reconstruction.

AIM

The aim is to initiate a scholarly dialogue on exploring the potential integration of physical literacy into social work practice and the possibilities for its implementation in helping to support diverse client groups, taking into account the experience of Ukrainian-German cooperation.

MATERIALS AND METHODS

A comprehensive review of English-language literature on physical literacy was conducted using three scientometric databases. Additionally, expert consultations were held with Prof. Dr. Olaf Huz, Head of the Sports Center at the Faculty of Humanities, Julius Maximilian University of Würzburg (Germany), and his assistant, Dr. M. Zimlich.

To obtain factual data about the target population, a questionnaire survey was administered. The empirical data were analyzed using a range of statistical methods, including descriptive statistics and correlation analysis. Statistical processing was carried out with the assistance of SPSS Statistics software, version 19.0.0.

ETHICAL PRINCIPLES OF THE STUDY

The research was organized and conducted in line with recognized ethical standards of empirical inquiry. Respondents participated voluntarily, with assurances of anonymity, confidentiality, and mutual trust. They were informed that no responses were considered "correct" or "incorrect," and their professional insights and subjective evaluations were valued. The collected data were used exclusively for scholarly purposes and presented in an aggregated, anonymized form.

ORGANIZATION OF THE STUDY

Between July 2024 and May 2025, the Department of Sociology and Social Work at Lviv Polytechnic University conducted a pilot sociological survey among participants of advanced training courses for social work professionals. Within the thematic module *"Health-Promoting Activities in Social Work,"* five specialist groups attended an online lecture by Prof. O. Khuz titled *"Perspectives of Physical Literacy for Health Promotion in Social Work."* A total of 99 valid questionnaires were collected. While the study is not representative, it serves to identify preliminary trends and formulate hypotheses for future in-depth research.

The generalized definition of physical literacy underlying the questionnaire for social workers conceptualizes PL as a key factor in personal development, facilitating effective and diverse engagement with both the social environment and the broader world. This definition emphasizes individuals' conscious and confident participation in various, including novel, forms of physical activity and interaction, which fosters self-discovery, realization of human potential, and the enhancement of physical abilities (such as coordination, balance, speed, flexibility, and endurance). Additionally, it supports the development of social competencies, including self-confidence, ethical and cultural sensitivity in communication, empathy, and a forward-looking perspective—thereby contributing to overall health and well-being. A physically literate individual is thus motivated to pursue bodily changes, recognizing their value and positive impact on adaptability in a changing environment.

FRAMEWORK

The work is a fragment of the research project «Research Initiative and Practical Implementation of Socio-political Projects to Resolve Social Problems in Ukraine During Wartime and Post-war Recovery.» No. 0123U103018.

RESULTS

The sample was predominantly female (96%), with most respondents living in Western Ukraine (95.1%). The majority are employed in government and municipal social service agencies (65.7%), while others work in public and charitable organizations (12.1%), local government (8.1%), educational institutions (3%), or outside the social sector but with relevant experience (11.1%). Over half of the participants (57.6%) have up to three years of professional experience, reflecting a relatively young group, while 18.2% have 4–10 years of experience. At the same time, a quarter (24.2%) already have more than 10 years of experience, which

ensures the presence of experienced specialists in the sample. Respondents most often work with persons with disabilities (48.5%), families in difficult situations (41.4%), older adults (40.4%), and internally displaced persons (34.3%). Less frequently, they engage with low-income individuals (28.3%), children from disadvantaged families (25.3%), and war veterans (24.2%), and least often with homeless individuals (3%), persons with mental health issues (5.1%), victims of human trafficking (6.1%), and survivors of gender-based violence (8.1%).

One of the objectives of the survey was to identify the qualities that respondents associate with physical literacy. While the majority of participants rated most of the proposed attributes as important, certain characteristics were selected with notably higher frequency, allowing for the identification of key features in the conceptualization of physical literacy. Foremost among these were awareness of the health benefits of physical activity (72.7%), motivation to engage in regular exercise (68.7%), and the ability to tailor physical activity to individual needs and capabilities (65.7%). Additionally, a positive attitude towards physical activity (61.6%) and the capacity to maintain a balance between physical activity and other life domains (52.5%) were also rated relatively highly.

The survey findings indicate that the concept of physical literacy, as understood by the surveyed social workers, is primarily centered around physical activity. While attributes such as adherence to safety guidelines (50.5%) and the capacity to independently plan and execute physical exercises (47.5%) were also recognized as important, they were less frequently emphasized. Social and communicative competencies—such as participation in team games (34.3%), conflict resolution during physical activities (31.3%), and the ability to motivate others to engage in physical activity (32.3%)—were among the least cited characteristics. This suggests that, in comparison to individual attributes, the social dimension of physical literacy is perceived as relatively less significant. Similarly, traits such as confidence in one's physical abilities (33.3%) and perseverance in pursuing physical goals (38.4%) were mentioned less often, yet they remain an important part of the overall image of a physically literate person.

The reviewed literature conceptualizes physical literacy as encompassing physical, psychological, cognitive, and social domains of learning. This multidimensional nature necessitates the simplification of existing knowledge and terminology to enhance its accessibility and practical application by educators, coaches, and public health professionals involved in physical activity promotion [9]. Concurrently, the concept of physical literacy is increasingly aligned with the framework of functional growth, which offers a holistic approach to fostering physical activity by integrating affective and cognitive dimensions alongside physical and motor development [5]. As a manifestation of personal development, PL is regarded as a foundational literacy through which other forms of literacy are mediated. It enables individuals not only to cultivate their own physical activity but also to contribute to broader holistic literacy, facilitating self-awareness, interpersonal understanding, and engagement with the surrounding world [17]. According to A. Chen and colleagues, acquiring PL requires familiarity with learning theories, mechanisms of motivational regulation, and principles of self-determination. These elements are essential for task completion and for fostering competence and self-regulation strategies that sustain motivation for movement [4]. Furthermore, Australian scholars emphasize the importance of critical competencies for PL educators and researchers, notably racial literacy and cultural competence [18].

Analysis of the data obtained revealed the most relevant indicators that social workers may utilize to assess the physical literacy of their clients. For further analysis, only those indicators that were endorsed by more than 50% of respondents were considered. Specifically, the findings highlight that the most frequently cited indicators of physical literacy include clients' awareness of the health benefits of physical activity (64.6%), the ability to perform basic physical exercises (58.6%), motivation to engage in physical activity (57.6%), the capacity to incorporate physical activity into daily routines (54.5%), emotional states and mood regulation (52.5%), and the ability to set and achieve realistic physical goals (51.5%). In contrast, indicators related to social interaction—such as teamwork, cooperation, leadership, and adherence to principles of fair play—received significantly lower ratings (ranging from 7.1% to 25.3%). This disparity underscores a prevailing tendency among social workers to prioritize individual aspects of physical literacy over its social components. This can be explained by the fact that specialists focus primarily on the individual physical activity indicators of clients and pay less attention to group or team forms of interaction.

These findings partially align with the analysis of international literature, which highlights a connection between physical literacy and the development of social skills – such as teamwork, group interaction, and cooperative play. However, this association is primarily observed within structured settings, including preschool groups, school classes, and groups of children with disabilities. In these contexts,

PL is implemented through preventive or educational programs (particularly physical education) aimed at enhancing self-regulation, prosocial behavior, interpersonal communication, a sense of belonging, and overall mental health and well-being across diverse populations [19]. The publications reviewed demonstrate the existence of causal links between PL and the mental health of children and adolescents [20]. PL promotes resilience and successful adaptation to challenges both within and beyond the school environment, extending throughout the lifespan. It is recognized as a factor that promotes the flourishing of authenticity of personality and contributes to an objectively good, comprehensive, individualized quality of life for a person as an «independent, self-reliant, socially formed subject» who achieves high social results, a high level of life satisfaction and well-being, is more resilient, has higher self-esteem, and a low level of susceptibility to depression, anxiety, and stress [16, 19, 21, 22].

The evaluation of experts' perspectives on the integration of physical literacy (PL) into their professional practice reveals a strong consensus regarding its relevance and applicability. A substantial majority of respondents (84.8%) expressed full agreement with the appropriateness of incorporating PL into their work, while 10.1% indicated partial agreement. Only 1% of participants disagreed, and 4% were undecided. These findings suggest that most professionals, irrespective of the specific client populations they serve, perceive the application of PL as both beneficial and suitable within the context of social work. This is further supported by the generally positive reception of PL as a concept, with only minor variations depending on client characteristics. The highest level of agreement ("Strongly agree") was observed among practitioners working with the most vulnerable groups—such as individuals with terminal illnesses, mental health issues, homeless people, behavioral disorders in children, survivors of human trafficking, and military veterans—where unanimous support (100%) was recorded. The lowest rate of complete agreement was found among those working with survivors of domestic violence (77.8%), although when combined with partial agreement, the overall support rises to 88.9%.

These findings are consistent with international literature, which positions physical literacy as a valuable framework for fostering lifelong engagement in physical activity as a means of unlocking human potential. PL is considered relevant across a wide range of populations, including children, school-aged youth, university students, adults, older individuals, and persons with disabilities. It addresses the needs of participants in preventive programs aimed at mitigating health risks and reducing the financial burden of treatment and rehabilitation. PL has been shown to support the rehabilitation of children with physical developmental disorders [23], coordination impairments of dystrophic origin [24], and intellectual disabilities [25]. It plays a role in promoting social inclusion among older adults [26], and contributes to the rehabilitation of veterans and combatants who have experienced various forms of injury [14].

The survey of social workers revealed a strong level of support for the integration of physical activity as a component of physical literacy within social work practice. More than three-quarters of respondents (75.8%) indicated that incorporating physical exercise is always or often appropriate in their professional activities. In contrast, only 2% considered it inappropriate, and an additional 3% emphasized objective barriers to implementation. Notably, 9.1% expressed interest in adopting such practices in the future, highlighting the potential for expanding physical activity programs within the social sector. Statistical analysis using the χ^2 criterion did not reveal a significant relationship between attitudes toward the integration of physical activity and work experience or the specifics of the client group ($p > 0.05$), indicating the universal nature of support for this idea.

Social workers' perceptions of the effectiveness of physical activity are predominantly positive. A total of 76.8% of respondents rated physical activity as either effective or highly effective in their professional context, while 18.2% adopted a neutral stance, and only 5% expressed skepticism. Consistent with previous findings, correlation analysis revealed no statistically significant relationship between perceptions of effectiveness and professional experience ($p > 0.05$). This outcome reinforces the notion of a broadly shared consensus among practitioners regarding the positive impact of physical activity on clients, irrespective of their length of service or specific area of practice.

These responses align with the perspectives found in scholarly literature, where physical literacy (PL) is widely recognized as a mechanism for enhancing personal health and well-being [10; 16; 18; 26; 27; 28]. The connection between PL and health literacy has also been substantiated [15]. In this context, health is defined in accordance with the World Health Organization's formulation as a state of complete physical, mental, and social well-being, and not merely the absence of disease or infirmity, adding to this its significance as a resource for everyday life, and a determinant of quality of life.

Respondents acknowledged the psychological dimension of physical literacy as highly significant. A

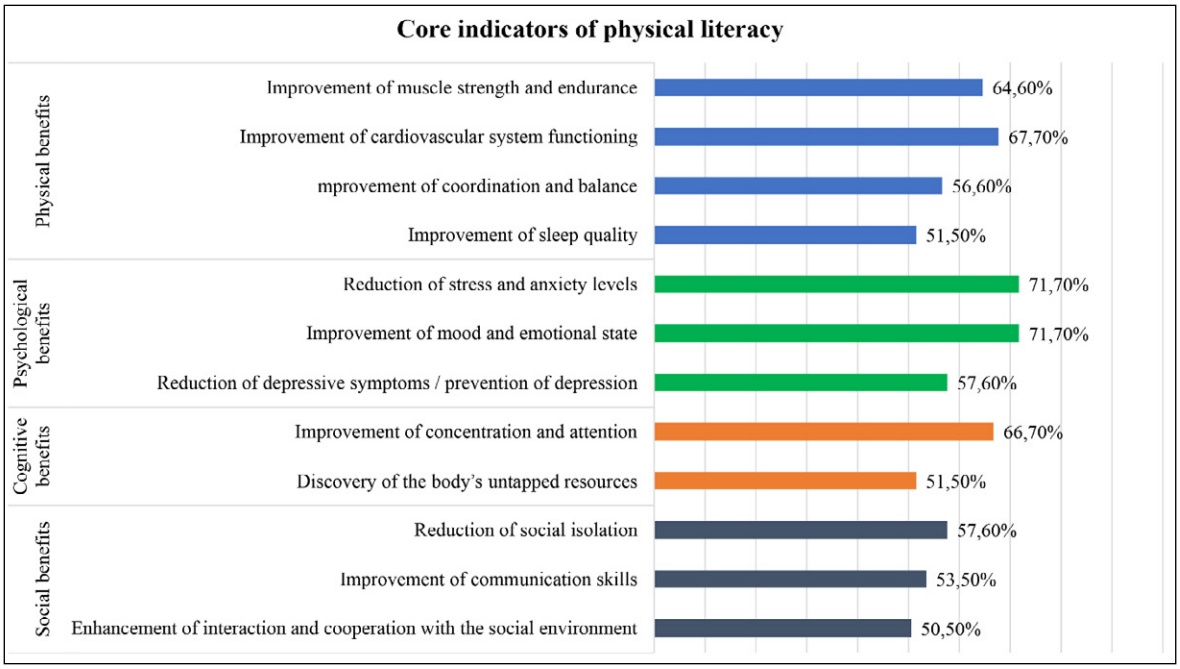

Fig. 1. Core indicators of physical literacy
Picture taken by the authors

substantial majority (96%) agreed that physical activity contributes to improving the psychological state of clients. Less experienced employees were more likely to express «complete agreement,» although no statistically significant differences in terms of experience were found (Pearson's χ^2 (8) = 8.057; p = 0.428).

Responses to questions regarding activities aimed at enhancing clients' motivation for physical activity revealed a degree of ambivalence among social workers. Only 21.2% reported frequently organizing such activities, while 23.2% indicated occasional engagement. Notably, 44.5% expressed an intention to implement motivational practices in the future, despite not having done so yet. Meanwhile, 11.1% did not perceive a need for such activities, which may reflect existing organizational or methodological barriers to integrating the psychophysical dimension into practice. Statistical analysis using Pearson's chi-square test (χ^2(16) = 16.707; p = 0.405) did not identify a significant relationship between professional experience and the implementation of motivational activities, suggesting that attitudes toward such practices are not dependent on years of service.

The majority of respondents (93.9%) believe that physical literacy contributes positively to clients' cognitive functioning, while only 6.1% were undecided. These findings reflect a high level of awareness among professionals regarding the connection between physical activity and mental processes. Although no statistically significant relationship was found between professional experience and these beliefs (p > 0.05), a trend was observed: less experienced practitioners were more likely to "strongly agree," whereas more experienced professionals tended to express their agreement with greater caution.

The link between physical literacy and psychosocial factors—such as resilience and the ability to adapt to challenges within educational settings and beyond—supports the view of physical literacy as a lifelong contributor to personal development, self-actualization, and the realization of an autonomous, holistic personality [21].

Although the social dimension of physical literacy received the lowest ratings among the four core dimensions, its significance was nonetheless affirmed. Specifically, 89.9% of respondents either fully or partially agreed that physical literacy contributes to enhancing clients' social well-being and functioning. Only 5% disagreed with this statement, and 5.1% were undecided. Work experience has no statistically significant effect on professionals' beliefs about the role of physical literacy in improving client well-being (p > 0.05).

At the same time, only 44.4% of respondents said that they always or often include teamwork exercises in their physical activity practice. Another 30.3% have never used such exercises but expressed a desire to integrate them in the future. Thus, almost three-quarters (74.7%) of social workers demonstrate real or potential readiness to implement team physical activities in their professional activities. Pearson Chi-Square did not find a statistically significant relationship (p > 0.05), and therefore there is a need for support and motivation

for both beginners and experienced professionals to integrate such techniques into their professional practice. This opens up opportunities for training, practical recommendations, and resource assistance for those who want to but do not yet have experience in this area. Ukrainian authors point to the application of PL in the social sphere, in particular, adaptive physical activity in the comprehensive rehabilitation of military personnel and war veterans who have suffered injuries, as well as to promote the social integration of persons with disabilities and other low-mobility groups of the population [14].

Overall, the experts' evaluations of the benefits of implementing physical literacy can be represented through a core set of physical literacy indicators—a set of aspects that respondents identified in the survey as most important for the development of physical literacy in their clients (Fig. 1). This core was established based on professional assessments by social workers across the physical, psychological, cognitive, and social dimensions of physical activity. Indicators selected by more than 50% of respondents represent the components deemed most essential for enhancing clients' health, social integration, and overall well-being. Indicators with a selection frequency below 50% are considered supplementary, playing a secondary role within the broader framework of professional evaluation. Thus, the core of physical literacy indicators reflects the agreed professional position of social workers on the effects of physical activity that have the greatest positive impact on the overall well-being of clients.

Within the physical dimension of physical literacy, social workers most frequently associate its development with improvements in fundamental physiological functions and motor abilities. The most commonly cited benefits include enhanced cardiovascular function (67.7%), increased muscular strength and endurance (64.6%), improved coordination and balance (56.6%), and normalized sleep patterns (51.5%). Additionally, nearly half of the respondents (49.5%) identified immune system strengthening as a relevant outcome. In contrast, indicators such as improved flexibility (29.3%), motor development (28.3%), reduced fatigue (27.3%), enhanced metabolic function (23.2%), and decreased susceptibility to infectious diseases (17.2%) were considered less significant. These findings are consistent with international research, which positions physical activity as a key determinant of health [13], positively correlated with embodied physical competence and various aspects of physical well-being, including activity levels, cardiorespiratory fitness, muscular strength and endurance, and healthy body composition [27].

Psychological Dimension. Social workers placed high value on the psycho-emotional potential of physical literacy. The most frequently cited outcomes included reduced stress and anxiety (71.7%), improved emotional state and mood (71.7%), and the prevention of depressive symptoms (57.6%). Additionally, 49.5% of respondents noted increased vitality as a key resource-related benefit for clients. Less commonly mentioned were enjoyment of physical activity (39.4%), enhanced motivation and confidence (31.3%), increased self-esteem (28.3%), reduced fatigue (27.3%), prevention of mental disorders (24.2%), and the promotion of goal-setting and self-efficacy (23.2%).

Cognitive Dimension. More than two-thirds of respondents (66.7%) identified improved concentration and attention as the primary cognitive benefit of physical activity. Over half (51.5%) emphasized the discovery of latent physical resources, while 46.5% noted a reduction in the risk of cognitive disorders. However, less emphasis was placed on the development of planning skills, speed of thinking, memory (30.3% each), decision-making ability (22.2%), strategic thinking (19.2%), and analytical skills (10.1%). These results suggest that while the cognitive benefits of physical activity are acknowledged, they are perceived as less central compared to the physical and psychological dimensions.

Social Dimension. Although the social benefits of physical literacy were rated lower than those of the physical, psychological, and cognitive dimensions, they remain relevant in professional practice. The most frequently cited outcomes include reduced social isolation (57.6%), enhanced communication skills (53.5%), and increased interaction with the surrounding environment (50.5%). Less commonly mentioned are the development of teamwork skills (39.4%), strengthening of social connections (38.4%), and the creation of a positive social environment (40.4%). Indicators such as empathy (13.1%), cultural competence (10.1%), and conflict resolution skills (9.1%) are noted with the least frequency.

The social context of physical literacy is closely linked to interpersonal interaction and is associated with increased vitality, energy, and dynamism. It contributes to overall well-being, fosters a sense of agency in working voluntarily with marginalized groups, and supports the development of additional personal capacities. PL enables individuals to feel well and flourish [15]. Its positive impact on the social functioning and social health of older adults has also been documented, particularly in reducing social isolation and loneliness, and enhancing social support networks [29].

DISCUSSION

Despite increasing scientific interest, the concept of physical literacy has not yet been adequately integrated into practice or policy in most European countries. At the global level, PL is recognized in the Global Action Plan on Physical Activity 2018–2030 (GAPPA) as a key construct for addressing physical inactivity and has been proposed by the United Nations as a relevant objective for the 2030 Sustainable Development Goals [13]. Recent Ukrainian scholarship has begun to engage with PL in the academic literature [29]. Owing partly to linguistic issues, the term PL in Ukraine is often rendered as "personal physical culture." Ukraine is among those countries where academic exchange about PL has occurred at the national level—most notably via networks and conferences, but the number of substantive publications remains limited and is largely confined to conference abstracts [13, 14].

European researchers emphasize that the advancement of PL depends primarily on the initiatives and achievements of individuals. In Ukraine, diagnostic instruments for assessing PL in physical education settings have been translated from English; however, PL is scarcely reflected in official health policy documents, and the Sports Federation of Ukraine has not formally endorsed the concept. National documents pertaining to sport, physical activity, health promotion, and education do not currently adopt PL as a coherent, holistic framework. Notwithstanding this absence and the uneven status of PL across Europe, experts anticipate a substantial growth in its prominence in the coming years [13].

Based on the results of an empirical study, the views of the experts surveyed on the implementation of physical literacy regarding key actors and obstacles were as follows. Most respondents place the main responsibility for the implementation and promotion of physical literacy in social work on state institutions. In particular, the most frequently mentioned were the Ministry of Social Policy of Ukraine (59.6%), the Ministry of Health (52.5%), state institutions and authorities (43.4%), as well as social services and organizations (42.4%). Respondents were much less likely to mention educational institutions that train social workers (27.3%), non-governmental organizations and charitable foundations (26.3%), the Ministry of Education and Science (20.2%), or professional associations and unions of social workers (12.1%). Only 28.3% of respondents believe that responsibility for implementing physical literacy should lie with social workers themselves. This pattern of responses demonstrates a prevailing view of the leading role of the state, while the potential of the professional community, the public sector, and the specialists themselves is rated lower.

Among the main barriers to the implementation of the concept of physical literacy in social work, respondents cited a lack of funding (73.7%), a lack of specially trained personnel (67.7%), and limitations in material and resource support (48.5%). Other significant obstacles include client resistance to change (44.4%), lack of time to include physical activity in the work schedule (33.3%), and insufficient awareness of the importance of physical literacy among colleagues and management (35.4%). At the same time, only 17.2% of respondents consider the difficulty of integrating physical activity into comprehensive care to be a critical issue, which may indicate recognition of the potential of this approach in social work. Other barriers mentioned included limited access to programs and training (24.2%), lack of support from clients' families (28.3%), and problems at the local community level (28.3%).

CONCLUSIONS

In the challenging circumstances of today, as Ukrainian society continues to fight for its independence and freedom of choice on the battlefield, and as the right to life for every Ukrainian depends on the outcomes of military actions, the issues of health and social inclusion acquire particular significance. Public health, as a strategic resource for national defense, is critically important both on the front lines and in the rear, far from the conflict zone. Moreover, individual health—understood as the unity of physical, mental, social, and spiritual components—is shaped, maintained, and developed not only through medical care but also through interdisciplinary collaboration and personal engagement.

Physical literacy (PL) is considered a vital tool for fostering health and developing a healthy personality. It serves as a means of personal growth through effective and diverse interaction with the social environment and the world, enabled by conscious and confident participation in various forms of physical activity. This engagement supports the development of individual potential, physical abilities, social competence, and overall well-being.

Social workers, as agents of social change, play a significant role in enhancing the personal potential of individuals, families, groups, and communities. By promoting social inclusion and preventing social dysfunction, they facilitate adaptation to rapidly changing and complex environments. Through the integration of physical literacy elements into their practice, social workers can substantially improve the health and well-being outcomes of their clients—especially those at the highest risk of experiencing difficult life circumstances and social exclusion.

The conducted research confirms that social work professionals recognize the potential of PL and express readiness to implement it, primarily as a tool for preserving clients' health. However, there is a noticeable lack of awareness regarding the social dimension of PL as a mechanism for individual growth and social development. This highlights the need to enhance the competencies of social workers in PL-related matters and to emphasize its role in holistic (biopsychosocial-spiritual) health and well-being.

Effective measures in this regard may include professional development courses for social workers and the introduction of new specialized educational and professional programs. Particularly relevant are the anticipated changes to the classifier of fields of knowledge and specialties, which will guide the training of professionals in higher education institutions starting from the 2025 admission cycle. Specifically, the unification of the fields of knowledge "Health Care and Social Welfare" and "Social Work and Counseling" under a common framework is expected to facilitate the adaptation of social work education programs to address health-related issues, including social problems based on health preservation principles. This integration may also accelerate the adaptation of legislative frameworks to support interdisciplinary collaboration between medical and social work professionals.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

COVID-19 aggravates well tolerated spinal cord injury – related neuropathic pain

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ABSTRACT

Aim: To compare the intensity of the pain, medication requirements and the evolution of symptoms of neuropathic pain patients with or without COVID-19 infection and to determine whether the severity of the infection influenced these variables.

Materials and Methods: In all, 400 adults with chronic neuropathic pain (≥3months duration) were included-200 positive and 200 negative for SARS-CoV-2. Baseline demographic and clinical characteristics were comparable between groups (age, sex, BMI, comorbidities, pain duration; all $p>0.22$) to meaningful outcome comparison. Pain severity, analgesic consumption, pain course and symptom deterioration were assessed pre-/post-COVID-19 or similar follow-up for the controls.

Results: In COVID-positive group score values raised from 4.6 ± 1.1 to 7.7 ± 1.4 ($p<0.05$), at a more significant extent in women (4.7-8.2, $p<0.01$) as in diabetic patients (4.6-8.1, $p<0.001$). The COVID-negative group had no significant change (4.2-4.3, $p=0.42$). Post-COVID, 72% of infected patients needed more analgesics and 65% of them other drugs, vs. 15% and 12%, respectively, in the negative group $p<0.001$. Pain curves trajectories evidenced steeper increments in COVID-positive (85% vs. 25% in severe vs. mild COVID-19 worsening, $p<0.001$, revealing a directly related pain severity to the infection.

Conclusions: SARS-CoV-2 infection is an aetiologic factor that independently leads to a substantial worsening of chronic neuropathic pain, an increase in analgesic needs, and a more severe symptom burden. The impact is most pronounced among women, diabetic patients and those with severe COVID-19. These results highlight the significance of proactive pain management approaches and longitudinal tracking in this population.

KEY WORDS: neuropathic pain; COVID-19, pain worsening, Visual Analog Scale, analgesic titration, sex; diabetes, disease severity

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ABBREVIATIONS

COVID-19: Coronavirus Disease 2019
CNS: Central Nervous System
ACE2: Angiotensin-Converting Enzyme 2
TMPRSS2: Transmembrane Protease Serine 2
DN4: Neuropathique 4
CRPS: Complex Regional Pain Syndrome
NRS: Numeric Rating Scale
ASIA: American Spinal Injury Association
AIS: Impairment Scale

INTRODUCTION

The severe acute respiratory coronavirus 2 (SARS-CoV-2) that causes coronavirus disease 2019 (COVID-19) has been known to infect and kill an increasing number of people in China starting December 2019. During the pandemic, the medical sector faced significant challenges. Of all the infectious respiratory diseases, COVID-19 emerged as one of the biggest public health threats. The neurological manifestation of COVID-19 is one of these. Three categories of pain exist: nociceptive pain, which is produced by tissue disease or damage (e.g., burns); neuropathic pain, which is caused by disease or injury to the sensorimotor system; and a mix of neuropathic and nociceptive pain [1]. Although neuropathic pain can result from a variety of nerve-damaging stimuli in the central or peripheral nervous systems, the clinical presentation of the pain is consistent across the many neuropathic syndromes and causes [2]. Patients usually have paradoxical sensory impressions, with lesion-induced decreased feelings and discomfort as the predominant pleasant symptom. Patients typically encounter these impressions for the first time [1]. When neurological disorders occur, such as when parkinsonian tremor develops following substantia nigra degeneration [3] or when spasticity develops following spinal cord injury [4], it is not uncommon for indications of hypersensitivity and allodynia to coexist. As a subjective sensory symptom, pain differs from these motor disruptions in that it is not apparent, is challenging to quantify, and contains psycho-

logical and emotional elements in addition to physical ones [1-2]. COVID-19 has been associated with a growing evidence of central nervous system (CNS) invasion [5-10]. Moreover, peripheral neurological signs have been linked with COVID-19 [11–13]. The spike (S) glycoprotein Naked,2,3 belongs to the refined structural organization of SARS-CoV-2. Virion tropism and endocytosis are enabled by the surface binding of SARS-CoV-2 to angiotensin converting enzyme 2 (ACE2) receptors, which are upregulated in some host cells [14–18]. The pathophysiology of COVID-19 neurological complications is associated with several pathways [7]. In the endosome, the SARS-CoV-2 spike (S) glycoprotein is cleaved by transmembrane protease serine 2 (TMPRSS2), furin, and cathe-sins B and L (Cat B and L), which enable SARS-CoV-2 to bind to ACE2 and mediate SARS-CoV-2 cell entry [10]. The nasal olfactory epithelium, a likely alternative site with increased receptor binding by SARS-CoV-2, and the olfactory nerve terminals and their peripheral nervous system are the entry points of the virus into the nervous system. Bilinska and co-workers showed ACE2 and TMPRSS2 expression in sustentacular cells of the olfactory epithelium, demonstrating that this sort of cells can resemble SARS-CoV-2 entry and anosmia [19]). Moreover, a new theory that reports an alternative potential transsynaptic route from nose through respiratory epithelium to the brain through the branch of the trigeminal nerve, but remains poorly proven was introduced [20]. Finally, retrograde dissemination through transsynatic passage was suggested as an alternative mechanism. This is achieved by internalisation or externalisation and a speedy axonal transport mechanism of vesicular transportation to transfer virus retroaxonally back to the cell bodies of neurons through microtubules [21]. There is also a lot of controversy on the concept of cytokine storm disorders, and whether some conditions, including COVID-19, shall be considered part of this spectrum [22]; nonetheless, the cytokine storm due to COVID-19 leads to thrombosis and coagulopathy [23]. In addition, SARS-CoV-2 may interact with toll-like receptors, and hence, interleukin (IL)-1 could be synthesized and released [24-25]. When activated, a cascade of biochemical reactions is initiated, involving caspase-1 to cleave pro-IL-1 and inflammasome activation. The aim is to ascertain whether the novel corona virus increased the risk of recurrent (old) episodes or deterioration of well-tolerated preexisting neuropathic pain in persons with spinal cord injuries.

AIM

The aim of this study is to compare the intensity of the pain, medication requirements and the evolution of symptoms of neuropathic pain patients with or without COVID-19 infection and to determine whether the severity of the infection influenced these variables.

MATERIALS AND METHODS

STUDY DESIGN

Type of study: Observational retrospective cohort study

Duration: 10 months (from November 2020 to September 2021)

Setting: Outpatient clinics and community health centers

Number of participants: 400

INCLUSION CRITERIA (GENERAL)

Adults aged 18-65 years [26-27].

Suffering from chronic neuropathic pain (duration ≥3–6 months) or fulfilling formal criteria (e.g., Douleur Neuropathique 4 (DN4) ≥4, Budapest criteria for Complex Regional Pain Syndrome (CRPS))

Pain intensity at baseline: mean Numeric Rating Scale (NRS) ≥4 [28].

DEMANDS SPECIFIC TO CONDITION

Spinal cord injury severity was based on American Spinal Injury Association (ASIA) Impairment Scale (AIS) grade with complete (AIS A or B)

Diagnosis of neuropathic pain (confirmed via clinical evaluation and validated scales)

Well-tolerated pain prior to COVID-19 infection

History of confirmed COVID-19 infection (PCR or antigen positive)

EXCLUSION CRITERIA

Severe psychiatric or neurological disorders

History of substance abuse

Recent surgery or hospitalization unrelated to COVID-19

Patients currently undergoing pain management interventions

Patients with untreated or severe pre-existing neuropathic pain.

Concurrent severe medical conditions that could confound results (e.g., advanced cancer).

Inability to provide informed consent.

DATA COLLECTION

Timeline: Baseline (pre-COVID), post-COVID (3, 6, and 10 months).

ETHICAL APPROVAL

This study was reviewed and approval by ethical committee within College of Pharmacy, University of Alkafeel.

METHODS

SURVEYS AND QUESTIONNAIRES

Brief Pain Inventory (BPI) for pain severity

Neuropathic Pain Symptoms Inventory (NPSI)

SF-36 Quality of Life Questionnaire

Clinical Assessments: In-person assessments conducted at baseline and follow-up visits.

DATA ANALYSIS

Data is presented as Means ± SEM. GraphPad Prism 9.3.1 was used for data analysis and visualization. The number of observations (n) is given in each figure legend. Control and infected individuals were compared using Student's t-test. Simple frequency for demographic information. Pre- versus post-COVID comparisons were tested for mean differences or significant differences with the paired t-test and Chi-square, respectively. Statistical significance was considered at $P < 0.05$.

RESULTS

DEMOGRAPHIC AND CLINICAL FEATURES

Four hundred chronic neuropathic pain patients, 200 with positive-SARS-CoV-2 and 200 with negative-SARS-CoV-2. There was no significant between-group difference in demographic parameters, including age p=0.84, sex ratio p=0.42, BMI (p=0.22), diabetes rate (p=0.77), hypertension rate (p=0.40), previous use of pain medicine p=0.62, and disease duration of neuropathic pain p=0.53, Table 1. These results indicate that the demographic data between the two groups were similar, thus the comparisons of pain-related outcomes were meaningful.

PAIN INTENSITY BEFORE AND AFTER COVID-19 INFECTION

As can be seen in Table 2, between pre- and post-COVID-19 positive patients, pain severity was significantly higher. The average Visual Analog Scale (VAS) score was 4.6 ± 1.1 before vs. 7.7 ± 1.4 (p<0.045) after infection. This decline was most marked for vulnerable subgroups like women (increase from 4.7 to 8.2, p<0.01) and patients with diabetes (increase from 4.6 to 8.1, p<0.001). By contrast, subjects in the COVID-19 negative group showed no significant change in pain intensity over the follow-up period (p=0.42 overall) and small and non-significant increases in pain were apparent in female subjects and those with diabetes. These findings suggest that there may be a formal relationship between COVID-19 and the worsening of neuropathic pain.

PAIN KILLER POST-COVID-19

A significant proportion of patients recruited in the COVID-19 + cohort required escalation in their pain management after the infection, as shown in Table 3. More specifically, 72% of them needed higher doses of medication and 65% had new medications added to their treatment. In comparison, only 15% and 12% of those negative for COVID-19 reported these changes, respectively (both p<0.001), highlighting an enhanced clinical burden among the post-COVID group.

PAIN COURSE AND SYMPTOM WORSENING

Figure 1 demonstrates that pain trajectory over time which is clearly separated between the two group and COVID-19 positive case experienced a sharp rise on pain severity. A higher percentage of patients in the COVID-19 positive group reported worsening of symptoms related to neuropathic pain when compared to the control group as depicted in Figure 2.

ASSOCIATION OF COVID-19 SEVERITY WITH PAIN WORSENING

Analysis depicted in Table 4 revealed that COVID-19 severity was positively associated with worsened pain. Among patients with mild COVID-19, VAS score increased by only a small amount (1.2 ± 0.6) and 25% of patients reported worsening of symptoms p=0.01. The increase in pain was significantly higher in moderate and severe ill patients (2.8 ± 1.2 and 4.3 ± 1.5, respectively), which was reported %65 and %85 as a symptom worsening p<0.001. This gradient implies a direct association between the degree of COVID-19 severity and worsening of neuropathic pain.

DISCUSSION

The current investigation's results are strong evidence for the fact that neuropathic pain perceptions highly escalate among COVID-19 positive patients, dealing with chronic pain. Of interest, the COVID-19 positive and negative group were comparable in demographic and clinical

Table 1. Demographic and clinical characteristics of the study population

Characteristic	COVID-19 positive	COVID-19 negative	p-value
Age (years) (mean ± SEM)	47.3 ± 3.4	45.98 2.3	0.84
Gender (% female)	48%	49%	0.42
BMI (mean ± SD) (kg/m^2)	26.7 ± 2.3	25.8 ± 2.5	0.22
Diabetes (% with diabetes)	41%	39%	0.77
Hypertension (% with hypertension)	52%	47%	0.40
Pre-COVID-19 pain medications (%)	86%	88%	0.62
Duration of pain (months)	17.9 ± 7.3	18.87± 2.9	0.53

Source: compiled by the authors of this study

Table 2. Pain severity before and after COVID-19 diagnosis (mean ± SEM)

Subgroup	Baseline VAS	Follow-up VAS	p-value
COVID-19 positive patients			
Overall	4.6 ± 1.1	7.7± 1.4	<0.045
Female patients	4.7 ± 1.2	8.2 ± 1.3	<0.01
Patients with diabetes	4.6 ± 1.4	8.1 ± 1.5	<0.001
COVID-19 negative patients			
Overall	4.2 ± 0.98	4.3 ± 1.1	0.42
Female patients	4.3 ± 1.1	4.8 ± 1.7	0.51
Diabetic patients	4.5 ± 1.3	4.6 ± 1.05	0.38

Source: compiled by the authors of this study

Table 3: Changes in pain medication usage post-COVID-19

Intervention	COVID-19 positive	COVID-19 negative	p-value
Increase in dosage (%)	72%	15%	<0.001
Addition of new medication (%)	65%	12%	<0.001

Source: compiled by the authors of this study

Table 4. Correlation between COVID-19 Severity and Pain Worsening

COVID-19 severity	Mean increase in VAS score	Reporting worsening symptoms [%]	p-value
Mild	1.2 ± 0.6	25%	0.01
Moderate	2.8 ± 1.2	65%	<0.001
Severe	4.3 ± 1.5	85%	<0.001

Source: compiled by the authors of this study

characteristics (i.e. age, sex, BMI, and co-morbidities), thus these variables are unlikely to confound the effects of pain outcomes on pain. This tends to support the speculation that COVID-19 infection per se may be involves in the modulation of neuropathic pain intensity. The most striking finding is the increase in the severity of pain in the post-infection phase in the COVID-19 positive group. The mean VAS score changed from 4.6 to 7.7 ($p<0.045$) and females and diabetic patients were especially affected. These subgroup discrepancies are consistent with prior literature arguing that females and participants suffering from metabolic comorbidities, such as diabetes, are more susceptible to processes that amplify pain [1, 29-30]. The lack of correlation between pain variation and COVID-19 negative implies a direct relationship between COVID-19 and the severity of neuropathic pain. The higher demand of escalating doses of pain medication in COVID-19 positive patients highlights more severe clinical status. Since 72% of such patients required increased medication dosages and 65% required additional medications, this also indicates that COVID-19 may impact the intensity and refractory characteristics of neuropathic pain. These findings are in line with the evidence of post-viremic pain syndromes and of neuroinflammatory pathways in response to systemic infections [31-32]. Crucially, the time profile for pain trajectory and percentage of patients reporting worsening symptoms show that COVID-19 patients are also at risk of new or progressive symptoms in

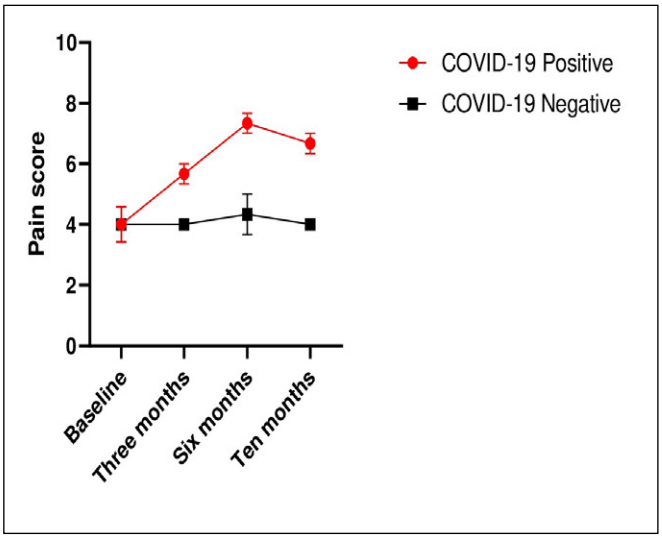

Fig. 1. Pain severity over time (COVID-19 positive vs. negative groups)
Source: Own materials

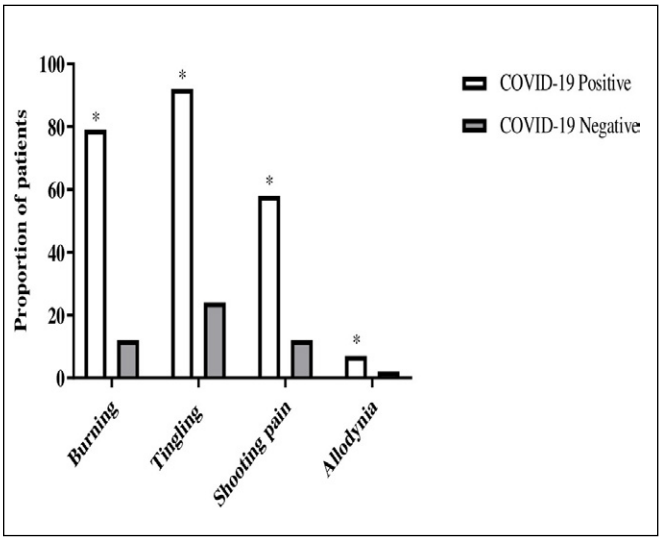

Fig. 2. Proportion of patients reporting exacerbation of neuropathic pain symptoms (n=200, *= significantly different compared to control, t-test)
Source: Own materials

addition to acute pain. The increasing severity of COVID-19 and the stronger pain exacerbation according to the dose-dependent relationship strengthen the pathophysiological relevance, as the more severe the infection the more severe the increase in the pain scores, that is, the worsening of the symptoms. This gradient indicates that mechanisms involving systemic inflammation, cytokine release, and neuroimmune activation might be the basis of the observed effects [32]. It has been reported by a comprehensive systematic review that COVID patients begin to experience neuropathic pain within weeks, and in some cases, months of an infection and that patients with neuropathic pain and COVID often have a worsening of neurologic complications and pain. Joshi and colleagues aimed in their systematic review to study neuropathic pain in both during and post-COVID-19 infection [33]. They presented data about patients who developed neuropathy or neuropathic pain in and around the disease after COVID. Data was extracted and synthesized qualitatively from 939 articles. They made the conclusion that there are neurologic associations with COVID-19 and other coronaviruses, however, new onset of neuropathic pain may be a consequence of subclinical inflammation. Levels of serum IL-6 and intercellular adhesion molecule-1 (ICAM-1) were significantly correlated with painful DSPN (after controlling for age and sex), while not with CRP, Radloff8, TNF-α, adiponectin and IL-1 receptor antagonist [34]. Furthermore, the associations remained significant, even after adjustment for height, waist circumference, blood pressure, cholesterol, smoking, alcohol intake, physical activity, history of previous MI and/or CVA (which was derived by combining the question with similar questions concerning cerebrovascular accident and CVA), history of other neurological diseases, and nonsteroidal anti-inflammatory drug use. Alternative pathogenetic mechanism of the postinfection peripheral neuropathic pain also was suggested. Oaklander and colleagues who studied people with long-term COVID reported that following moderate SARS-CoV-2 infection, the most common, the longest lasting, and disabling small-fiber neuropathy started within 30 days of COVID-19 onset. Many lines of evidence suggest that immune dysregulation is a common sequel to infections [35]. Among 17 tested persons, 59% had at least one test result that favored neuropathy. It consisted of 50% 4/ 8 of autonomic function tests, 17 % 2/ 12 of electrodiagnostic testing, and 63 % 10/ 16 of skin biopsies. Twenty-one days after moderate COVID, one patient was diagnosed with critical illness axonal neuropathy, another with multifocal demyelinating neuropathy, and ten or more with small-fiber neuropathy. No patients had complete recovery of their disease, and the mean follow-up improvement was 52%. Of treated patients, 65% (11/17) had been treated with immunotherapy (corticosteroids, intravenous immunoglobulin (IVIG) or both). In another review article by Attal and co-workers, the most typical viral infections that have been connected to neurological sequelae were reported [36]. These included enteroviruses, poliovirus, HIV, herpes zosters, and a few tropical viruses. the neurological symptoms of COVID-19, specifically myelitis, stroke, and Guillain-Barré syndrome. To precisely quantify the probability of neuropathic pain after COVID-19, longitudinal patient cohorts are necessary. Given the significance of COVID-19's neurological side effects, it is advised that patients who experience neuropathic pain for the first time within a few weeks or months or who do not report worsening neurological symptoms or discomfort do so. When combined, these findings add to the body of information indicating that COVID-19 may be a contributing or precipitating factor for neuropathic

pain, particularly in groups that are already at risk. There are also relevant clinical implications to be made in light of these findings, stressing the relevance of tight pain management in post-COVID subjects and further investigations of the implicated underlying neuroinflammatory pathways. Future research must integrate mechanistic understanding (immunologic, neurovirology, epigenetic) with the best in therapeutics (pharmacologic, neuromodulator, cellular) and trial design.

CONCLUSIONS

SARS-CoV-2 infection is an aetiologic factor that independently leads to a substantial worsening of chronic neuropathic pain, an increase in analgesic needs, and a more severe symptom burden. The impact is most pronounced among women, diabetic patients and those with severe COVID-19. These results emphasize the importance of proactive pain management approaches and longitudinal tracking in this population.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Healthcare providers' proficiency, challenges, and attitudes toward genetic testing integration in psychiatric practices: The influence of an educational initiative

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ABSTRACT

Aim: This study examines the impact of an educational initiative on healthcare providers' proficiency and attitudes toward genetic testing.

Materials and Methods: A quasi-experimental design with a single group pre/posttest was employed. Convenience sampling was used to recruit 227 participants. Four valid and reliable tools were used to assess demographic characteristics, knowledge, attitudes, and challenges related to pharmacogenetic testing.

Results: Among the 227 participants, 36% were nurses, 17.7% were physicians, 17.7% were psychologists, 14.8% were pharmacists, and 5.4% were social workers. Most participants had 1--5 years of experience and lacked prior education or workshop attendance related to genetic testing. Most agreed that genetic testing is unused in psychiatric diagnosis but acknowledged its crucial role in guiding medication selection, dosage, and personalized treatment. After the educational program, significant improvements in participants' knowledge and attitudes toward genetic testing were observed.

Conclusions: The educational program effectively enhanced healthcare providers' knowledge and attitudes regarding integrating genetic testing into psychiatric practice. Substantial improvements between pre- and posttest scores indicate increased proficiency in addressing challenges related to genetic testing. The findings reveal a significant knowledge gap, as many participants lacked prior education on genetic testing during their undergraduate studies. This underscores the need for ongoing training and support. Encouraging collaboration between genetic specialists and psychiatric practitioners and ensuring adequate resources are essential for successfully implementing genetic testing in routine psychiatric care.

KEY WORDS: genetic testing, psychiatric care, personalized treatment, healthcare providers, pharmacogenetics, educational initiative, knowledge enhancement, attitudes, quasi experimental design, clinical practice integration

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LIST OF ABBREVIATIONS

KAIMRC: King Abdullah International Medical Research Centre
IRB: International Review Board
NHG: National Guard Health Affairs
KKH: King Khalid Hospital
WHO: World Health Organization
MOH: Ministry of Health
ASD: autism spectrum disorder
HCPs: health care providers

INTRODUCTION

Traditional psychiatric treatment usually focuses on symptom control with psychotropic drugs, which may overlook root causes and produce inconsistent results. These restrictions emphasize the necessity for more personalized therapy approaches. Genetic components are crucial in numerous psychiatric conditions, making genetic testing a valuable resource for tailoring treatment, reducing negative medication effects, and enhancing clinical results. Nonetheless, the incorporation of genetic testing into psychiatric practice is still restricted because of issues like a lack of pharmacogenomic understanding, ethical dilemmas, and doubts about its clinical usefulness.

Progress in genomics has hastened the implementation of precision medicine in various medical fields, such as psychiatry. Genetic testing has proven useful in detecting variants associated with disorders like schizophrenia, bipolar disorder, and depression, facilitating more precise diagnoses and customized treatments. For example, pharmacogenomic information can inform medication choices and dosage, improving treatment

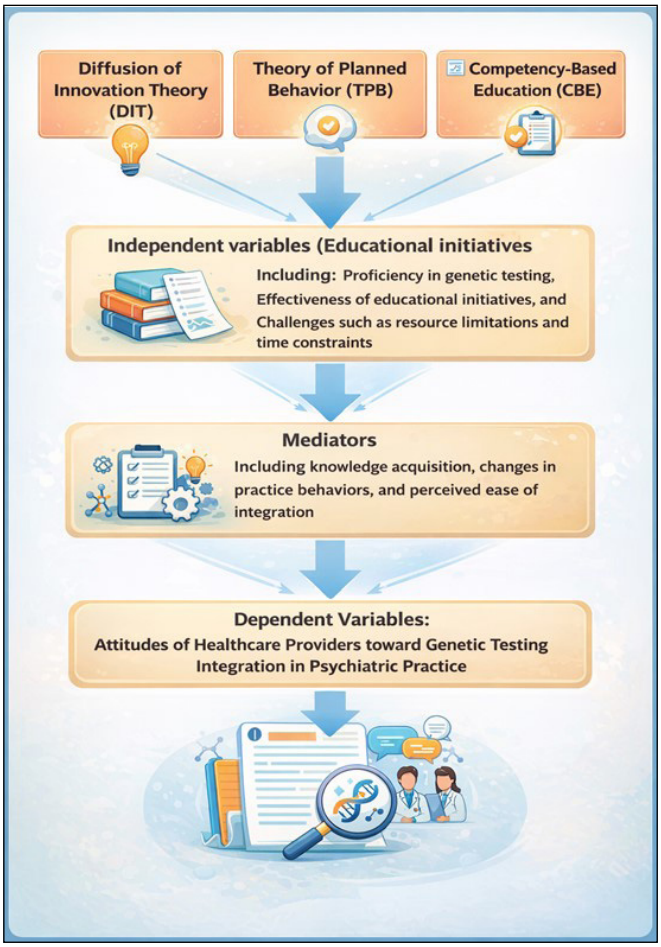

Fig. 1. The theoretical framework interconnections for genetic testing integration Diffusion of Innovation Theory (DIT): Examine the perception and acceptance of genetic testing among healthcare professionals. Theory of Planned Behavior (TPB): Examine how attitudes, social norms, and perceived control affect the adoption of genetic testing. Competency-Based Education (CBE): Stress the importance of specific skills and knowledge acquisition
Source: compiled by the authors of this study

outcomes for individuals with major depressive disorder. However, the successful utilization of these tools relies on healthcare providers' capacity to interpret and implement genetic information a proficiency that is still inadequately developed in numerous psychiatric environments [4–7].

Aside from pharmacotherapy, genetic testing can aid in evaluating the risk for neurodevelopmental and hereditary psychiatric disorders like Huntington's disease, fragile X syndrome, and autism spectrum disorder [5, 8, 9]. Although it holds promise, its execution is obstructed by ethical and logistical challenges such as privacy issues, complexities surrounding informed consent, and a lack of trained personnel [10–13]. These challenges are further complicated by ongoing knowledge deficiencies among healthcare providers, especially in psychiatric settings.

In Saudi Arabia, current pharmacogenomic studies have primarily emphasized pharmacists, showing reduced clinical confidence stemming from insufficient training. Nonetheless, there is limited knowledge regarding how psychiatric healthcare professionals view and utilize genetic testing in their practice. Gaining insight into their existing skills, perceived obstacles, and viewpoints is crucial for creating effective implementation strategies [6,16].

This study explores the baseline proficiency, challenges, and attitudes of psychiatric healthcare providers regarding genetic testing integration. It also evaluates the impact of a targeted educational initiative aimed at enhancing their competence in this area. The findings are intended to inform policy and practice by identifying actionable gaps and supporting the development of precision psychiatry in the region.

THEORETICAL FRAMEWORK

The conceptual structure combines the diffusion of innovation theory (DIT), the theory of planned behavior (TPB), and competency-based education (CBE) to investigate the implementation of genetic testing in psychiatric settings. DIT elucidates how medical professionals view and incorporate genetic testing as an innovative practice, considering factors such as competence, obstacles, and perspectives [17]. The TPB investigates how attitudes, social influences, and perceived control over behavior shape practitioners' intentions and actions regarding genetic testing [18]. CBE focuses on acquiring the specific knowledge and abilities essential for implementing this practice and informing the creation of educational programs [19]. These theoretical approaches

collectively guide the development of interventions to address identified obstacles, with educational initiatives serving as the primary means of enhancing healthcare providers' expertise. Intermediary factors, including knowledge gain, alterations in practice behaviors, and perceived ease of incorporation, influence the relationship between educational programs and the outcome variable, which encompasses providers' attitudes and practices. The figure (1) of the framework illustrates how these components interact to promote the adoption of genetic testing, enhance patient outcomes, and facilitate more methodical integration of genetic tools in psychiatric care.

The relationships between these theories collectively guide educational initiatives designed to enhance understanding and implementation. Intermediary factors such as knowledge gain and behavioral shifts facilitate the impact of educational programs on outcomes. The outcome variables (e.g., perspectives and behaviors) are directly affected by intermediary factors and indirectly shaped by theoretical frameworks.

SIGNIFICANCE OF THE STUDY

This study holds significant importance within mental healthcare. It contributes to integrating genetic testing into psychiatric practice by providing insights into healthcare providers' competence and attitudes. The research can inform targeted educational initiatives to enhance diagnostic and treatment strategies. As personalized medicine gains prominence in mental healthcare, this study aligns with utilizing genomics in psychiatry, making it relevant to evolving psychiatric practice. By examining providers' challenges and attitudes toward genetic testing, this study can impact patient outcomes, enhance recovery, and improve psychiatric care quality. It emphasizes educational interventions to bridge proficiency gaps and foster innovation in psychiatric genomics. Furthermore, this study may be the first in Saudi Arabia to target healthcare providers working in the Erada complex for mental health and addiction; thus, the findings will contribute to psychiatric practice and broader understanding of genetics in healthcare.

ETHICAL APPROVAL

The study received ethical approval from the CON-J research committee, KAIMARC (IRB No. SP23J/167/11), and the Ministry of Health. Approval was also obtained from the Erada Complex for Mental Health and Addiction in Jeddah. Informed consent was secured from all participants, who were given unique passcodes to ensure data privacy. Participants were assured of confidentiality, with data access limited to the PhD researcher and coauthors and were informed of their right to withdraw at any time. All data were securely stored within MNGHA premises in compliance with privacy policies.

AIM

This study aimed to investigate the impact of an educational initiative on enhancing health care providers' proficiency and attitudes in relation to incorporating genetic testing in dealing with psychiatric patients.

RESEARCH QUESTIONS

RQ 1≠ What are the relationships among healthcare providers' knowledge, attitudes, and the barriers they face in integrating genetic testing in psychiatric care?
RQ2≠ Is there a relationship between healthcare providers' demographic characteristics (professional role, years of experience, or specific training in genetics) and their attitudes, knowledge, and clinical practices regarding the integration of genetic testing in psychiatric patient care?

MATERIALS AND METHODS

RESEARCH DESIGN

This study used a quasi-experimental research design with a single group (pre/post) to meet its objectives. We gathered data in pretest and posttest phases and compared results (as discussed in "Tutorial on Integration of Genetic Testing in Psychiatric Practice" [20]). To mitigate possible confounding variables, we gathered demographic and professional information (such as years of experience and knowledge of genetic testing) to evaluate their impact. When necessary, these variables were statistically adjusted to isolate the effects of the educational program. The assumptions underlying statistical analyses were examined. The Shapiro-Wilk test assessed data normality, and Levene's test checked for homogeneity of variance. For assumption violations, we used nonparametric tests or bootstrapping methods to ensure reliable results.

RESEARCH SETTING

This study was conducted at the Erada Complex for Addiction and Mental Health in Jeddah, Saudi Arabia. The complex consists of six facilities including male patients, psychiatric wards for female patients, outpatient departments, and emergency departments. Located in

the Al-Mahjar neighborhood in southern Jeddah, it is the only psychiatric hospital in the city, providing comprehensive services for psychiatric patients and families, including emergency, outpatient, and inpatient care.

RESEARCH SUBJECTS

Healthcare professionals working in Erada for Mental Health and Addiction were approached for data collection. This included physicians, social workers, and psychiatric nurses at the Psychiatric Disorders Hospital in Jeddah. The hospital has 264 healthcare providers: 247 nurses, 60 doctors, and 20 social workers. Convenience sampling selected participants who met the criteria: individuals aged 20 years and older, with at least two years of patient experience, who were willing to participate.

SAMPLE SIZE

The Rao software program (www.reosoft.com) was used to calculate the sample, with a minimum sample size of 178 out of 330 healthcare providers working in the selected setting at 5%. The margin of error is the amount of error that can be tolerated, and the 95% confidence interval is the amount of uncertainty that can be tolerated. A higher confidence level would require a larger sample size. In terms of the numbers selected above, the sample size n and margin of error E are given by:

$x = Z(c/100)2r(100-r)$

$n = N\,x/((N-1)\,E2 + x)$

$E = Sqrt\,[(N - n)\,x/n(N-1)]$

SAMPLING TECHNIQUE

Convenience sampling was used to gain insight into the level of efficacy of genetic testing in psychiatric practice among health providers in psychiatric hospitals. This method facilitates the inclusion of health providers who are easily accessible considering their difficult work schedules and limited availability. This approach was adopted to ensure that the data collection was practical and effective. A total of 227 healthcare providers were recruited from the Erada Complex for Addiction and Mental Illnesses: 36% nurses, 17.7% physicians, 17.7% psychologists, 14.8% pharmacists, and 5.4% social workers.

STUDY TOOLS (DATA-COLLECTION INSTRUMENTS)

Four valid tools were used to achieve the study objectives:

1. **The first section** concerned demographic characteristics, consisting of participants' age, sex, profession, years of experience, level of education, and questions regarding any training in genetic testing usage in psychiatric practice.
2. **Knowledge scale:** Participants used a scale developed by Khalil A., the principal investigator of the current study, on the basis of an extensive literature review. A knowledge scale was developed to assess the knowledge of healthcare professionals (HCPs) regarding genetic testing.

The initial tool, consisting of 40 statements, was distributed to a panel of nursing experts for feedback. The experts evaluated each statement for relevance, clarity, and comprehensiveness. On the basis of their feedback, the tool was refined to 30 statements. This process enhanced the content validity of the tool. Construct validity was addressed through expert review and refinement. Although detailed statistical analysis for construct validity was not performed at this stage, the rigorous expert review process provided a strong basis for the tool's construct validity.

Reliability of the scale: Translation and back-translation were performed to ensure accuracy because the participants' primary language was Arabic. The Arabic version was then reviewed by a panel of nursing experts, who provided feedback for refinement. To assess the reliability of the tool, a pilot study was conducted with a sample of healthcare providers. The tool demonstrated good internal consistency, with a Cronbach's alpha coefficient of .87.3."

The final version of the reviewed valid and reliable scale consists of 30 statements covering various aspects of genetic testing, including its overview, advantages, disadvantages, types, patient benefits, challenges, ethical considerations, and communication of results to patients and families. Responses were rated on a 3-point Likert scale: "agree" (2), "do not know" (0), and "disagree" (1). Eight statements (1, 18, 21, 22, 23, 24, 25, and 26) were reverse scored. The total score ranges from 30--90 and is categorized into low knowledge (30-50), moderate knowledge (51-70), and good knowledge (71-90).

3. **Attitudes scale:** The scale consists of 15 statements aimed at assessing participants' attitudes toward pharmacogenomics and its implications. The first seven questions were adapted from Elewa et al. [21], and the subsequent eight questions were adapted from Jessel et al. (2022). The participants responded on a 3-point Likert scale: "agree" (2), "neutral" (0), and "disagree" (1).

• Scoring: The scale assesses both positive and negative attitudes toward pharmacogenomics. Higher scores indicate more positive attitudes, whereas lower scores indicate more negative attitudes.

• Positive Attitudes: Statements indicating positive views on pharmacogenomics were scored as "agree" (2), "neutral" (0), or "disagree" (1).

Table 1. Educational workshop sessions on genetic testing integration

Day	Session	Topic	Key Content
Day 1	1	Introduction to Genetic Testing in Psychiatry	Relevance, history, and current integration in psychiatric care
	2	Fundamentals of Genetic Testing	Basic genetics, types of tests, and interpretation of results
	3	Ethical and Legal Considerations	Ethical principles, informed consent, data privacy, and legal frameworks
	4	Challenges and Barriers	Common barriers, provider challenges, and case-based discussions
Day 2	5	Clinical Decision-Making	Using genetic data in treatment planning and clinical decision-making tools
	6	Interactive Workshops	Group case analysis, role-play on patient-provider interactions
	7	Patient Communication and Counseling	Strategies for discussing genetic testing and addressing patient concerns
	8	Integrating Genetic Testing into Practice	Best practices, implementation strategies, and support resources
	9	Feedback and Discussion	Participant reflections, posttest evaluation, and program wrap-up

Source: compiled by the authors of this study

• Negative Attitudes: Statements indicating negative views were reversed as "agree" (1), "neutral" (0), or "disagree" (2).

Validity and Reliability: The reliability of the translated attitude scale was tested in a pilot study, resulting in a Cronbach's alpha of .926, reflecting high internal consistency and reliability.

4. **Challenges faced by health care providers (HCPs):** Developed by Jessel et al. [22], this scale includes eight statements addressing challenges associated with implementing genetic testing in psychiatry, such as lack of clinical evidence, reimbursement issues, inadequate training, and potential benefits. The respondents rated their responses on a 3-point Likert scale: "agree" (2), "neutral" (0), and "disagree" (1).

o Score interpretation: A higher average score indicates stronger agreement with the statement, and a lower average score indicates stronger disagreement.

o Validity and reliability: The reliability of the translated challenge scale was tested in a pilot study, resulting in a Cronbach's alpha of .781, reflecting good internal consistency and reliability.

o Overall reliability of all scales: The reliability of the total scale, including all the translated scales, was examined in the pilot study, resulting in a Cronbach's alpha of .934.

DATA COLLECTION PROCESS

The data collection process received approval after submission to KAIMRC, IRB. The study was conducted at Erada for the Addiction and Mental Health Complex in Jeddah, Saudi Arabia, with MOH permission. Hospital managers of nurses, pharmacists, social workers, psychologists, and psychiatrists were contacted to inform participants about the study's purpose and methodology. Participants were assigned unique code numbers for pretest and posttest. The participants were divided into five groups, and each group was contacted separately. Six sessions of the program were completed with specific groups. The training was conducted by researchers and the principal investigator (PI) with co-author support, in both in-person and virtual settings. Nurses participated in two in-person sessions, while social workers, psychologists, pharmacists, and psychiatrists engaged in four online meetings via Microsoft Teams. Each session included three hours for surveys and program content presentation. The program aims to promote the integration of genetic testing into psychiatric care. Through developing competencies, addressing challenges, and shaping attitudes among healthcare providers, this initiative aims to increase precision in mental health care. The educational initiative is interactive and practical to maximize impact on participants' proficiency toward integrating genetic testing into psychiatric practice, while promoting a collaborative learning environment tailored to the audience's expertise level.

Educational program components and guidelines

Table 1 presents a two-day workshop which was developed for psychiatrists, psychologists, nurses, social workers, and pharmacists at the Eradah Complex for Psychiatry and Addiction. The aim was to enhance participants' knowledge, attitudes, and confidence in integrating genetic testing into psychiatric care. The program combined lectures, case discussions, and interactive activities covering theoretical and practical aspects of genetic testing.

The sessions emphasized ethical conduct, practical utility, and interdisciplinary collaboration. Participants were encouraged to apply the knowledge gained in their clinical practice and were provided with resources for continued learning.

Table 2. Distribution of the studied participants according to their demographic characteristics (N=227)

Variables	n=227	%
Gender		
• Female	122	53.7
• Male	105	46.3
Age (years) Mean ±SD	31.7±7.90	
Marital status		
• Single	107	47.1
• Married	108	47.6
• Divorced	12	5.3
Profession		
• Nurse	81	35.7
• Physician	53	23.3
• Pharmacist	31	13.7
• Psychologist	39	17.2
• Social worker	11	4.8
• Others	12	5.3
Experience		
• No experience	32	14.1
• Less than 1 year	3	1.3
• 1 year to 5 years	96	42.3
• 6 years to 10 years	35	15.4
• 11 years to 15 years	35	15.4
• More than 15 years	26	11.5
Education level		
• Undergraduate	54	23.8
• Graduate	136	59.9
• Master	21	9.3
• PhD	16	7.0
Did you study anything related to genome testing in your undergraduate years		
• No	185	81.5
• Yes	42	18.5
If yes, which level (n=43)		
• High school	1	2.3
• University	40	93.0
• Others	2	4.7
Did you attend any workshop regarding genetics		
• No	213	93.8
• Yes	14	6.2
If yes, where(n=13)		
• University	6	46.2
• Workplace	2	15.4
• Others	5	38.5
What is the source of information you learned about genetic testing		
• Internet	121	53.3
• TV	12	5.3
• Conference	18	7.9
• Others	76	33.5
Do you have any extra credentials postgraduate study apart from your bachelor's degree		
• No	213	93.8
• Yes	14	6.2
If yes, please specify(n=14)		
• Graduate	4	28.6
• Master	5	35.7
• Board	5	35.7

Source: compiled by the authors of this study

Table 3. Distribution of the participants according to their pre-post knowledge of the application of pharmacogenetics testing in the psychiatric field (N=227)

Knowledge statements		PRE		POST	
		n=227	%	n=277	%
KQ11. Genetic testing is not commonly used as a direct diagnostic tool for psychiatric disorders.	Disagree	67	29.5	50	22.0
	I do not know	49	21.6	7	3.1
	Agree	111	48.9	170	74.9
KQ2. Genetic testing is commonly used in autism spectrum disorder and schizophrenia diagnostics	Disagree	47	20.7	39	17.2
	I do not know	57	25.1	15	6.6
	Agree	123	54.2	173	76.2
KQ3 Genetic testing plays a crucial role in guiding medication selection, dosage decisions, and addressing factors related to potential treatment resistance	Disagree	41	18.1	19	8.4
	I do not know	50	22.0	8	3.5
	Agree	136	59.9	200	88.1
KQ4 Genetic testing assists in personalized treatment plan development, predicting medication response and side effects	Disagree	32	14.1	23	10.1
	I do not know	49	21.6	11	4.8
	Agree	146	64.3	193	85.0
KQ5 Minor genetic variations can significantly impact an individual's response to antidepressant medications.	Disagree	41	18.1	22	9.7
	I do not know	57	25.1	14	6.2
	Agree	129	56.8	191	84.1
KQ6 Genetic testing can both pinpoint specific genetic causes of psychiatric disorders and assess the risk of developing certain psychiatric conditions.	Disagree	28	12.3	24	10.6
	I do not know	50	22.0	7	3.1
	Agree	149	65.6	196	86.3
KQ7 In the context of genetic testing in psychiatric practice, it is essential to adhere to ethical guidelines, encompassing informed consent, privacy, and results communication.	Disagree	29	12.8	12	5.3
	I do not know	37	16.3	7	3.1
	Agree	161	70.9	208	91.6
KQ8 Genetic testing in psychiatric practice should ensure patient autonomy and prevent discrimination.	Disagree	31	13.7	22	9.7
	I do not know	51	22.5	10	4.4
	Agree	145	63.9	195	85.9
KQ9 Common genetic tests in psychiatric practice include DNA sequencing and SNP analysis	Disagree	30	13.2	17	7.5
	I do not know	82	36.1	19	8.4
	Agree	115	50.7	191	84.1
KQ10 Interpretation of genetic test results in psychiatry considers the family history of psychiatric disorders	Disagree	26	11.5	17	7.5
	I do not know	44	19.4	14	6.2
	Agree	157	69.2	196	86.3
KQ11 Challenges in psychiatric genetic testing involve high costs and limited access to genetic counselors	Disagree	28	12.3	21	9.3
	I do not know	64	28.2	11	4.8
	Agree	135	59.5	195	85.9
KQ12 Confidence in interpreting genetic test results can vary	Disagree	48	21.1	39	17.2
	I do not know	84	37.0	23	10.1
	Agree	95	41.9	165	72.7
KQ13 Whole Exome Sequencing (WES) is frequently used for diagnosing and assessing the risk of mental disorders	Disagree	33	14.5	27	11.9
	I do not know	106	46.7	29	12.8
	Agree	88	38.8	171	75.3
KQ14 Genome-Wide Association Study (GWAS) is a common tool for assessing genetic predisposition to bipolar disorder and schizophrenia.	Disagree	24	10.6	16	7.0
	I do not know	100	44.1	32	14.1
	Agree	103	45.4	179	78.9
KQ15 Next-Generation Sequencing (NGS) is commonly used to analyze genetic variations associated with psychiatric disorders	Disagree	26	11.5	29	12.8
	I do not know	101	44.5	28	12.3
	Agree	100	44.1	170	74.9

Table 3. cont.

KQ16 A limitation of genetic testing in psychiatric practice is the potential to raise false hopes and anxiety in patients	Disagree	38	16.7	40	17.6
	I do not know	69	30.4	26	11.5
	Agree	120	52.9	161	70.9
KQ17 Respecting patient autonomy and securing informed consent is crucial when conveying genetic test results to psychiatric patients	Disagree	25	11.0	25	11.0
	I do not know	43	18.9	9	4.0
	Agree	159	70.0	193	85.0
KQ18 Genetic test results should not be disclosed to family members of psychiatric patients	Disagree	68	30.0	84	37.0
	I do not know	45	19.8	19	8.4
	Agree	114	50.2	124	54.6
KQ19 Healthcare providers should ideally communicate genetic test results to psychiatric patients with clear, understandable explanations, counseling, and support	Disagree	31	13.7	23	10.1
	I do not know	42	18.5	7	3.1
	Agree	154	67.8	197	86.8
KQ20 An ethical approach involves clear communication of negative genetic test results indicating an elevated risk for severe mental disorders, along with available support options	Disagree	35	15.4	25	11.0
	I do not know	51	22.5	10	4.4
	Agree	141	62.1	192	84.6
KQ21 Genetic factors do not always contribute to treatment resistance in individuals with psychiatric disorders	Disagree	70	30.8	108	47.6
	I do not know	58	25.6	17	7.5
	Agree	99	43.6	102	44.9
KQ22 Identifying genetic factors does not always lead to alternative treatment options for individuals with psychiatric disorders	Disagree	65	28.6	105	46.3
	I do not know	67	29.5	19	8.4
	Agree	95	41.9	103	45.4
KQ23 Genetic testing cannot predict with absolute certainty the development of psychiatric disorders in asymptomatic individuals	Disagree	52	22.9	98	43.2
	I do not know	62	27.3	13	5.7
	Agree	113	49.8	116	51.1
KQ24 Early intervention or preventative measures are not always recommended solely based on genetic testing	Disagree	73	32.2	106	46.7
	I do not know	56	24.7	16	7.0
	Agree	98	43.2	105	46.3
KQ25 Genetic information alone cannot provide a complete and definitive subtype of psychiatric disorders	Disagree	43	18.9	91	40.1
	I do not know	58	25.6	15	6.6
	Agree	126	55.5	121	53.3
KQ26 The development of targeted therapies based on genetic information is not guaranteed and may not always result in effective treatments	Disagree	55	24.2	91	40.1
	I do not know	60	26.4	21	9.3
	Agree	112	49.3	115	50.7
KQ27 Genetic research can lead to the identification of drug targets or pathways involved in psychiatric disorders	Disagree	25	11.0	26	11.5
	I do not know	64	28.2	14	6.2
	Agree	138	60.8	187	82.4
KQ28 Clinical trials can be influenced by genetic information, but treatments are not always tested on a uniform population	Disagree	33	14.5	23	10.1
	I do not know	68	30.0	21	9.3
	Agree	126	55.5	183	80.6
KQ29 Tailoring antidepressant treatments based on genetic profiles can lead to improved outcomes by reducing trial-and-error prescribing	Disagree	26	11.5	33	14.5
	I do not know	64	28.2	15	6.6
	Agree	137	60.4	179	78.9
KQ30 Genetic testing in children and adolescents should be considered, especially when dealing with treatment-resistant conditions and when dosing guidelines are available for specific medications	Disagree	30	13.2	29	12.8
	I do not know	59	26.0	15	6.6
	Agree	138	60.8	183	80.6

Source: compiled by the authors of this study

Table 4. Distribution of the participants according to their attitudes toward the application of pharmacogenetic testing in the psychiatric field (N=227)

Statements		PRE		POST	
		n=227	[%]	n=277	[%]
AQ1 It would be important for me as a health care professional to identify medications that require pharmacogenetic testing	Disagree	13	5.7	9	4.0
	I do not know	87	38.3	69	30.4
	Agree	127	55.9	149	65.6
AQ2 I believe that a patient's genetic variation may influence his/her response to drug therapy in terms of efficacy and safety	Disagree	13	5.7	4	1.8
	I do not know	84	37.0	59	26.0
	Agree	130	57.3	164	72.2
AQ3 It is my responsibility to apply pharmacogenetic testing to drug therapy selection, dosing, and monitoring	Disagree	25	11.0	11	4.8
	I do not know	96	42.3	56	24.7
	Agree	106	46.7	160	70.5
AQ4 It is my responsibility to counsel the patients on the results of their pharmacogenetic testing	Disagree	20	8.8	7	3.1
	I do not know	80	35.2	53	23.3
	Agree	127	55.9	167	73.6
AQ5 Pharmacogenetic testing will improve our ability to effectively control drug therapy expenditures	Disagree	16	7.0	2	.9
	I do not know	72	31.7	50	22.0
	Agree	139	61.2	175	77.1
AQ6 It is my responsibility to make the patients aware of relevant pharmacogenetic tests for their prescribed medication.	Disagree	18	7.9	5	2.2
	I do not know	71	31.3	50	22.0
	Agree	138	60.8	172	75.8
AQ7 I am interested as a health care professional to become involved in pharmacogenetic testing training sessions/workshops	Disagree	17	7.5	9	4.0
	I do not know	65	28.6	50	22.0
	Agree	145	63.9	168	74.0
AQ8 In children and adolescents, pharmacogenetics testing improves the efficacy of psychotropic medications	Disagree	15	6.6	2	.9
	I do not know	76	33.5	51	22.5
	Agree	136	59.9	174	76.7
AQ9 In children and adolescents, pharmacogenetic testing reduces the risk for adverse drug reactions related to psychotropic medications	Disagree	17	7.5	8	3.5
	I do not know	82	36.1	53	23.3
	Agree	128	56.4	166	73.1
AQ10 In children and adolescents, pharmacogenetics testing is clinically useful	Disagree	10	4.4	5	2.2
	I do not know	89	39.2	56	24.7
	Agree	128	56.4	166	73.1
AQ11 In children and adolescents, pharmacogenetics testing should be used to inform psychotropic medication selection	Disagree	18	7.9	4	1.8
	I do not know	84	37.0	47	20.7
	Agree	125	55.1	176	77.5
AQ12 In children and adolescents, pharmacogenetics testing should be used to inform psychotropic medication dosing	Disagree	17	7.5	4	1.8
	I do not know	95	41.9	41	18.1
	Agree	115	50.7	182	80.2
AQ13 In children and adolescents, pharmacogenetics testing should be used to inform psychotropic medication switching	Disagree	16	7.0	3	1.3
	I do not know	93	41.0	48	21.1
	Agree	118	52.0	176	77.5
AQ14 In children and adolescents, pharmacogenetics testing should be used to inform psychotropic medication augmentation	Disagree	16	7.0	3	1.3
	I do not know	93	41.0	47	20.7
	Agree	118	52.0	177	78.0
AQ15 In children and adolescents, pharmacogenetics testing should be used to inform psychotropic medication deprescribing	Disagree	16	7.0	5	2.2
	I do not know	94	41.4	54	23.8
	Agree	117	51.5	168	74.0

Source: compiled by the authors of this study

Table 5. Distribution of the participants in overcoming challenges related to the application of pharmacogenetic testing in the psychiatric field (N=227)

Statements		PRE		POST	
		n=227	[%]	n=277	[%]
CQ1 There is not enough clinical evidence for me to use pharmacogenetics testing in my practice	Disagree	25	11.0	35	15.4
	I do not know	106	46.7	79	34.8
	Agree	96	42.3	113	49.8
CQ2 Lack of reimbursement for pharmacogenetics testing is a barrier for use in my practice	Disagree	14	6.2	3	1.3
	I do not know	96	42.3	59	26.0
	Agree	117	51.5	165	72.7
CQ3 If I wanted to order a pharmacogenetics test, I could identify an appropriate laboratory to perform the testing	Disagree	52	22.9	58	25.6
	I do not know	80	35.2	64	28.2
	Agree	95	41.9	105	46.3
CQ4 I have the necessary training to interpret pharmacogenetics testing results	Disagree	74	32.6	69	30.4
	I do not know	79	34.8	60	26.4
	Agree	74	32.6	98	43.2
CQ5 I have access to experts that can assist me in interpreting or implementing pharmacogenetics-testing results	Disagree	56	24.7	52	22.9
	I do not know	89	39.2	67	29.5
	Agree	82	36.1	108	47.6
CQ6 It takes too long to get pharmacogenetics testing results for it to be clinically useful in my practice	Disagree	19	8.4	34	15.0
	I do not know	104	45.8	58	25.6
	Agree	104	45.8	135	59.5
CQ7 Pharmacogenetics testing results would assist me in discussing psychotropic medication options with my patients	Disagree	16	7.0	8	3.5
	I do not know	91	40.1	46	20.3
	Agree	120	52.9	173	76.2
CQ8 Clinical practice guidelines for the use of pharmacogenetics testing in children and adolescents would be helpful to me	Disagree	16	7.0	4	1.8
	I do not know	83	36.6	48	21.1
	Agree	128	56.4	175	77.1

Source: compiled by the authors of this study

DATA MANAGEMENT AND STATISTICAL ANALYSIS

The collected data were coded and analyzed via the latest version of the statistical software SPSS. Descriptive statistics such as frequencies, percentages, means, and standard deviations were calculated to summarize the data. Independent sample t tests were performed to compare the item means of knowledge evaluation between the pre- and post-program among the study participants. Additionally, paired t tests were conducted to analyze the changes in total knowledge, attitudes, and challenge scores within the participants' responses from the pretest to the posttest. Statistical tests appropriate for assessing the associations between the participants' sociodemographic variables and the study variables were employed, with a significance level set at $p < 0.05$.

RESULTS

A total of 227 healthcare providers participated in the survey. Table 2 provides demographic variables of participants. Most participants were female (74.9%) or nurses (35.7%). The mean age was 31.7 years (SD=7.90). Most participants were graduates (59.9%) with a master's degree (9.3%) or PhD (7.0%). The majority had 1--5 years of experience (42.3%), followed by 6--10 years (15.4%) and 11--15 years (15.4%). Few had no experience (14.1%) or less than 1 year (1.3%). When asked about genome testing studies during undergraduate years, 81.5% answered "No," while 18.5% responded "Yes," mostly studying it at the university level (93.0%). Regarding genetics workshops, 93.8% responded "No," with 6.2% answering "Yes," mainly attending at university (46.2%). Information about genetic testing came primarily from the internet (53.3%), conferences (7.9%), and other sources (33.5%). Most participants (93.8%) had no additional postgraduate credentials beyond their bachelor's degree, while 6.2% had additional degrees, mainly graduate (28.6%) or master's (35.7%).

Table 3 highlights significant improvements in healthcare providers' knowledge about genetic testing in psychiatric practice following an educational interven-

tion. The most notable gain was in understanding the role of genetic testing in guiding medication selection, dosage, and addressing treatment resistance (KQ3), with agreement rising from 59.9% to 88.1%. Another substantial improvement was observed in awareness that genetic testing is not commonly used as a direct diagnostic tool for psychiatric disorders (KQ11), where agreement increased from 48.9% to 74.9%.

However, some knowledge areas showed only slight changes. The belief that genetic test results should not be disclosed to family members rose modestly from 50.2% to 54.6%. Awareness that identifying genetic factors does not always lead to alternative treatment options increased slightly from 41.9% to 45.4%. The belief in the ability to predict psychiatric disorders in asymptomatic individuals using genetic testing changed minimally from 49.8% to 51.1%. Understanding that early intervention or prevention should not be solely based on genetic testing increased marginally from 43.2% to 46.3%. Notably, recognition that genetic information alone cannot provide a complete subtype of psychiatric disorders slightly declined from 55.5% to 53.3%, and awareness that targeted therapies based on genetic information are not guaranteed to be effective rose only slightly from 49.3% to 50.7%.

Before the intervention, Table 4 highlights negative attitudes toward pharmacogenetic testing, with 42.3% unsure about its application and 35.2% unsure about counseling patients. Post-intervention, attitudes shifted dramatically, with agreement increasing to 70.5% for testing and 73.6% for counseling. Additionally, belief in pharmacogenetic testing's role in controlling drug therapy costs rose from 61.2% to 77.1%, and its perceived efficacy and safety in psychotropic medications for children and adolescents increased from 59.9% to 76.7%.

In Table 5, the findings highlight the main challenges healthcare providers face when implementing pharmacogenetic testing in psychiatric settings. The primary obstacle identified was the perceived lack of reimbursement for testing, noted by 51.5% of participants (CQ2). Additionally, 46.7% expressed uncertainty about the clinical evidence for pharmacogenetics (CQ1), and 39.2% lacked access to experts for result interpretation (CQ5). After the intervention, improvements were seen in perceptions of clinical evidence (agreeing increased from 42.3% to 49.8%) and access to experts (from 36.1% to 47.6%). However, challenges like finding appropriate laboratories (CQ3) and delays in receiving results (CQ6) persist, indicating that issues related to reimbursement and timeliness continue to hinder the integration of pharmacogenetics in psychiatric practice, highlighting the need for targeted interventions.

Table 6 presents the Wilcoxon test results, indicating significant improvements in all evaluated parameters (knowledge, attitudes, and challenges) between pre- and postintervention assessments, with p values less than 0.001. Knowledge increased, with median values changing from 72.0 to 76.0 and a mean rank shift from 116.45 to 97.48, reflecting a medium effect size ($r = -0.401$). Attitude also improved, with median values rising from 38.0 to 42.0 and mean ranks declining from 117.49 to 76.32 (medium effect size, $r = -0.458$). Additionally, the challenge variable showed progress, with median values increasing from 18.0 to 20.0 and mean ranks shifting from 109.70 to 92.54 (small effect size, $r = -0.253$).

Figure 2 compares mean values of knowledge, attitude, and challenge before and after an intervention. Knowledge increased from 71.3 (preintervention) to 76.6 (postintervention), attitudes rose from 37.3 to 40.8, and the Challenge Overcoming domain improved from 18.4 to 19.6. These increases are statistically significant ($p<0.001$), indicating the intervention effectively enhanced knowledge, attitudes, and obstacle management.

Table 7 indicates that the Mann–Whitney test revealed significant differences in knowledge, attitudes, and perceived challenges based on gender, undergraduate genome study experience, and workshop participation, while postgraduate qualifications had minimal impact. Men had notably higher knowledge scores both pre- and post-intervention ($p = 0.021$, $p = 0.005$) and perceived greater challenges ($p = 0.016$, $p = 0.046$). Individuals with undergraduate genome study experience showed higher initial knowledge ($p = 0.017$), but this difference disappeared after the intervention ($p = 0.683$). Workshop attendance positively influenced preintervention attitudes ($p = 0.030$), though this effect faded postintervention ($p = 0.214$). Overall, gender, prior genome study experience, and workshop attendance were key factors in knowledge and attitudes, while postgraduate qualifications showed little relevance.

Table 8 summarizes the mean values for knowledge, attitudes, and overcoming challenges before and after the intervention, categorized by education level and experience. While experience and knowledge showed no significant differences pre- ($p = 0.103$) or post-intervention ($p = 0.141$), most groups had modest gains. Attitude changes were significant before the intervention ($p = 0.001$) but not afterward ($p = 0.155$). Challenge overcoming approached significance before the intervention ($p = 0.059$) but showed no significant changes after ($p = 0.781$). Education level saw higher post-intervention means, though knowledge did not significantly change pre- ($p = 0.276$) or post-intervention ($p = 0.175$). Significant attitude differences were noted before the intervention ($p = 0.009$) but not after ($p = 0.643$). Challenge overcoming showed no changes from pre- ($p = 0.266$) to post-intervention ($p = 0.736$).

Table 6. Wilcoxon test (pre-post comparisons) between scores on knowledge, attitudes, and perceived challenges among participants (N=227)

Variable Pair	N	Mean Rank (Pre)	Median (Pre)	Mean Rank (Post)	Median (Post)	p value	r
Knowledge	151	116.45	72.0	97.48	76.0	<0.001	-0.401
Attitude	136	117.49	38.0	76.32	42.0	<0.001	-0.458
Challenge	125	109.70	18.0	92.54	20.0	<0.001	-0.253

r: Effect size (small effect size 0.2, medium effect size 0.5, large effect size 0.8)
Source: compiled by the authors of this study

Table 7. Mann–Whitney test of gender; type of education; workshop attendance; and knowledge, attitudes, and challenges regarding the use of genetics testing in the psychiatric field (N=227)

Variable	Metric	Group 1	N1	Mean Rank1	Median1	Group 2	N2	Mean Rank2	Median2	p value
Gender	Pre Knowledge	Female	122	104.65	70.5	Male	105	124.87	73.0	0.021
	Post Knowledge	Female	122	102.67	76.0	Male	105	127.17	78.0	0.005
	Pre Attitude	Female	122	108.52	38.0	Male	105	120.36	39.0	0.174
	Post Attitude	Female	122	109.07	41.0	Male	105	119.73	42.0	0.213
	Pre Challenge	Female	122	104.30	17.0	Male	105	125.27	19.0	0.016
	Post Challenge	Female	122	105.97	19.5	Male	105	123.33	21.0	0.046
Undergrad Genome Study	Pre Knowledge	No	185	109.03	72.0	Yes	42	135.90	75.0	0.017
	Post Knowledge	No	185	114.85	76.0	Yes	42	110.27	76.0	0.683
	Pre Attitude	No	185	110.64	38.0	Yes	42	128.79	39.0	0.105
	Post Attitude	No	185	117.14	42.0	Yes	42	100.15	41.0	0.122
	Pre Challenge	No	185	117.14	19.0	Yes	42	100.19	17.0	0.128
	Post Challenge	No	185	112.33	20.0	Yes	42	121.37	20.0	0.418
Workshop Attendance	Pre Knowledge	No	213	112.42	72.0	Yes	14	138.04	77.5	0.157
	Post Knowledge	No	213	113.89	76.0	Yes	14	115.68	76.5	0.921
	Pre Attitude	No	213	111.59	38.0	Yes	14	150.71	42.5	0.030
	Post Attitude	No	213	115.36	42.0	Yes	14	93.32	40.5	0.214
	Pre Challenge	No	213	112.14	18.0	Yes	14	142.36	21.0	0.093
	Post Challenge	No	213	111.98	20.0	Yes	14	144.71	22.0	0.069
Postgrad Credentials	Pre Knowledge	No	213	113.45	72.0	Yes	14	122.36	74.5	0.623
	Post Knowledge	No	213	113.53	76.0	Yes	14	121.21	76.0	0.670
	Pre Attitude	No	213	115.36	38.0	Yes	14	93.29	35.0	0.221
	Post Attitude	No	213	114.31	42.0	Yes	14	109.36	40.5	0.780
	Pre Challenge	No	213	114.74	18.0	Yes	14	102.68	17.0	0.502
	Post Challenge	No	213	113.70	20.0	Yes	14	118.50	20.5	0.790

Source: compiled by the authors of this study

ANOVA

Table 9 shows the statistical analysis for varying predictive impacts of participant attributes on pre- and postintervention outcomes. For preintervention measures, older age positively correlated with initial knowledge (B=0.345, p=0.013) and attitudes (B=0.218, p=0.011). In contrast, greater experience negatively influenced pre knowledge (B=-1.662, p=0.020), pre attitude (B=-1.329, p=0.003), and pre challenge (B=-0.536, p=0.023). Gender significantly predicted pre attitude (B=1.886, p=0.048) and pre challenge (B=1.354, p=0.008), while marital status predicted lower pre attitude scores (B=-1.894, p=0.030). Postinter-

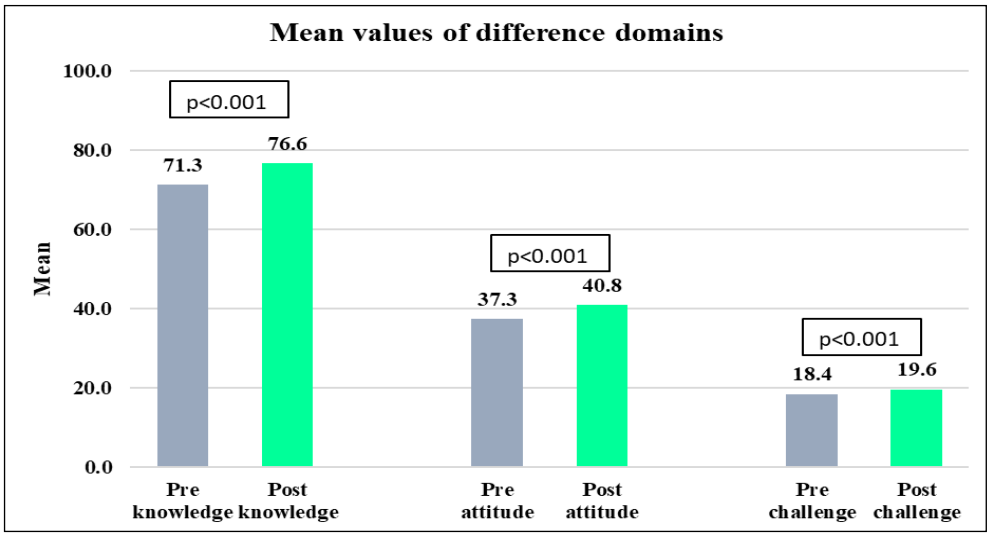

Fig. 2. Comparison between knowledge, attitudes, and challenges in pre- and postintervention assessment programs, N=227
Source: compiled by the authors of this study

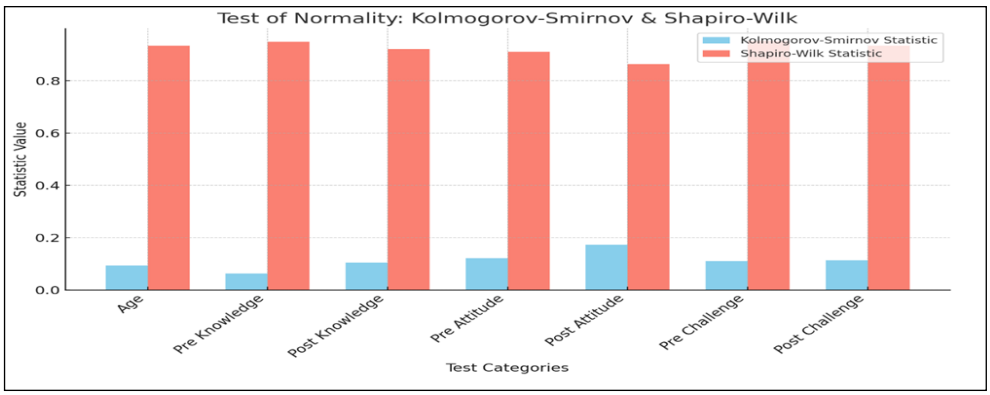

Fig. 3. Test of normality: Kolmogorov–Smirnov & Shapiro–Wilk tests
Source: compiled by the authors of this study

vention models showed no statistical significance ($p > 0.05$) and accounted for minimal variance (R^2: 1.8–3.1%). Age and gender trends were generally positive, with experience reducing preintervention scores. Preintervention models explained 6–9% of the variance, indicating limited predictive value. Notably, gender approached significance for pre knowledge (p=0.063) and post challenge (p=0.076), suggesting potential trends for further exploration. In summary, preexisting characteristics weakly predict baseline measures but do not effectively explain postintervention outcomes.

Figure 3 displays The Kolmogorov-Smirnov (KS) statistic ranges from 0.063 (pre knowledge) to 0.174 (post attitude), indicating the greatest deviation from normality at post attitude. The Shapiro–Wilk (SW) statistics vary from 0.864 (Post Attitude) to 0.952 (Pre challenge), with lower values indicating greater departures from normality. Both Post Attitude (0.864) and Post Knowledge (0.920) show significant nonnormality, while Pre challenge (0.952) is closest to normal distribution. All p values for the KS and SW tests are ≤ 0.05, indicating a non-normal distribution at the 5% significance level. Therefore, nonparametric tests like the Mann–Whitney U test and Wilcoxon signed-rank test may be more appropriate than parametric tests like t tests or ANOVA.

DISCUSSION

This research explored the obstacles to integrating genetic testing into psychiatric practice and assessed the impact of a specific educational intervention on healthcare providers in Saudi Arabia. As one of the first studies in this field, it showed notable enhancements in genomic knowledge and moderate changes in attitudes, consistent with earlier studies by Meiser et al., Bauer et al., and Quinn et al., which indicated that education enhances clinical confidence in utilizing genetic testing [23–25]. Nevertheless, persistent issues - such as genetic determinism, difficulties in data interpretation, and ethical challenges - continue to hinder adoption. These results align with those of Wright et al. and Byres et al., who found that increased knowledge does not always translate into attitudinal change [26–27]. Wondrasek et al. [28] emphasized that existing beliefs and perceived relevance significantly affect receptivity. The intervention led to a slight decrease in perceived barriers (mean difference = 1.17, $p < 0.001$, $r = -0.253$), suggesting that while knowledge improved, participants became more aware of systemic and ethical complexities. This paradox - enhanced literacy paired with increased recognition of limitations—has been observed in similar studies. Ethical concerns included

Table 8. Comparison between healthcare providers in relation to knowledge, attitudes, and challenges pre- and postintervention (N=227).

			N	M±SD	95% CI		p
Profession							
Preknowledge	Nurse	81	68.9±11.36	66.35	71.38	0.060
	Physician	53	72.7±7.45	70.66	74.77	
	Pharmacist	31	74.9±13.81	69.84	79.97	
	Psychologist	39	70.9±10.08	67.60	74.14	
	Social worker	11	75.2±8.32	69.59	80.77	
	Others	12	69.8±8.94	64.15	75.52	
Postknowledge	Nurse	81	76.3±7.22	74.74	77.93	0.381
	Physician	53	75.9±9.77	73.25	78.64	
	Pharmacist	31	78.8±6.86	76.32	81.35	
	Psychologist	39	75.5±5.03	73.91	77.17	
	Social worker	11	79.5±9.59	73.02	85.89	
	Others	12	76.5±8.42	71.15	81.85	
Preattitude	Nurse	81	35.9±7.40	34.23	37.50	0.063
	Physician	53	38.1±4.04	36.94	39.17	
	Pharmacist	31	38.7±7.74	35.90	41.58	
	Psychologist	39	37.3±6.64	35.13	39.43	
	Social worker	11	41.2±5.56	37.44	44.92	
	Others	12	35.8±5.89	32.09	39.58	
Postattitude	Nurse	81	40.1±4.84	39.05	41.19	0.537
	Physician	53	41.0±4.02	39.91	42.13	
	Pharmacist	31	41.8±3.95	40.39	43.29	
	Psychologist	39	41.1±3.80	39.87	42.34	
	Social worker	11	41.2±5.46	37.52	44.85	
	Other	12	41.0±4.29	38.28	43.72	
Prechallenge	Nurse	81	17.7±3.73	16.87	18.52	0.158
	Physician	53	18.8±2.75	18.07	19.59	
	Pharmacist	31	19.4±3.96	17.97	20.87	
	Psychologist	39	18.3±3.45	17.14	19.38	
	Social worker	11	19.5±3.36	17.20	21.71	
	Others	12	18.1±3.29	15.99	20.17	
Postchallenge	Nurse	81	19.0±3.67	18.21	19.84	0.091
	Physician	53	20.0±3.37	19.11	20.97	
	Pharmacist	31	19.4±3.37	18.15	20.62	
	Psychologist	39	19.4±3.18	18.35	20.42	
	Social worker	11	22.2±3.12	20.08	24.28	
	Others	12	19.8±3.19	17.72	21.78	

Source: compiled by the authors of this study

privacy protection, informed consent, and psychological impact, as outlined in international frameworks like GINA [29] Practical challenges - such as cost, workflow integration, and limited access to genetic counseling - remain significant hurdles. Therefore, educational initiatives must be supported by institutional reforms to facilitate sustainable integration. Demographic analysis (Table 1) showed a predominantly young, female, nursing-based group with limited prior exposure to genomics. Most participants had not studied genetics during their undergraduate education (81.5%) or attended relevant workshops (6.2%) [30, 31]. The internet (53.3%) was the main source of information, while conference attendees achieved the highest post-inter-

Table 9. Multiple regression results for the knowledge, attitude, and challenge (pre and post) interventions

Predictor	Pre-Knowledge B (p)	Post-Knowledge B (p)	Pre-Attitude B (p)	Post-Attitude B (p)	Pre-Challenge B (p)	Post-Challenge B (p)
Gender	2.892 (0.063)	1.952 (0.090)	1.886* (0.048)	0.703 (0.282)	1.354** (0.008)	0.915 (0.076)
Age (years)	0.345* (0.013)	0.106 (0.300)	0.218* (0.011)	0.010 (0.867)	0.072 (0.116)	-0.004 (0.932)
Marital Status	-0.479 (0.734)	-0.135 (0.897)	-1.894* (0.030)	-0.113 (0.849)	-0.445 (0.339)	-0.201 (0.668)
Profession	0.535 (0.262)	0.112 (0.751)	0.355 (0.226)	0.263 (0.191)	0.128 (0.417)	0.206 (0.195)
Experience	-1.662* (0.020)	-0.195 (0.711)	-1.329** (0.003)	-0.140 (0.640)	-0.536* (0.023)	0.076 (0.748)
Education Level	-1.299 (0.154)	-0.479 (0.476)	0.457 (0.413)	-0.367 (0.338)	0.194 (0.517)	0.115 (0.703)
Model Significance	F=2.566* (p=0.02), R^2=6.5%	F=1.187 (p=0.314), R^2=3.1%	F=3.518* (p=0.02), R^2=8.8%	F=0.674 (p=0.671), R^2=1.8%	F=2.313* (p=0.029), R^2=6.1%	F=1.066 (p=0.384), R^2=2.8

* - Significant results ($p < 0.05$) are marked with an asterisk

Source: compiled by the authors of this study

vention knowledge scores (mean = 74), highlighting the importance of structured, targeted education. Notably, advanced degrees did not consistently predict better outcomes, underscoring the need for contextual, practice-oriented content [32, 33]. While knowledge gains were evident, attitudinal changes were less consistent. This discrepancy may reflect differences in intervention design or unaddressed psychological factors, as suggested by Byres et al. [34] and Hull et al. [35], who stressed that professional context - not personal demographics - determines intervention effectiveness [27, 30]. Professionally, social workers and pharmacists showed the most significant improvements, likely due to the direct relevance of pharmacogenomics to their roles [36–38]. However, social workers also reported increased perceived challenges post-intervention, possibly due to greater awareness of systemic constraints. Table 8 showed knowledge improvements across experience levels, though these were not statistically significant. This suggests that short-term interventions may be insufficient for lasting attitudinal change. Similarly, postgraduate education and conference attendance improved baseline knowledge but did not significantly influence post-intervention attitudes, reinforcing the need for interventions that address institutional and psychological dimensions [39, 40]. Multivariate regression revealed that demographic traits predicted baseline scores but not post-intervention outcomes. This indicates that successful implementation depends more on external factors such as institutional support, reflective learning environments, and interdisciplinary collaboration. Without reinforcement and follow-up, initial gains may diminish, highlighting the importance of continuous professional development and mentoring [41, 43].

In summary, while the educational initiative effectively enhanced genomic literacy, deeper systemic, ethical, and logistical challenges must be addressed to achieve meaningful integration. Future genomic education in Saudi Arabia should be tailored to diverse professional roles and supported by institutional frameworks to foster sustainable change in psychiatric practice [44, 45].

WHAT WAS ADDED BY THIS STUDY

This study on integrating genetic testing into psychiatric practice through education is both innovative and timely, offering valuable insights into personalized medicine. By evaluating healthcare providers' knowledge, attitudes, and barriers, it highlights key factors influencing adoption. The development of a reliable, expert-validated educational tool (Cronbach's alpha = .87) demonstrates methodological strength and practical relevance. The findings can guide targeted interventions and policy development, promoting wider use of genetic testing to enhance diagnostic accuracy and individualized treatment. Overall, the study provides a strong foundation for future research and advances both scientific and clinical applications in psychiatry.

LIMITATIONS

This study offers valuable insights but has several limitations affecting its generalizability. The use of convenience sampling may have introduced bias, as participants could have had a preexisting interest in genetic testing. The limited sample size and demographic scope further restrict the applicability of the findings. The short-term nature of the intervention prevents assessment of long-term impact, and reliance on self-reported data raises concerns about response bias. Additionally, findings may not be transferable to different cultural or healthcare settings. Future research should use probability sampling, include more diverse participants, and explore broader factors like institutional support and interprofessional collaboration.

CONCLUSIONS

This research demonstrated that a targeted educational program significantly improved healthcare professionals' understanding of genomics and their readiness to integrate genetic testing into psychiatric practice. The notable enhancements in knowledge and attitudes observed before and after the intervention highlight the effectiveness of structured training, particularly in environments with limited prior exposure to genomics. Two primary conclusions emerge:

1. While educational efforts are essential, they are insufficient on their own - despite the increase in knowledge, persistent challenges such as ethical concerns, systemic limitations, and inadequate institutional support remain. Addressing these issues requires coordinated policy and infrastructure reforms
2. Tailored, profession-specific training is crucial - the variations in outcomes across different fields underscore the need for education that aligns with specific clinical roles and responsibilities. These findings advocate for the development of ongoing, interdisciplinary genomic education programs to support the meaningful and ethical integration of genetic testing in psychiatric care.

RECOMMENDATIONS

- Implement regular, mandatory training for healthcare providers on genetic testing in psychiatry
- Integrate genetic testing education into undergraduate and graduate healthcare curricula.
- Encourage collaboration between genetic specialists and psychiatric professionals
- Provide sufficient resources and support systems for routine use of genetic testing
- Advocate for supportive legislation and funding to promote genetic testing in psychiatry
- Urge healthcare administrators to offer infrastructure and education for implementation
- Engage institutional and national stakeholders to support system-wide adoption of genetic testing in mental healthcare.

CLINICAL IMPLICATIONS

- Continuing education enhances nurses' proficiency in genetic testing, improving patient outcomes in psychiatry.
- Increased knowledge and positive attitudes enable better clinical decision-making and patient counseling.
- Integrating genetic testing enriches psychiatric care by incorporating genetic insights.
- Ongoing training supports nurses' professional growth and keeps them current with advancements.
- Addressing systemic barriers like cost and access to genetic counseling—through institutional and policy changes is essential for successful implementation.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Association of calcitonin gene-related peptide levels with cardiovascular disease in hypertensive male patients

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ABSTRACT

Aim: The current study aimed to investigate the association between CGRP, lipid profile, and atherogenic indices in hypertensive male patients with respect to cardiovascular risk.

Materials and Methods: Fifty-four male HTN patients and 45 healthy controls (ages 51–69) were enrolled. The subjects were divided into two subgroups according to LAP: Group 1 was HTN patients with LAP (≤50), and Group 2 was HTN patients with LAP >50. Serum CGRP (pg/mL) was measured via ELISA; lipid profiles and atherogenic indices were assessed spectrophotometrically.

Results: The mean values of WHR, VAI, LAP, SBP, DBP, atherogenic indices, and lipid profile in the HTN patients showed a statistically significant increase compared to the control, except CGRP and HDL-C, which revealed a significant decrease ($P < 0.001$). Also, the G2 displayed a decrease in CGRP compared to the G1. After adjusting for main hazard factors, the relation between CGRP and CVD remained significant in the multivariate analysis. The curve of ROC based on CGRP levels to indicate the existence of CVD in HTN patients was 0.80. Additionally, the AUC of CGRP was 0.82 in patients with a LAP greater than 50, compared to those with a LAP less than 50.

Conclusions: These findings indicate that reduced CGRP levels are significantly associated with higher CVD risk in hypertensive men, suggesting that CGRP may be explored as a potential biomarker for cardiovascular risk assessment in future longitudinal studies.

KEY WORDS: AIP, vasodilator, LAP, VAI, BF%, HTN

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INTRODUCTION

Cardiovascular disease (CVD) encompasses a wide spectrum of disorders concerning the blood vessels and heart, such as coronary artery disease, heart failure, and stroke [1]. It remains the leading cause of mortality worldwide, with marked regional variations in prevalence and mortality rates. Unhealthy lifestyle factors, such as poor dietary habits, physical inactivity, and psychological stress, are strongly associated with these diseases [2]. The World Health Organization estimates that, lifestyle modification could prevent more than 75% of CVD-related deaths [3]. Hypertension (HTN) is a main risk contributing to the progression of CVD, leading to severe complications such as left ventricular hypertrophy (LVH), heart failure, and arrhythmias caused by impaired diastolic filling [4].

Understanding this link is essential for designing effective prevention and management strategies. Recent research has highlighted the complex role of neuropeptides in cardiovascular regulation. A new, secure, and useful index to represent excessive cerebral lipid accumulation is being proposed by Lipid Accumulation Product (LAP), which depends on a combination of two straightforward and cost-effective measurements: waist circumference (WC) and triglyceride concentration. This index was later referred to as the LAP. In women, LAP is closely associated with different metabolic and cardiovascular risk factors. Previous investigations performed in various populations of both male and female have revealed that LAP displays a high predictive value of other indicators such as BMI, WHR, WHtR, and visceral adiposity index (VAI), in diagnosing metabolic syndrome [5].

The multifunctional peptide Calcitonin gene-related peptide (CGRP) plays a pivotal role in a wide range of physiological processes. It exists in two primary isoforms, α-CGRP and β-CGRP, in sensory neurons; they are widely expressed and are also present in neuroendocrine cells and motor neurons [6]. The effects of CGRP are mediated through G protein-coupled receptors (GPCRs) that are in complex with receptor activity-mod-

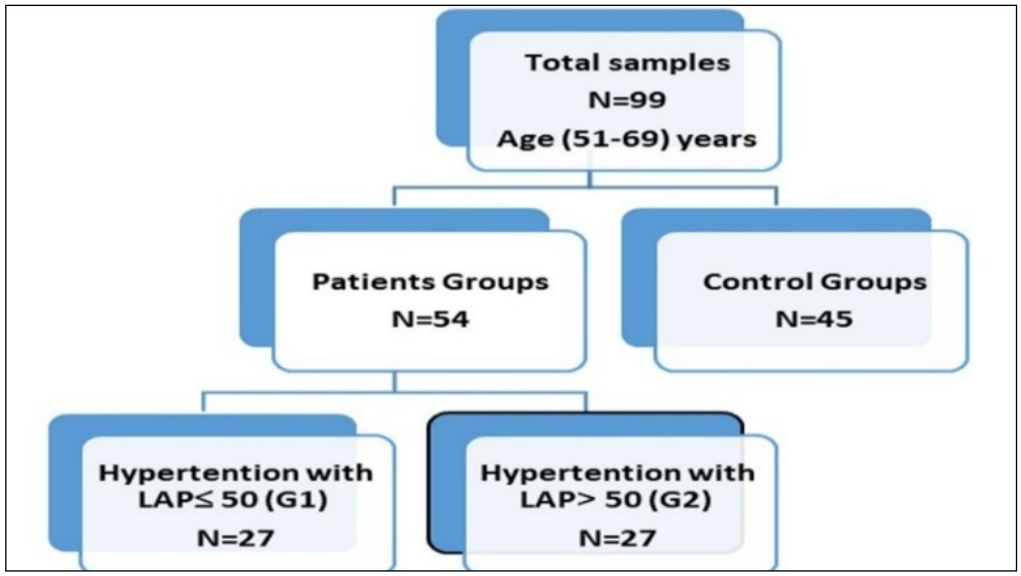

Fig. 1 Study design flowchart of participant groups
Source: compiled by the authors of this study

ifying proteins (RAMPs). The receptor of CGRP and receptor of Amylin 1 (AMY1) are the most prominent, while additional receptor subtypes have been identified in rodents. Functionally, CGRP regulates multiple organ systems, including the central and peripheral nervous systems, where it mediates pain signaling and vascular tone; the cardiovascular system, as a potent vasodilator and cardioprotective agent; the respiratory and gastrointestinal systems; the hematopoietic and immune systems; and metabolic regulation in adipose and muscle tissues.

The CGRP peptide is implicated in various pathological conditions, most notably migraine, in which it contributes to peripheral vasodilation and nociceptive signaling. Therapeutically, targeting the CGRP pathway through monoclonal antibodies against CGRP or its receptor represents a benchmark bench-to-bedside success. Ongoing research into CGRP biology offers opportunities for the development of novel pharmacological interventions or combinatorial strategies targeting this pathway in diseases beyond migraine [7].

Despite extensive experimental evidence, the clinical relevance of CGRP in hypertension and its potential role as an associated biomarker for cardiovascular complications remains poorly understood. This knowledge gap highlights the need for a comprehensive investigation of CGRP in conjunction with other metabolic and vascular indices to improve risk stratification in hypertensive patients.

MATERIALS AND METHODS

Seven milliliters of venous blood were drawn from each subject after 12 hours of fasting. The blood was collected in a gel tube, allowed to clot for 10 minutes at 37 °C, and then centrifuged at 1200 × g for 15 minutes. The serum was aliquoted into labeled Eppendorf tubes and then frozen at -80 °C until analysis. A study was carried out at Al-Sadder Hospital. Fifty-four male patients with hypertension were enrolled and subdivided based on the Lipid Accumulation Product (LAP) into two groups: G1 (LAP ≤ 50) and G2 (LAP > 50). LAP values were categorized according to the thresholds reported by Milla et al. (2023) [8], based on their analysis of adults at high cardiovascular risk. This approach allowed the identification of participants with elevated LAP levels relative to population-based cardiovascular risk. It should be noted that population-specific thresholds may vary, and future studies should validate the optimal cut-off in local populations.

All participants were non-smokers, newly diagnosed hypertensive males who had not received any antihypertensive or lipid-lowering medications. Dietary habits and physical activity were carefully controlled, as all participants followed comparable lifestyle patterns. Forty-five age-matched healthy males served as the control group. Participant ages ranged from 51 to 69 years (Fig. 1). Sample collection was carried out from November 2024 to the end of March 2025. Individuals with chronic kidney disease, cardiovascular disease, diabetes mellitus, cancer, and liver disease were excluded.

Cholesterol, high-density lipoprotein (HDL-C), and triglycerides (TG) were determined using colometric methods (Biolabo, France). CGRP levels were measured using a commercially available ELISA kit (Elabscience Biotechnology Co., Ltd, EKHU-0679). The assay sensitivity was < 1 pg/mL, with intra-assay and inter-assay coefficients of variation of < 10% and < 15%, respectively, indicating acceptable reproducibility and reliability

Table 1. Comparison of Anthropometric measurements and biomarkers of the studied groups

Variables	Control n=45	G1 n=27	G2 n=27	LSD	F	df	P value
Age (Years)	61.0 ± 4.1	60.6 ± 5.1	63.01± 3.5	2.357403	2.685	2	0.073
Duration of Disease (Years)	-	4.74 ± 1.9	5.81 ± 1.7	-	-	-	-
Weight (g)	76.28 ± 6.47	75.74± 4.39	78.00± 4.86	3.001	1.253	2	0.290
BMI (kg/m^2)	25.82± 2.10	25.81 ± 1.70	26.56± 1.51	1.000	1.587	2	0.210
WHR	0.91 ± 0.01	0.93 ± 0.01	0.92 ± 0.01	0.000	17.171	2	< 0.001
BF%	39.51± 2.62	39.53± 2.55	41.06± 2.26	1.356	3.691	2	0.29
VAI	1.382 ± 0.16	3.21 ± 0.64	3.80 ± 0.68	0.271	229.401	2	< 0.001
LAP	34.21 ± 8.24	31.73 ± 5.84	58.71 ± 7.98	4.103	110.957	2	< 0.001
SBP (mmHg)	120.06± 1.54	138.77 ± 4.5	137.34 ± 6.7	2.356	209.522	2	< 0.001
DBP (mmHg)	80.87 ± 1.72	91.00 ± 3.49	91.48 ± 2.42	1.352	210.884	2	< 0.001
TG (mg/dl)	134.1 ± 5.62	177.0 ± 7.54	178.5 ± 7.38	3.611	23369.2	2	< 0.001
TC (mg/dl)	145.33 ± 7.21	156.03 ± 6.0	157.64 ± 4.7	3.412	1642.6	2	< 0.001
HDL (mg/dl)	54.22 ± 3.94	28.81 ± 5.13	28.37 ± 4.62	2.420	8062.9	2	< 0.001
LDL (mg/dl)	63.98 ± 7.79	91.82 ± 6.79	93.55 ± 5.88	3.810	10130.5	2	< 0.001
VLDL (mg/dl)	26.97 ± 1.21	35.40 ± 1.50	35.71 ± 1.47	0.742	904.8	2	< 0.001
AC	1.68 ± 0.19	4.57 ± 0.97	4.74 ± 1.00	0.400	108.948	2	< 0.001
AIP	0.394 ± 0.02	0.794 ± 0.08	0.806 ± 0.07	0.034	2.020	2	< 0.001
CRI-I	2.68 ± 0.19	5.57 ± 0.97	5.74 ± 1.00	0.400	198.258	2	< 0.001
CRI-II	1.18 ± 0.18	3.30 ± 0.74	3.43 ± 0.77	0.308	58.742	2	< 0.001
CGRP (pg/ml)	33.60 ± 5.06	30.62 ± 6.91	23.48 ± 5.07	3.042	871.1	2	< 0.001

Source: compiled by the authors of this study

of the measurements. Low-density lipoprotein (LDL-C) and very low-density lipoprotein (VLDL-C) were estimated using $LDL_C = T.C - HDL_C - VLDL\_C$ [9], $VLDL_C = \left(\frac{TG}{5}\right)$ [10] respectively.

The atherogenic index of plasma (AIP) was calculated as:

$$AIP = LOG\left(\frac{TG}{HDL_C}\right)$$ [11].

The atherogenic coefficient (AC) was determined using:

$$AC = (TC - HDL_C)/HDL\_C$$ [12].

Castelli's Risk Indexes (CRI I and CRI II), also referred to as cardiac risk indexes, were calculated using:

$$CRI_I = TC/HDL\_C,\ CRI_{II} = LDL_{\_C}/HDL\_C$$ [13]

$$VAI = \left(\frac{waist(cm)}{39.68} + (1.88 * BMI)\right) * \text{TG}\left(\frac{mmol}{L}\right)/1.03*1.31/\text{HDL}$$ [14]

$$LAP = (waist(cm) - 65) * TG\left(\frac{mmol}{L}\right)$$ [8].

Dividing the weight by height squared in meters is used to calculate body mass index (BMI) [15]. Waist-to-hip ratio (WHR) was determined [16]. Body fat percentage **,** where Gender = '1' for men and '0' for women [17].

For all statistical studies, SPSS software 27 was used. The Kolmogorov–Smirnov test was used to evaluate variable distribution. The mean and standard deviation (SD) were used to represent variables. Differences between continuous variables were examined using one-way analysis of variance (ANOVA) with a significance threshold of $p < 0.05$. Pearson's correlation coefficient was used to evaluate linear correlations between variables.

ETHICAL APPROVAL

Permission to conduct the study was given by the University of Kufa's Institutional Review Board (IRB) and the Sadr Teaching Hospital Committee of Ethics (IRB reference number 7547 R/2023). The IRB adheres to the International Guidelines for the Protection of Human Subjects of Research as mandated by the Declaration of Helsinki 2024. Prior to enrolment, each subject provided written, informed consent to take part in the trial.

RESULTS

The anthropometry and biomarker findings for the HTN and control groups are displayed in Table 1. There was non-significant difference in weight, BMI, age, and body

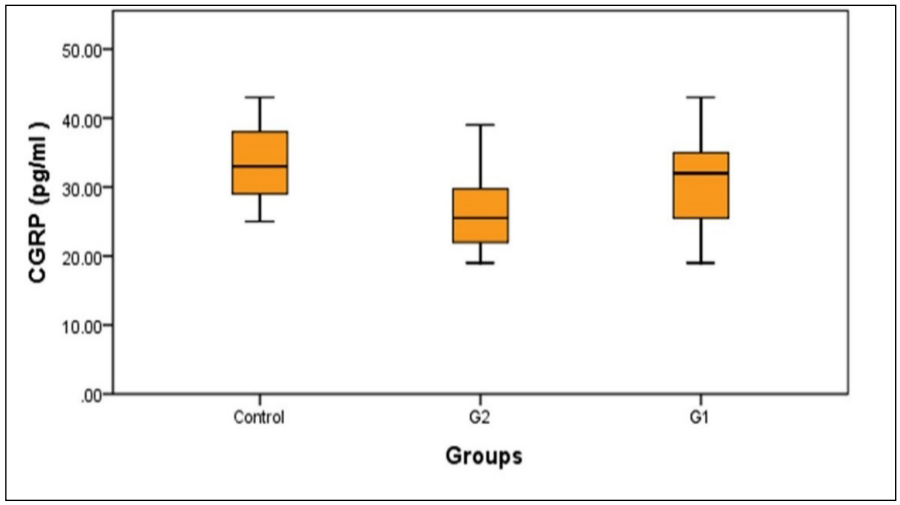

Fig. 2. Comparison of serum CGRP between HTN (G1, G2) and the control group
Source: compiled by the authors of this study

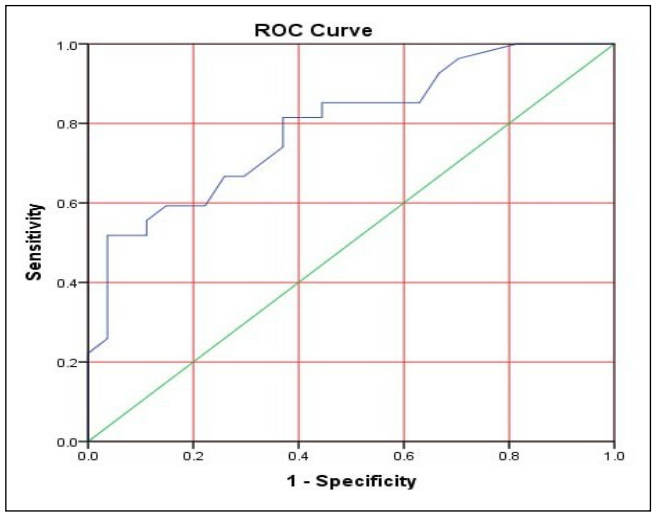

Fig. 3. ROC curve of CGRP in differentiating between control and HTN patient groups
Source: compiled by the authors of this study

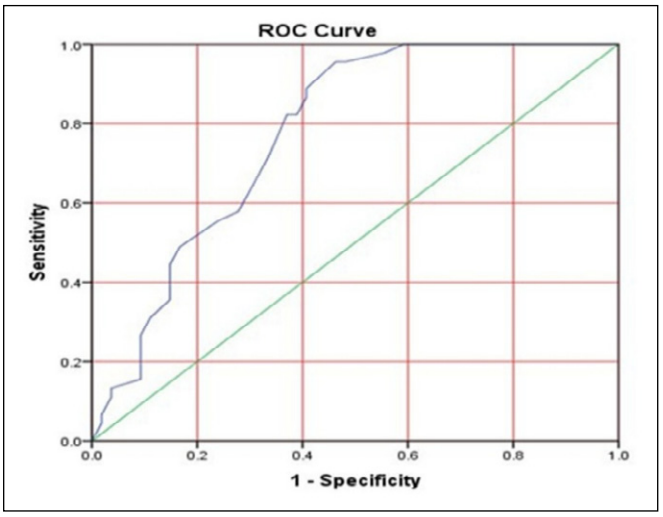

Fig. 4. Receiver operating characteristic curves of CGRP for differentiating between two groups of hypertensive (HTN) patients
Source: compiled by the authors of this study

fat percentage (BF%); whereas it found differences among the groups regarding WHR, VAI, LAP, SBP, DBP, TG, TC, HDL, LDL, VLDL, AC, AIP, CRI, and CGRP (P< 0.001). In addition to a significant difference in age, BF%, VAI, LAP, and CGRP between G1 and G2 (P< 0.001). Fig. 2 illustrates.

Figure 2 shows that an increase in CGRP concentrations occurred in the control group compared to both hypertensive groups (G1 and G2). Among the hypertensive groups, G2 exhibited the lowest CGRP levels, while G1 showed intermediate levels. This demonstrates a marked reduction of CGRP in hypertensive patients, particularly in G2 (HTN patients with LAP >50).

The correlations between the CGRP levels and the anthropometric and clinical data are presented in Table 2. The search revealed a negative relation between CGRP and LAP (r = -0.453, p = 0.001), TC (r = -0.382, p = 0.004), duration (r = -0.332, p = 0.014), and DIA (r = -0.276, p = 0.044).

Table 3 shows The incidence of CVD using a multivariable stepwise logistic regression considering the common risk variables and CGRP

The sensitivity and specificity of the detected CGRP in identifying patients with HTN from healthy people were assessed using a receiver operating characteristic (ROC), and the results were displayed in Table 4 and Figure 3

Using a receiver operating characteristic (ROC) analysis, the sensitivity and specificity of CGRP in two groups of HTN were shown in Table 5 and Fig. 4.

DISCUSSION

Patients with hypertension decrease the risk of cardiovascular diseases because of their dyslipidemia and an inflammatory state [18]. Various studies have shown how the lipid profile affects CVD development, impacted by higher concentrations of TG and TC. Because LDL-C accumulates in the artery's intima-media, it may promote thrombocytopoiesis, which is another way that elevated LDL-C might lead to atherosclerosis. On the other hand, people with higher HDL-C levels might be less likely to develop CVD [19]. The constriction and abstraction of cardiac arteries, which are

Table 2. Correlation of bivariate between serum CGRP and parameters in HTN patients

Parameters	CGRP	
	r	p-value
Age	- 0.076	0.583
Duration	- 0.332*	0.014
Weight	0.006	0.968
BMI	- 0.092	0.508
WHR	- 0.027	0.848
BF	- 0.13	0.350
VAI	- 0.032	0.018
LAP	- 0.453**	0.001
SYS	0.195	0.158
DIA	-0.276*	0.044
TC (mg/dl)	- 0.382**	0.004
LDL (mg/dl)	- 0.148	0.286
HDL (mg/dl)	- 0.215	0.119
VLDL (mg/dl)	- 0.066	0.634
TG (mg/ ml)	- 0.066	0.634
AIP	0.171	0.216
AC	0.104	0.454
CRI-I	0.104	0.454
CRI-II	0.089	0.522

* Significant correlation ($p < 0.05$), ** significant correlation ($p < 0.01$)
Source: compiled by the authors of this study

Table 3. The incidence of CVD using a multivariable stepwise logistic regression considering the common risk variables and CGRP

Parameters	B	Wald	df	P-value	Exp(B)	95% CI for EXP(B)	
						Lower	Upper
LAP	0.046	4.339	1	0.037	1.048	1.003	1.094
CGRP	-0.131	10.110	1	0.001	0.877	0.809	0.951

Source: compiled by the authors of this study

Table 4 Area under the curve (AUC) and ROC of determined CGRP for the diagnose HTN patients

Parameter	Cut-off	Sensitivity %	Specificity %	Youdin's J statistic	AUC (95 % CI)	P
CGRP	< 28.75	0.822	0.630	0.452	0.82(0.684-0.867)	<0.0001

Source: compiled by the authors of this study

Table 5. ROC and AUC of determined CGRP for HTN patients (G1 and G2)

Parameter	Cut-off	Sensitivity %	Specificity %	Youdin's J statistic	AUC (95 % CI)	P
CGRP	< 31.5	0.52	0.96	0.48	0.80(0.674-0.911)	<0.0001

Source: compiled by the authors of this study

strongly connected with the risk of CVD [20], the logarithm of (TG/HDL) ratio, often referred to as AIP, which are identified as a risk for the emergence of myocardial infarction, stroke, and CVD [21], Studies show that AC values are higher significant in patients with CAD, indicating its potential as a risk marke [22]. CRI I and II demonstrated significant relationships with CVD mortality, emphasizing their role in long-term risk assessment [23]. VAI serves as an indirect marker of adipose tissue dysfunction, which is closely linked to cardiometabolic risks, particularly in type 2 diabetes patients. Research indicates that dietary interventions, such as Gum Arabic supplementation, can significantly reduce VAI and improve blood pressure, thereby potentially mitigating CVD risk [24]. A recent study found that higher LAP levels were strongly correlated to higher mortality from CVD and all causes. Participants in the highest LAP quartile had a

55% higher risk of CVD mortality compared to those in the lowest quartile [25]. In the Isfahan Cohort Study, LAP was correlated with metabolic variables and showed a significant association with CVD incidence, although it was not independently predictive of CVD mortality in multivariate analysis [26].

According to the present study, results in Table 1 showed a significant increase in all lipid markers, consistent with previous reports, as well as an increase in WHR. Importantly, CGRP levels were significantly lower in HTN patients with LAP > 50 compared to those with LAP ≤ 50 (Table 1, Fig. 2), a novel finding since no prior research has investigated the link between CGRP and CVD in the context of hypertension based on LAP. CGRP has a complex role in cardiovascular disease, with evidence suggesting both protective and neutral effects [27, 28]. Given its vasodilatory and cardioprotective properties [29, 30], our results highlight that lower CGRP levels may reflect greater disease severity. Correlation analysis in Table 2 revealed significant negative correlations of CGRP, particularly with disease duration, suggesting that CGRP levels may decline over time in patients, which is crucial for understanding its temporal dynamics in disease contexts. Moreover, the negative correlation of CGRP with diastolic blood pressure indicates a potential role in cardiovascular regulation and blood pressure management. Additionally, its negative correlation with total cholesterol and LAP highlights its involvement in lipid metabolism, underscoring the multifaceted role of CGRP in the pathophysiology of cardiovascular disease [6]. These findings emphasize the importance of CGRP as both a biomarker and a therapeutic target in cardiovascular research. Furthermore, results from Table 3 indicate that CGRP had a stronger and more statistically significant effect than LAP, suggesting a potential protective role. Nonetheless, LAP remains an important risk factor. Together, these markers may provide complementary insights: LAP as a risk indicator and CGRP as a protective factor.

Finally, ROC findings demonstrated that CGRP has diagnostic value in identifying hypertensive patients (Table 4, Fig. 3) and showed potential in diagnosing CVD in this population based on the LAP marker, as indicated by ROC curve analysis (Table 5, Fig. 4). Serum CGRP levels may be associated with cardiovascular risk in hypertensive patients with LAP >50 and could be explored as a potential biomarker in future longitudinal studies.

A limitation of the current work is the need for longitudinal data to confirm these findings and to establish whether reduced CGRP levels can reliably serve as a biomarker for CVD in hypertensive patients.

CONCLUSIONS

Reduced CGRP levels are associated with higher cardiovascular risk in hypertensive men with LAP >50. CGRP may serve as a potential biomarker for cardiovascular risk assessment; however, longitudinal studies are required to confirm its predictive value.

LIMITATIONS

This study has several inherent limitations. The sample size was relatively small (n = 54) and included only male participants, which may reduce the generalizability of the findings. The restriction to males was intentional to minimize sex-related biological variability and to obtain a more homogeneous study population. Future studies including both sexes are recommended to evaluate possible sex-specific differences. Although CRP and other inflammatory markers were not measured, cardiovascular risk assessment was based on detailed lipid profile parameters and atherogenic indices, including the recently proposed lipid accumulation product (LAP), which has been reported in recent studies to have a strong association with cardiovascular diseases [26].

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ORIGINAL ARTICLE

CONTENTS

Molecular markers of endogenous neuroprotection in the brain of rats with experimental Parkinson's disease treated with various pharmacotherapy regimens

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ABSTRACT

Aim: To study apoptotic processes and their role in the formation of premature dopaminergic neurodegeneration, to identify key biomarkers for early diagnosis and implementation of complex measures towards braking the progression of PD in the early stages, to develop possible treatment regimens with a specific neuroprotective effect on the dopaminergic system.

Materials and Methods: The experiment involved 90 Wistar rats (6 months old, 220–290 g). Parkinsonism was induced by the neurotoxin MPTP (N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). Intact animals received saline (1 ml/100 g, i.p.); the control group received MPTP followed by saline.

Rats were divided into nine groups: I – intact; II – PD control; III – PD + Amantadine (AM); IV – PD + AM + Cerebrocurin; V – PD + AM + Pramistar; VI – PD + AM + Gliatilin; VII – PD + AM + Noophen; VIII – PD + AM + Pronoran; IX – PD + AM + Melatonin.

Results: The data obtained indicate that neuroprotective therapy of PD with drugs such as melatonin, cerebrocurin, pronoran, and gliatilin in combination with amantadine leads to an increase in the expression of the HIF-1α, HIF-3α, and HSP70 genes, and can also serve as a molecular marker for the activation of endogenous neuroprotection mechanisms under experimental PD conditions.

Conclusions: We have experimentally demonstrated a new target of neuroprotection in PD conditions – apoptosis of dopamine-producing neurons and substantiated modulators of this process – drugs for combined therapy with amantadine (melatonin, cerebrocurin, pronoran, and gliatilin) as promising drugs for PD treatment.

KEY WORDS: caspase-3, HIF-1α, HSP70, BCL-2, c-Fos, HIF-3α

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INTRODUCTION

Parkinson's disease (PD) is one of the most common neurodegenerative diseases among the elderly. Motor symptoms are primarily due to significant dopamine depletion caused by degeneration of dopaminergic neurons in the compact substantia nigra. Apoptosis, considered one of the main mechanisms of neuronal death in PD, is mediated by a number of initiator and executioner caspases, occurring either intrinsically or extrinsically. The activation of initiator caspase-9 mediates the intrinsic pathway, which is also called the mitochondrial-mediated pathway [1]. Alternatively, the activation of initiator caspase-8 mediates the extrinsic pathway of apoptosis, which is the pathway mediated by the cell death receptor. Both initiator caspases converge on a common pathway of executioner caspases that include caspase-3 and caspase-6. The activation of "caspase killers" leads to the development of morphological features characteristic of apoptosis, such as DNA cleavage and its subsequent fragmentation. Pro-apoptotic factors, i.e. Bax, are involved in neuronal cell death in PD, and there is evidence that both intrinsic and extrinsic apoptotic pathways can play a significant role in this process [2].

Neuronal death occurs during normal development and in response to a variety of pathological factors,

such as traumatic injury, ischemia, infectious agents, or genetic aberrations. The main mechanisms of neuronal death are apoptosis and necrosis. Apoptosis is the predominant mode of neuronal death in many neurodegenerative diseases, including PD. While the pathogenic processes in PD are not fully understood, convergent mechanisms result in neuronal death via apoptosis, making the apoptosis pathway an interesting potential therapeutic target. Cell death via apoptosis is observed in cell culture models of animal with PD, as well as in the nigrostriatal regions of the brains of PD patients at autopsy [3].

Apoptosis, the main pathway of programmed cell death, can be initiated by a number of broad classes of cell death stimuli, including abnormal intracellular calcium concentrations (exitotoxicity), deprivation of afferent or efferent trophic factors, activation of death receptors, and stress. Neuronal apoptosis, which is common observed during development and maturation, is essential for the formation of the nervous system and the development of appropriate neural circuits. This process involves a certain sequence of events that are energy-dependent [4]. It is characterized by specific morphological and biochemical changes, including cell contraction, chromatin compaction, nuclear DNA fragmentation, and the formation of apoptotic bodies containing nuclear material. The cell membrane retains its integrity. Apoptotic bodies are removed by phagocytosis without further inflammatory response. Biochemically, apoptosis is characterized by an increased rate of protein degradation and increased caspase activity. These biochemical components of apoptosis are a group of molecules called the B-cell lymphoma family (BCL-2), apoptotic peptidase activation factor (Apaf-1) and caspases [5].

Evidence from in vitro, in vivo and postmortem studies in humans suggests that apoptotic cell death is involved in PD pathogenesis. Identification of the main triggers of the apoptotic process in PD may contribute to a better understanding of the sequence of events leading to programmed cell death. Consequently, it would be possible to determine potential factors that could be targeted therapeutically to stop or slow down the progression of the disease as well as to recognize people who are predisposed to develop Parkinson's disease at the early and preclinical stages.

AIM

Aim of the study: to study apoptotic processes and their role in the formation of premature dopaminergic neurodegeneration, to identify key biomarkers for early diagnosis and implementation of complex measures towards braking the progression of PD at the early stages, to develop possible treatment regimens with a specific neuroprotective effect on the dopaminergic system.

MATERIALS AND METHODS

The study was conducted in accordance with Directive 2010/63EU of the European Parliament and of the Council of September 22, 2010 on the protection of animals used for scientific purposes, as well as with the national regulations, such as "General Ethical Principles for Animal Experimentation" (Ukraine, 2001) and "Basic Principles for Studying the Toxicity of Potential Pharmacological Agents" (the State Pharmacopoeia of Ukraine (SPhU), Kyiv, 2000). The experiment was approved by the Bioethics Committee of Zaporizhzhia State Medical University (ZSMU). This study is an expanded presentation and continuation of the study DOI:10.21303/2585-663.2020.001491.

Experimental groups of animals were formed after a 2-week quarantine period. The experimental groups were created based on the age and weight of the rodents. All experiments were conducted at the Educational Medical and Laboratory Center of ZSMU, certified by the Ministry of Health of Ukraine (certificate No. 039/14). The experiments were carried out in a well-lit room in complete silence. During the experiments, the influence of external and internal visual, olfactory and auditory stimuli was excluded.

The study was conducted on 90 Wistar rats aged 6 months and weighing 220-290 grams. The animals were kept in standard vivarium conditions (12-hour light cycle, temperature 22^0C). To run the experiments, the animals were subjected to food deprivation, described below in detail. In order to tame the rats, before the experiment, they were hand-handled for 2-3 minutes for 5 days, which facilitated further experimental studies.

Parkinsonism was induced by injection of the neurotoxin MFTP (N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) into experimental rats. The intact group received a single intraperitoneal (parenteral) injection of saline 1 ml per 100 g of body weight, whereas the control group received a single intraperitoneal injection of saline in a similar dosage after the administration of MFTP.

We have developed a concept for PD treatment, which is based on selective effects on the leading neurodegeneration targets: nitrosative stress, deprivation of endogenous neuroprotective factors (HSP70), disruption of the thiol-disulfide system,

mitochondrial dysfunction, inhibition of dopamine transmission, neuroapoptosis, and activation of IL-1b pathways involved in neurodegeneration. To confirm the prospects of the selected target pathways, we selected drugs whose mechanism of action impacts these particular target pathways. Cerebrocurin contains neurotrophic factors and rilin protein, reduces signs of primary and secondary mitochondrial dysfunction, regulates transcriptional processes; Noophen can increase dopamine levels, activates Roberts' compensatory shunt; Pramistar is a nootropic of the racetam class, exhibits dopamine agonist properties, increases the density of nicotinic cholinergic receptors, stimulates energy metabolism; Pronoran has the properties of a dopamine agonist; Melatonin in therapeutic doses stimulates HSP70 expression, normalizes the thiol-disulfide system, modulates the mitochondrial pore, activates the compensatory malate-aspartate shunt, regulates NO \ SN mechanisms of gene transcription; Gliatilin is a nootropic with specific mitoprotective properties and cholinomimetic effects.

The rationale for a PD therapy strategy was based on the study of drug activity in the following groups of animals: I - intact (passive control); II – animals with experimental Parkinson's disease (PD, active control); III – PD + Amantadine (AM); IV – PD + AM + Cerebrocurin; V – PD + AM + Pramistar; VI – PD + AM + Gliatilin; VII – PD + AM + Noophen; VIII – PD + AM + Pronoran; IX – PD + AM + Melatonin.

The treatment course with the investigated drugs in experimentally substantiated doses was as follows: Amantadine Sulfate – 1 mg/kg, Cerebrocurin - 150 µl/kg, Pramistar – 10 mg/kg, Gliatilin – 250 mg/kg, Noophen – 100 mg/kg, Pronoran – 2 mg/kg, Melatonin – 10 µg/kg in animals with experimental PD [4]. All of the above drugs were administered intragastrically, and Cerebrocurin was administered intraperitoneally (parenterally).

All steps in the preparation of brain homogenate were carried out in the cold at +2°C, using the Silent Crusher S homogenizer (Heidolph). The medium used for homogenization of brain tissue was characterized by neutral pH value and the required osmotic pressure which protect the particles from swelling and rupture.

The concentration of HSP70 protein in the cytoplasmic fraction of the brain was determined by Western blot analysis. The proteins were separated in a 10% polyacrylamide gel (PAGE). The separation of protein fractions was performed by electrophoresis: initially at 100 V (to allow stacking), then at 200 V after the samples reached the separating gel, continuing until the dye front reached the end of the gel.

The real-time reverse transcription polymerase chain reaction (RT-PCR) was used to analyze the expression of HSP70, HIF-1α, HIF-3α, and BCL-2 genes. The object of the study was brain homogenate. The molecular genetic study consisted of several stages.

Total RNA was isolated from rodent brain tissue using the TRIzol RNA Prep 100 kit. RNA was isolated according to the kit protocol. Reverse transcription (cDNA synthesis) was performed using the Reverse Transcription Reagent Kit (RT-1). To determine the expression level of the studied genes, the CFX96™-Real-Time PCR Detection Systems amplifier (Bio-Rad Laboratories, Inc., USA) and a set of reagents for PCR-RV in the presence of SYBR Green R-402 were used. The final reaction mixture for amplification included SYBR Green dye, SynTaq DNA polymerase with enzyme-inhibiting antibodies, forward and reverse specific primers, dNTPs, and a cDNA template. Specific primer pairs (5'-3') for the analysis of the studied and reference genes were selected by using PrimerBlast software (www.ncbi.nlm.nih.gov/tools/primer-blast) as well as manufactured by Thermo-Scientific (USA). Amplification was performed under the following conditions: initiating denaturation at 95°C for 10 min; then 50 cycles: denaturation at 95°C for 15 s, primer annealing at 58-63°C for 30 s, elongation at 72°C for 30 s. The fluorescence intensity was recorded automatically at the end of the elongation stage of each cycle using the SybrGreen channel. The beta-actin (Actb) gene was used as a reference gene to determine the relative value of changes in the expression level of the studied genes. The comparative Ct method ($_{\Delta\Delta}$Ct method) was used to quantify the relative level of gene expression. Negative controls were included in the experiment: no addition of cDNA matrix in the PCR reaction, no addition of mRNA matrix in cDNA synthesis, no addition of enzyme in cDNA synthesis. Each amplification reaction was performed in triplicate on individual samples.

The beta-actin (Actb) gene was used as a reference gene to determine the relative value of changes in the expression level of the studied genes. The comparative Ct method ($_{\Delta\Delta}$Ct method) was used to quantify the relative level of gene expression. The statistical analysis of real-time PCR data was carried out with the help of CFX Manager™ software (Bio-Rad, USA). Each amplification reaction was performed in triplicate on individual samples. The results of the real-time PCR analysis of Hif-1α, Hif-3α, and HSP70 were presented as the relative normalized expression of these mRNAs.

To detect the expression of c-Fos and VCL-2 proteins in the brain of the animals, the immunohistochemical method of indirect immunofluorescence was used

Table 1. Influence of the studied drugs on the expression of endogenous neuroprotection genes under conditions of PD formation in rats (M ± m; Q50,(Q25;Q75), n = 20)

Marker	HIF1a mRNA expression, a.u./g protein	HIF-3α mRNA expression, a.u./g protein	HSP_{70} mRNA expression, a.u./g protein	Number of Fos-positive neurons per 1 mm²	c-Fos mRNA expression, a.u./g protein
Intact	45.37±4.51	6.26±0.57	39.51±4.48	42.14±2.85	26.81±0.45
Control (PD)	33.70±2.61	1.62±0.12	30.69±2.26	89.18±6.72	48.33±0.39
PD+AM	32.11±2.52*	5.54±4.92	45.04±4.25*	82.71±8.43*	38.75±1.49
PD + Cerebrocurin + AM	32.76±2.77	9.18±7.31*	36.14±4.01*	61.32±6.19*	31.09±1.27
PD+Pramistar +AM	32.88±2.62	5.02±3.19	44.12±3.02	71.63±10.11	35.48±0.56
PD + Gliatilin +AM	32.16±2.81*	7.88±7.11*	36.65±3.56	57.21±7.83	30.15±3.09*
PD +Noophen +AM	34.41±2.95	4.98±3.23	40.26±3.53	65.37±7.02	32.50±1.22
PD +Pronoran+AM	34.07±3.07	8.83±7.65	40.36±4.72*	70.15±9.14*	32.62±0.53*
PD +Melatonin +AM	35.89±3.75*	9.49±3.41	46.97±3.48*	55.43±8.12*	28.89±4.27

Notes: p – the level of statistical significance when comparing samples using ANOVA (Kruskal-Wallis test), *–p≤0.05 relative to the control group
Source: compiled by the authors of this study

after preparation of 15 μm tissue sections. First, primary antibodies to c-Fos and VSL-2 protein (Sigma Chemical, USA) were applied to the sections and incubated at +400°C for 24 hours. After the incubation, the sections were washed three times with 0.1 M phosphate buffer. Then, the samples were coated with secondary antibodies (fluorescein-conjugated goat IgG) (Sigma Chemical, USA) and incubated at room temperature for 60 min. After the incubation, the sections were washed with 0.1 M phosphate buffer. Fos-immunopositive neurons and BCL-2-immunopositive neurons were examined using an Axioskop fluorescence microscope (Ziess, Germany). Images of Fos- and BCL-2-immunopositive neurons of the CA-1 region of the hippocampus were acquired using a fluorescence microscope and captured with a high-sensitivity video camera COHU-4922 (COHU Inc., USA). The images were then processed with the VIDAS digital image analysis system.

IL-4, TNF-α, and caspase-3 were determined by ELISA, an enzyme-linked immunosorbent assay of a sandwich type, based on the specific binding of an antibody to an antigen, with one of the components being conjugated to an enzyme. Samples and standards are incubated in microplate wells coated with antibodies up to the component to be detected. The biotinylated indicator antibody binds to the captured component which is to be detected. The unbound sample material is removed by washing. The streptavidin-peroxidase conjugate binds to the biotinylated indicator antibody. The streptavidin-peroxidase conjugate reacts with the substrate tetramethylbenzidine (TMB). As a result of the reaction with the chromogenic substrate, a colored, detectable product is formed. The enzymatic reaction is stopped by the addition of acid, and the absorbance is measured using a Sirio-S microplate immunoassay reader (seac Radim Company, Italy) at 450nm/630nm. The concentration of the components is calculated from the calibration curve based on the absorbance of known standards. IL-4 was determined using the eBioscience™ IL-4 Platinum ELISA Kit (USA) according to the instructions in the kit.

Statistical processing of the results was performed using Microsoft Excel 2016 with the statistical processing package AtteStat 12. To assess the significance of differences in the study groups, the Kruskal-Wallis test with Dunn's correction was used. Differences at $p < 0.05$ were considered significant.

RESULTS

HIFs are key regulators of neuroprotection and neuroplasticity under the conditions of nervous system pathology, in particular in PD. They stimulate reparative processes in nervous tissue, increasing the pool of free radical scavengers and angiogenesis factors. Additionally, under conditions of PD formation, HIFs increase the energy resources of the neuron, participating in glucose and key glycolysis enzymes metabolism, thereby increasing ATP synthesis. HIFs have pronounced antiapoptotic effects that lead to a decrease in neuronal death in PD.

In the control group of our study, in the rats with PD, under conditions of increased reactions of oxidative, nitrosative stress and deficiency of energy resources in brain tissues, a 25.72% decrease in the synthesis of HIF1a was observed compared to the intact group

of animals, whereas HIF3a synthesis decreased by 74.12%. These processes are associated with the activation of the ubiquitin-independent degradation pathway of oxidatively modified HIF-1α and HIF3a, and the suppression of its synthesis at the stage of translation.

The administration of Amantadine, as well as its combination with Cerebrocurin, Gliatilin, Noophen, Pronoran, and Pramistar, under conditions of PD, had almost no effect on the level of HIF1a mRNA expression compared with the control group, except for the Melatonin group, where a statistically significant increase in the expression of this gene was observed (6.10%). The increase in HIF3a mRNA expression compared with the control group in the Amantadine group was 70.76%, in the Cerebrocurin group – 82.35% ($p \leq 0.05$), Pramistar – 67.72%, Gliatilin – 79.44% ($p \leq 0.05$), Noophen – 67.47%, Pronoran – 81.65%, Melatonin – 82.92% (Table 1).

Protein quality control is an important component of neuronal survival under conditions of PD. We investigated the neuroprotective activity of small chaperones, heat shock proteins HSP70, whose synthesis is increased in response to hypoxia, ischemia, metabolic disorders, and stress. HSP70 has an antiapoptotic effect by inhibiting the action of apoptosis activator protease-1, Bax family proteins; prevents translocation of apoptosis-inducing factor (AIF) to the nucleus and capture of procaspase-3 by the apoptosome; and increases the activity of the antiapoptotic protein BCL-2.

The anti-inflammatory mechanisms of HSP70 in PD involve limiting the local inflammatory response by reducing the synthesis of pro-inflammatory cytokines, inhibiting the release of matrix metalloproteinases and inducible nitric oxide synthase, decreasing NADPH oxidase activity while simultaneously increasing SOD activity, and suppressing the activity of the transcription factor NF-kB along with the kinases, i.e. IkB, JNK, and p38. HSP70 also functions as a signaling molecule, interacting with Toll-like receptors 2 and 4 as well as promoting the activity of immunocompetent cells.

The discovered abnormalities that occur in neurons during PD are accompanied by a deficit in the functioning of the endogenous cytoprotection factor, the HSP70 protein. All this is the result of structural changes and a decrease in the synthesis of the latter under conditions of severe energy deficiency and in the context of reduced levels of neuronal antioxidant defense enzymes. HSP70 deficiency in a neuron against the background of systemic glutathione deprivation is associated with hyperproduction of reactive oxygen species and cytotoxic forms of nitric oxide, which leads not only to modification of various molecules, including HSP70 itself, but also to a reduced activity of genes encoding the synthesis of the latter. HSP70 deficiency is one of the causes of mitochondrial dysfunction. Thus, we found a 22.32% decrease in HSP70 mRNA expression in the control group, compared to the intact group.

The neuroprotective effects of the drugs under study are linked to the normalization of endogenous HSP70-mediated neuroprotection against the background of activated expression of antioxidant enzyme genes, compared to the control group: in the Amantadine group – by 31.86% ($p \leq 0.05$), in the Cerebrocurin group – by 15.08% ($p \leq 0.05$), in the Pramistar group – by 30.44%, in the Gliatilin group – by 16.26%, in the Noophen group – by 23.77%, in the Pronoran group – by 23.96% ($p \leq 0.05$), in the Melatonin group – by 34.66% ($p \leq 0.05$). The antioxidant properties of the studied drugs, combined with the ability to activate the transcription of HSP70 genes and antioxidant enzymes, enhances the resistance of neurons to PD, which indicates an important contribution of these drugs to neuroprotection.

The brains of rats of the control group in the PD model revealed an increased expression of c-Fos (c-Fos-positive neuronal cells) by almost 52.75%, compared to the intact group of the animals, which reflects the progressive activation of apoptotic processes and the death of dopamine-producing neurons. The expression of c-Fos mRNA in the control group grew by 44.53% compared to the intact group. The increased c-Fos gene expression does not always have a protective physiological function. In case of this gene overexpression, c-Fos already plays a negative role, significantly raising the amount of c-Fos protein in neurons, where it is directly involved in DNA fragmentation and initiation of apoptotic cell death.

Given the extremely important role of c-Fos gene expression in physiological and pathological processes, an urgent task of experimental medicine is to find ways of pharmacological correction of various pathological conditions, which will be aimed at correcting hyper- or insufficient c-Fos gene expression. In our study, the following dynamics of Fos-positive neurons was observed: Amantadine administration decreased expression by 7.25% ($p \leq 0.05$), Cerebrocurin – by 31.24% ($p \leq 0.05$), Pramistar – by .68%, Gliatilin – by 35.85%, Noophen – by 26.70%, Proran – by 21.34% ($p \leq 0.05$), Melatonin – by 37.84% ($p \leq 0.05$).

A similar statistically reliable dynamics was also observed in the study of c-Fos mRNA expression against the background of therapeutic effects in rats with PD: a decrease in c-Fos mRNA expression in all groups, but

Table 2. Influence of the studied drugs on the expression of proapoptotic factors and the level of inflammatory process markers under conditions of PD formation in rats (M ± m; Q50,(Q25;Q75), n = 20)

Marker	Density of BCL-2-positive neurons per 1 mm²	BCL-2 mRNA expression, a.u./g protein	Caspase-3, ng/ml	IL-1β, pg/ml	TNF-α, pg/ml
Intact	310.20±9.43	0.23±0.01	2.56±0.12	6.75±0.54	0.15±0.02
Control (PD)	223.80±17.87	0.17±0.01	5.15±0.19	11.35±0.95	0.89±0.09
PD+AM	249.16±22.41*	0.18±0.01*	4.84±0.22*	10.03±0.99*	0.82±0.07*
PD + Cerebrocurin + AM	293.37±2.55*	0.21±0.006*	3.18±0.30	8.15±0.74	0.54±0.07
PD+Pramistar +AM	271.41±3.16	0.20±0.01	4.73±0.41*	9.47±1.31*	0.76±0.05*
PD + Gliatilin +AM	285.87±1.99*	0.22±0.02*	3.05±0.24*	7.54±0.75	0.48±0.03
PD +Noophen +AM	274.94±3.11	0.19±0.01	4.09±0.35	8.89±0.90	0.65±0.05
PD +Pronoran+AM	278.82±3.64	0.21±0.009*	3.69±0.38	7.94±0.72*	0.52±0.05*
PD +Melatonin +AM	302.96±3.42	0.22±0.02	2.83±0.40	7.18±1.03*	0.39±0.06*

Notes: p – the level of statistical significance when comparing samples using ANOVA (Kruskal-Wallis test), *p≤0.05 relative to the control group
Source: compiled by the authors of this study

a particularly pronounced depression of the apoptotic activity of this early response gene was observed when treated with Cerebrocurin, Gliatilin, Noophen, Pronoran, and, especially, Melatonin, with the values being close to the intact group.

We also investigated the changes in the activity of specific regulatory proteins that prevent the implementation of the apoptotic program. One of them, the BCL-2 protein, has a protective effect on many types of cells that are under unfavorable conditions. BCL-2 promotes cell survival, and the antiapoptotic effect of this protein is associated with the normalization of mitochondrial function, which is involved in the initiation of apoptosis. First of all, BCL-2 blocks the release of cytochrome *c* from mitochondria, forms transmembrane mitochondrial pores, which determines the transmembrane potential, as well as the release of various active compounds and ions from mitochondria.

In the control group during PD, the density of BCL-2-positive neurons decreased by 27.85%, compared to the intact group, and BCL-2 mRNA expression decreased by 26.09%. After rats with PD had been treated with Amantadine, the density of BCL-2-positive neurons increased by 10.18% (p≤0.05), compared with the control; with Cerebrocurin – by 23.71% (p≤0.05); with Pramistar – by 17.54%; with Gliatilin – by 21.71% (p≤0.05); with Noophen – by 18.60%; with Pronoran – by 19.73%; and with Melatonin – by 26.13%. There was a synchronous increase in BCL-2 mRNA expression in all experimental groups compared with the control group, especially with the administration of Melatonin, Cerebrocurin, Gliatilin, and Pronoran within the range of statistical significance Table 2.

A 50.29% increase in caspase-3 activity in the control group, compared with the control group, was also a sign of neuronal cell apoptosis activation during PD. The therapeutic effect of Amantadine led to a decrease in this marker by only 6.01% (p≤0.05), while the combination of Amantadine with Cerebrocurin reduced caspase-3 by 38.25%, with Gliatilin – by 40.78% (p≤0.05), with Noophen – by 20.58%, with Pronoran – by 28.35%, and with Melatonin – by 45.05%.

Localized inflammation in brain tissue is an unavoidable consequence of oxidative and nitrosative stress during PD, and results in pathological activation of microglia structures. The following markers served as signs of inflammatory process in our experimental study: IL-1β and TNF-α with a stable elevation in the control group, compared to the intact group, by 40.53 and 83.15%, respectively. The resultant vector of the changes described is a cytokine-mediated increase in the activity of inducible NOS, which leads to an even greater growth in the production of free radicals, as well as to the induction of an apoptotic scenario of neuronal death in experimental PD.

When treating rats with PD with Amantadine, the levels of IL-1β and TNF-α decreased, compared with the control group, by 11.63 and 7.87% (p≤0.05), with Cerebrocurin – by 28,19 and 39.33% (p≤0.05), with Pramistar – by 16.56 and 14.61%, with Gliatilin – by 33.57 and 46.07% (p≤0.05), with Noophen – by 21.76 and 26.97%, with Pronoran – by 30.04 and 41.57%, with Melatonin – by 36.74 and 56.18%.

DISCUSSION

Apoptosis is the main mechanism of neuronal loss in PD, as evidenced by the identification of DNA

fragmentation and apoptotic chromatin changes in dopaminergic neurons of PD patients in pathological studies [6]. Similar approaches to clinical and instrumental evaluation of degenerative and neurofunctional changes have been presented in studies of athletes with intervertebral disc pathology [7]. In addition, the role of apoptosis in PD pathogenesis has been confirmed via in vitro studies showing increased caspase-3 activity and increased expression of active caspase-3 in the substantia nigra pars compacta. Moreover, dopaminergic neuronal death is characterized by a decrease in the expression of antiapoptotic proteins, such as BCL-2, in cellular models of PD [8]. It has also been revealed that caspase inhibitors rescue neurons from death in cellular models of PD, which further supports the idea that apoptosis is the main mechanism of neuronal death in PD. Increased levels of pro-apoptotic proteins, such as Bax, have also been observed in postmortem brain tissue of patients with PD [9].

Although there are some suggestions that the extrinsic apoptotic pathway may be active in PD, its role remains unclear. It is believed that the predominant mechanism of neuronal death is the intrinsic apoptosis pathway [10]. Mitochondria-mediated apoptosis has been extensively studied in PD. It involves a sequence of events: increased formation of reactive oxygen species, cytochrome *c* release and ATP depletion, as well as activation of caspase-9 and caspase-3. It remains unclear how the multiple pathogenic processes of PD, such as alpha-synuclein aggregation and mitochondrial dysfunction, for example, interact with each other to cause apoptotic cell death.

Alpha-synuclein is widely expressed in the central nervous system, especially presynaptically. It is prone to aggregation of fibrils that form the main component of Lewy bodies, which are a pathological feature of Parkinson's disease. During PD, aggregation and inclusion of α-synuclein occurs in the brain, as well as in rodent cells and cells treated with mitochondrial toxins [11]. The accumulation of wild-type α-synuclein in dopaminergic neurons leads to a decrease in mitochondrial complex I activity and an increase in the generation of reactive oxygen species, an effect that is more pronounced during the expression of the aggregation-prone α-synuclein A53T mutant. It has also been shown that α-synuclein localizes on the mitochondrial membrane in SHSY cells and in isolated rat brain mitochondria. This interaction was assumed to lead to oxidative stress and release of cytochrome *c* into the cytosol in in vitro systems. After the release into the cytoplasm, cytochrome *c* interacts with antiapoptotic proteins that promote survival by triggering mitochondrial-mediated apoptosis [12].

Mitochondrial dysfunction can undoubtedly be an early phenomenon in humans and in animal PD models. Impaired mitochondrial complex I activity has been found in the substantia nigra of patients with PD. Dopamine metabolism leads to the formation of reactive oxygen species, which can lower the threshold for apoptotic cell death. Dopamine is enzymatically metabolized by monoamine oxidase (MAO), which triggers the production of H^2O^2, which subsequently results in the formation of reactive oxygen species. The breakdown products of dopamine undergo auto-oxidation, which causes an increase in the formation of reactive oxygen species. Thus, dopaminergic neurons are particularly susceptible to dysfunction of mitochondrial complex I, which is considered one of the main sources of reactive oxygen species in PD. Therefore, the production of reactive oxygen species may be a potentially important mechanism contributing to the death of dopaminergic neurons through apoptosis. It is assumed that impaired mitochondrial complex I activity increases the susceptibility of dopaminergic neurons to degeneration by lowering the threshold for activation of the intrinsic apoptotic pathway [13].

Given that the end point of PD pathogenesis is apoptotic neuronal death, treatments targeting the molecular and biochemical events that allow apoptosis to progress may protect against the loss of dopaminergic neurons. As already discussed, apoptosis depends on the activation of caspases. Thus, inhibition of caspases is considered to be a new therapeutic approach to neurodegenerative diseases that arise due to apoptosis. Indeed, caspase inhibition prevents the death of dopaminergic neurons in the substantia nigra pars compacta induced by MPTP or its active metabolite MPP+ in vitro and in vivo [14].

MPTP is a neurotoxin that selectively damages dopaminergic neurons in the compact part of the substantia nigra. MPTP is a lipophilic substance that actively penetrates the blood-brain barrier and enters the central nervous system, where it is converted into its active metabolite, the so-called MPP+ (1-methyl-4-phenylpyridinium). This conversion is carried out with the help of MAO, which is present in glial cells. After being reuptaken by the dopamine carrier, MPP+ accumulates in the mitochondria of dopaminergic neurons, inhibiting mitochondrial complex I, which leads to ATP depletion and increased generation of reactive oxygen species. As a result, nigrostriatal dopaminergic neurons die via a caspase-dependent apoptotic pathway. Apoptosis induced by MPTP

is characterized by the generation of reactive oxygen species, cytochrome *c* release, p53 expression, caspase-3 cleavage and DNA fragmentation, as well as other morphological features characteristic of apoptosis. The apoptosis induced by MPTP is attenuated by BCL-2 overexpression [15].

However, although dopaminergic neurons could be rescued, nigrostriatal terminals were already damaged, suggesting that this approach may simply ensure the survival of dysfunctional neurons, which means that apoptosis inhibition alone may not be that promising for the treatment of PD. At the same time, the simultaneous administration of glial cell line-derived neurotrophic factor (GDNF) allowed us to circumvent this problem by restoring dopamine concentration in the striatum. Thus, the literature suggests that caspase inhibition in combination with specific growth factors may play a notable role in treating PD in the future [16].

Before caspase activation, intervention in the induction phase of the apoptosis has been considered as a strategy to prevent the death of dopaminergic neurons and restore their function. For example, Bax is activated in dopaminergic neurons to treat MPTP. In addition, genetic deletion of Bax prevented dopaminergic neurodegeneration in a mouse model of MPTP-induced nigrostriatal degeneration. Furthermore, Bax inhibition could reduce the loss of nigral dopaminergic neurons induced by intrastriatal injection of 6-OHDA (6-hydroxydopamine), suggesting that Bax-inhibitory peptides are a possible therapeutic agent for the treatment of PD [17].

The propargylamine derivative CGP 3466 (dibenzo[b,f]oxepin-10-ylmethyl-methyl-prop-2-ynylamine) has been shown to have neuroprotective and antiapoptotic characteristics. CGP 3466 B inhibits neuronal apoptosis by activating protein-L-isoaspartate (D-aspartate) O-methyltransferase (PCMT1, protein-L-isoaspartate (D-aspartate) O-methyltransferase), an enzyme that repairs damaged L-isoaspartyl residues in intracellular proteins. Up-regulation of PCMT1 leads to overexpression of the anti-apoptotic protein BCL-2, as well as under-expression of the pro-apoptotic Bax and active caspase-3, inhibiting mitochondrial-dependent apoptosis, as a result. At the same time, it prevents the death of dopaminergic cells in vitro in PD models of rodents, and thus suppresses the development of motor symptoms caused by MPTP and 6-OHDA. Therefore, therapies that prevent the development of apoptotic pathways may represent promising therapeutic strategies aimed to protect against the loss of dopaminergic neurons and the subsequent pathogenesis of PD in patients [18]. Similar physiological and biochemical adaptation patterns have been observed in studies of vascular and respiratory regulation in athletes [19, 20].

Having discussed these approaches, it should be noted that there are concerns regarding apoptosis being a specific target in neurodegenerative diseases. It is believed that apoptosis in PD is triggered by a number of intracellular pathological processes, with mitochondrial dysfunction being particularly important. So, inhibition of apoptosis can prevent the programmed removal of dysfunctional, non-viable neurons, which can ultimately lead to necrosis and the potential development of an inflammatory response. In cell culture models of PD, treatment with caspase inhibitors did trigger a shift from neuronal apoptosis to necrosis [21]. In addition, despite the fact that genetic deletion of Bax inhibited the death of dopaminergic neurons in response to 6-OHDA in transgenic mice, it failed to improve the behavioral deficits associated with PD, whereas the surviving dopaminergic neurons showed severe neuronal atrophy. In addition, the systemic administration of antiapoptotic drugs can promote prolonged survival and accumulation of dysfunctional and potentially neoplastic cells in many tissues, which will undoubtedly be detrimental to the body as a whole [22].

The site of HSP70 action in this complex cascade of reactions initiating neuroapoptosis is not precisely determined. An alternative pathway for triggering apoptosis via Fas/Apo1 includes the Daxx protein. The mechanism of action of this protein is poorly understood. Normally, Daxx is localized in the nucleus, where it is associated with certain proteins, but it is able to move into the cytoplasm and play the role of an adaptor protein responsible for triggering the JNK kinase cascade by activating Fas/Apo [23]. It is assumed that HSP70 is able to move to the nucleus, where it interacts with Daxx, preventing its release into the cytoplasm and activation of the receptor. It has been previously stated that HSP70 may be involved in the regulation of apoptosis not only at the level of the Fas/Apo1 receptor, but also at the level of certain intracellular target proteins. HSP70 has been shown to prevent mitochondria-induced apoptosis [24]. We have experimentally substantiated for the first time the pathogenetic effect of complex neuroprotection on the damaged brain, which is the ability to modulate the expression of 70kDa heat shock proteins in neurons. An increase in the concentration of HSP proteins in brain tissues was found. Due to chaperone activity and stabilization of actin filaments, these proteins prevent the development of neuroapoptosis and progression of PD.

Cerebrocurin has a complex neuroprotective effect due to its ability to stabilize the functional state of mitochondria and suppress the development of mitochondrial dysfunction; prevent the formation of energy deficiency; block the development of lactate acidosis against the background of activated compensatory mitochondrial-cytosolic shunts of energy production, particularly the malate-aspartate shuttle; reduce the manifestations of oxidative and nitrosative stress; modulate the expression of all NOS isoforms, as well as HIF and HSP proteins; enhance the activity of the antioxidant and thiol-disulfide system enzymes; morphologically stabilize neuronal and glial cells with parallel activation of RNA synthesis in them, as well as restore the morphological ultrastructure of mitochondria; and affect the processes of apoptosis / necrosis, also through regulatory influence on c-Fos expression. Cerebrocurin increases HSP70 expression by activating NFkB. Melatonin in PD increases the level of HSP70 by activating the melatonin receptors MT1 and MT2 [25]. In addition, the unique structure of the melatonin molecule makes it an effective scavenger of ROS / RNS and prevents total damage to polypeptide bonds, inactivation of enzyme systems, and antioxidant links of endogenous protection, including HSP70. Melatonin is also known to have cytoprotective effects by supporting the activity of glutathione peroxidase, Cu, Zn- and Mn-superoxide dismutase, and γ-glutamylcysteine ligase. At the same time, the drug is also capable of inhibiting a number of prooxidant enzymes, such as lipoxygenase and NO synthase, which reduces the production of ROS during PD. The positive effect of melatonin on energy metabolism is explained by its ability to prevent damage to aconitate hydrolase and thus maintain the Krebs cycle at the citrate-isocitrate stage. Pramistar restores thiol-disulfide balance in the brain during PD and limits iNOS expression. By increasing the level of reduced glutathione, Pramistar is able to upregulate the expression of HSP70. Gliatilin promotes synaptogenesis in cholinergic structures, has a mitoprotective effect, increases intra-mitochondrial glutathione levels and is able to elevate HSP70 levels [26].

Thus, although apoptosis is the final stage of the pathogenetic pathway in PD, it remains to be seen whether apoptosis inhibition in PD can be effective and safe. Additionally, a thorough evaluation of the literature data and experimental findings is needed.

CONCLUSIONS

1. The results obtained indicate that neuroprotective therapy of PD with such drugs as Melatonin, Cerebrocurin, Proran, and Gliatilin in combination with Amantadine leads to an increase in the expression of HIF-1α, HIF-3α and HSP70 genes and can serve as a molecular marker indicating activation of endogenous neuroprotection mechanisms in experimental PD.
2. The study of the mechanisms of programmed neuronal death via apoptosis in PD under oxidative stress conditions and pharmacological correction of apoptosis execution mechanisms is a pathogenetically justified target for the treatment of socially important diseases. We propose c-Fos and BCL-2 proteins, along with effector caspase-3, as markers of apoptosis.
3. We have experimentally demonstrated a new target of neuroprotection in PD, i.e. apoptosis of dopamine-producing neurons, and substantiated this process modulators – Amantadine therapy combined with other drugs (Melatonin, Cerebrocurin, Pronoran, and Gliatilin), which can be seen as promising treatment of PD.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Blockchain as a tool for resolving psychological and professional conflicts

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ABSTRACT

Aim: To substantiate the idea and to develop and test an experimental program for the use of BGP as a component of blockchain technology to improve existing strategies for resolving pedagogical conflicts in educational institutions.

Materials and Methods: The research methodology synthesizes two components. The first is based on the experience of researching psychological and professional conflicts (essence, categories of participants, forms of behavior in conflict, etc.). The second component i.e. the BGP phenomenon is presented through the prism of conflict theory.

Results: At the preparatory stage, three groups of experiment participants were identified, i.e. experimenters who developed its theoretical and technological principles; the control group (hereinafter CG) consisting of 56 undergraduate students of the 4th year of studying at the Pedagogical Faculty of Vasyl Stefanyk Precarpathian National University (Ivano-Frankivsk, Ukraine), who participated in the ascertainment experiment; experimental group (hereinafter EG) consisting of 22 students separated from the CG, who participated in the formula and control stages of the experiment. To achieve consensus understanding in the BGP conflict allows to create a system that ensures clear transmission of information; protects against non-fulfillment of agreements; stipulates actions if one of the parties violates them. On this basis, the core idea is substantiated, according to which, to reach a consensus it is enough for the majority of the participants in the conflict to take an agreed compromise position, then the minority, by force of internal conviction, agrees and accepts the agreements reached.

Conclusions: The presented results of the research generally confirmed the hypothesis that BGP, as a component of blockchain technology, can become a productive tool for resolving interpersonal and group conflicts in in various social and professional environments. The conceptual and methodological idea based on the analysis of the BGP phenomenon assumes that in order to resolve the conflict, it is not necessary to reach complete agreement between all its participants. To achieve understanding and consensus, it is enough for the majority to take an agreed compromise position, then the participants in the conflict who are in the minority, due to their internal moral convictions, will be forced to agree and fulfill the agreements reached by the majority.

KEY WORDS: psychological conflict, professional conflict, blockchain technology, types of conflict behavior, methods of conflict resolution, barrier-free competence

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INTRODUCTION

A famous researcher and adept of the Byzantine General's Problem (BGP) testifies: "My son is almost 10 years old, but I have shared this problem with him. He struggled for a long time with how to untie it... And it's not surprising! This is a fictitious problem, but one of the most difficult that has ever existed. Its essence is to find out how different parties in "complete disagreement" can find a way to achieve "complete consensus" [1].

This opinion clearly conveys the essence of our studio. The modern world is constantly filled with many innovations. Many of them, thanks to their originality and perspective, are actively implemented in various spheres of social life, which, despite the subject specificity, are always filled with interpersonal and group disputes and conflicts [2; 3].

The integration of various fields of knowledge became the master of the development of science in the 21st century. It is natural that the greatest scientific discoveries are made at the intersection of related humanitarian or natural sciences [4; 5]. However, it is no less promising to conduct research at the junction of various conventionally distant scientific directions. In our case, we are talking about the connection of

medicine and education with cryptography i.e. the science of mathematical methods that ensures confidentiality, integrity, authenticity of information storage and transmission [6-8].

AIM

The aim of the study is to substantiate the idea and to develop and test an experimental program for the use of BGP as a component of blockchain technology to improve existing strategies for resolving pedagogical conflicts in educational institutions.

MATERIALS AND METHODS

The research methodology synthesizes two components. The first is based on the experience of researching psychological and professional conflicts (essence, categories of participants, forms of behavior in conflict, etc.). The second component i.e. the BGP phenomenon is presented through the prism of conflict theory.

The design of the research work is outlined in four stages: 1) preparatory – determination of the participants of the experiment and the main parameters and theoretical foundations of the research; 2) ascertainment – development and testing of prognostic tools; 3) formative – implementation of the author's curriculum; 4) control – analysis of the results of research work.

Preparatory stage. Three groups of experiment participants and their functions are defined. The first group contained the experimenters (authors of this article) who developed scientific-theoretical, technological and organizational principles of scientific research work; the second one was the control group (CG) consisting of 56 undergraduate students of the 4th year of studying at the Faculty of Pedagogy of the Vasyl Stefanyk Precarpathian National University (Ivano-Frankivsk, Ukraine). They participated only in the ascertainment experiment. The third experimental group (EG) of 22 students who participated in the formula and control stages of the experiment was singled out from them.

The basic parameters of research work - hypothesis, goal, methodological principles - have been formulated. The hypothesis is the assumption that BGP, as a component of blockchain technology, can become a productive tool for resolving interpersonal and group conflicts in various social and professional environments. The purpose is to substantiate the idea and develop a methodology for resolving conflicts in social and professional environments using blockchain technology and their testing in the process of scientific research.

Constructive resolution of psychological conflicts is a key factor in shaping the barrier-free competence of students. Psychological barrier-freeness is understood as an integral characteristic of the socio-psychological environment that enables every individual to freely express themselves, feel acceptance, respect, and safety without prejudice or discrimination [9]. It implies overcoming stereotypes, developing empathy, tolerance, and communicative culture, as well as ensuring access to psychological support. By mastering these skills, future specialists form barrier-free competence — the readiness for effective interaction, professional activity in diverse environments, and the creation of a humanistically oriented educational and social space.

The development of the research methodology involves an organic synthesis of two components i.e. the theoretical foundations of psychological and professional conflictology and BGP as a component of blockchain technology. While developing the first component, they relied on representative studies on the theory of psychological and professional conflict [2; 8]. During their analysis, in line with the raised problem, emphasis was placed on the following provisions. Conflict is an important natural component of human interaction. Applying the principles of conflict theory to various components of the education system (medical, pedagogical, etc.) creates a theory of conflict in education. The use of conflict theory allows us to better understand the behavior of different social groups in educational institutions of different levels and types.

Psychological conflict is understood as an open or hidden clash, confrontation between two or more subjects of interpersonal interaction. They are based on different ideas, views, goals, needs, interests, values, attempts to resolve such conflicts are accompanied by high emotionality. Psychological conflicts arise in the process of professional and interpersonal interaction of participants in the educational process as a manifestation of subject-subject contradictions and involve a review of their relationships.

In the structure of psychological conflict, we distinguish four categories of participants: 1) main subjects (conflicting parties) who carry out direct active / passive and overt / covert offensive or defensive actions, actions against each other; 2) instigators, organizers who initiate, plan and directly or indirectly influence the development of the conflict; 3) support groups that indirectly influence the course and consequences of the conflict through their active or passive actions; 4) middlemen (mediators, facilitators) who occupy a neutral position, called to help the conflicting parties reach an agreement, understanding.

The core of the research methodology is the model of conflict regulation [9], which will be discussed further.

The essence of the second component of the research methodology, i.e. Byzantine General's Problem, is determined through the prism of the theory of conflict theories in educational and professional activities [10-12]. Understanding and using the complex phenomenon of BGP in professional activities facilitates its visual representation.

Let's imagine that a group of generals besieged a city and have a common goal in capturing it. Generals command their armies, which are located in different places and do not have a common command. Under the conditions of decentralization, generals need to determine a common strategy: when, with what forces to attack or refrain from active actions. The situation is complicated by the fact that the generals are subordinate to their kings and have different views on war, there is no trust between them, and there is a hidden conflict of interests. The messengers that the generals send to each other and to their kings can turn out to be traitors, so there is no guarantee that they will deliver the message as intended and not distort its meaning.

So, the essence of BGP is that in order to resolve conflicts (possible, existing; overt or hidden) and to achieve interaction and consensus, generals must communicate directly with each other and with their overlords. This requires a clear system that would: a) ensure reliable transmission of information (when and how to interact); b) ensured against non-fulfillment of agreements; c) stipulated actions in case one of the parties violates them.

According to the blockchain theory, solving these tasks requires a reliable, authoritative intermediary (facilitator) recognized by all parties. The basis of its actions can be the Byzantine Fault Tolerance (BFT) protocol as the optimal way of making effective decisions under conditions of uncertainty and the existence of potential or real contradictions and conflicts. He takes into account many nuances of how to act if one of the parties may disagree with something, oppose himself to others, or fail to fulfill agreements and commitments.

To resolve BGP, the BFT protocol offers a model of the recursive algorithm expressed in the formula: $n + m = 4$, where: n is the total number of generals, m is the number of traitors, 4 is the number of steps to reach consensus. Each of these steps provides multiple options for when generals and traitors can pass on the right or wrong information. But in the end, it is proved that in a system where m elements work incorrectly, agreement can be reached when $2m+1$, that is, when there are more than two-thirds of loyal generals [11].

This is the core conceptual and methodological idea of our research work: it is not necessary to reach a complete consensus among all participants to resolve the conflict. For understanding, it is enough for the majority (conditionally 2/3) to take an agreed compromise position. Then the participants in the conflict, who make up the minority (conditionally 1/3), must accept the consensus and consciously, by force of internal conviction, agree and fulfill the agreements reached by the majority.

The conceptualization of this approach to the resolution of pedagogical conflict is expressed by the "leader-follower" scheme as a component of BGP [11]. We interpret it through the prism of the investigated problem. To reach a consensus, all generals and their subordinate lieutenants (participants in the conflict) must agree on a certain decision. Meanwhile, the following conditions are taken into account: a) among them there may be "traitors" who are not interested in positive resolution of contradictions, have their own hidden interests, and may sabotage the search for consensus; b) generals for various reasons do not want or cannot communicate directly, so messengers (in our case, a facilitator) are needed to establish a dialogue.

Thus the question arises is: "Under such conditions, how can consensus and trust be achieved for the performance of joint tasks?" To cut this "Gordian knot", BGP relies on an algorithm that can guarantee that: 1) most generals and lieutenants (advocates of productive conflict resolution) share a similar point of view and adopt a coherent plan of action to achieve it; 2) traitors (opponents of a positive resolution of the conflict) are in the minority, so they can only slow down, but are unable to prevent the adoption of a plan to achieve consensus and understanding.

Such a component of BGP as Distributed Ledger Technology (hereinafter DLT) will contribute to the implementation of this approach. It requires all DLT nodes to agree on a specific set of rules (agreements) and be able to move forward by agreeing on a specific valuation of a transaction before it is added to the database. Agreements reached about the validity of new information are added to a common database, which prevents bad actors from sabotaging them and revising the agreements reached [12]. In other words, the use of the consensus algorithm allows the facilitator to coordinate the conflict resolution process purposefully through the gradual reconciliation of individual misunderstandings and contradictions between its participants. Meanwhile, most importantly, the reached agreements are clearly recorded, so they cannot be revised or falsified. This creates a favorable basis for reaching a consensus among the majority of the participants in the conflict.

CONFIRMATORY EXPERIMENT

The main goal of the ascertainment experiment is to develop and test prognostic tools to determine the level

Table 1. Questionnaire for identifying typical forms of behavior in conflict

Answer "A"	Answer «B"
1. I provide an opportunity for others to take responsibility for conflict resolution	Instead of discussing what we disagree with, I'll focus on what we do agree on
2. I always try to find a compromise in controversial issues	I try to settle the case, taking into account my interests and the interests of the other party
3. I usually persistently strive to achieve my goal (at any cost)	I try to understand and calm the other and save our relationship
4. I always try to find a compromise, mutually beneficial solution	I can sacrifice my interests for the sake of another person
5. When fixing a controversial situation, I look for support from another person	I try to do everything to avoid tension
6. First of all, I try to avoid trouble for myself	I try to achieve my goal at any cost
7. I try to postpone the resolution of the disputed issue in order to finally resolve it later	I consider it possible to give in temporarily in order to achieve my goal later
8. I usually strive to achieve my goals	First of all, I try to clearly understand what the essence of the dispute, conflict is
9. I think that you should not worry too much about minor disagreements in a relationship	I make every effort (means, resources) to necessarily achieve my goal
10. I firmly and steadfastly strive to achieve my goals and interests	I always try to find a compromise
11. First of all, I try to clearly find out what is the essence of the case, the dispute	I try to calm the other person down and thus maintain a good relationship.
12. I mostly avoid situations that could cause an argument	I give the other person the opportunity to stick to his opinion, if he also goes to meet
13. In any disputes, I look for (offer) the "golden mean"	I insist that my position prevails in the dispute
14. I express my position and try to find out the other person's opinion	I try to convince others of the merits of my views
15. I try to calm the other and keep our friendly relationship	I do everything necessary (depending on me) to avoid tension, arguments
16. I try not to hurt the feelings of another person	Trying to convince the other of the advantages of my position
17. I try hard to achieve my goal	I do everything possible to avoid tension in the relationship
18. If my concession makes another person happy, I will give him that opportunity	I give the opportunity to the other to stick to his opinion, if he also goes to meet
20. I try to immediately overcome misunderstandings that arise	I am looking for optimal ways (options) that harmonize my interests and those of others
21. During negotiations, I treat the other person's wishes and aspirations with respect	I always strive for frank discussion of the problem
22. I try to find a "golden mean" between my position and the interests of the other	I persistently defend my wishes
23. I strive to satisfy the wishes and interests of all parties to the conflict	Sometimes I give the opportunity to another to take the initiative and responsibility for solving a controversial issue
24. If the other person's position is important to him, I try to meet him, agree on positions	Trying to convince the other that my position is better
25. I try to prove to others the logic and merits of my views	During negotiations, I try to be attentive to the wishes of the other
26. I always look for a mutually beneficial solution to the case	As a rule, I try to satisfy my interests and desires and those of others
27. I avoid a position that could cause disputes	If it makes the other person happier, I make concessions
28. I usually persistently and uncompromisingly strive to achieve my goals	Fixing a case, a dispute, I am looking for the support of another
29. In a difficult situation, I look for and offer a solution that should satisfy all parties	In conflicts, all parties should not show excitement
30. I try never to hurt other people's feelings	I take a position on a controversial issue to achieve understanding and success

Source: compiled by the authors of this study

of readiness of future teachers to resolve pedagogical conflicts in the context of BGP postulates. Its development is based on the Thomas-Kilmann Conflict Regime Instrument (Thomas-Kilmann Instrument –hereinafter TKI), which assesses human behavior in conflict situations that arise due to incompatibility of the interests of individuals or groups. It is described according to two dimensions: 1) persistence - characterizes the behavior of a person who focuses on his own interests and ignores the needs of others; 2) readiness for cooperation (consensus), when opponents consider each other's interests and try to satisfy them. On this basis, scientists identified five ways to resolve conflicts: competition; cooperation; compromise; avoidance; adaptations [7; 9], which we consider further.

Based on the basic version of the TKI model [9] and the experience of its use [2; 3; 13], the "Questionnaire for identifying typical forms of conflict behavior" was developed (Table 1). CG members were asked to choose options "A" or "B" from 60 statements divided into 30 pairs. Processing of the results was carried out according to the following scales: rivalry: 3A; 6B; 8A; 9B; 10A; 13B; 14B; 16B; 17A; 22A; 25A; 28A; cooperation: 2B; 5A; 8B; 11A; 19A; 20A; 21B; 23B; 26B; 28B; 30B; compromise: 2A; 4A; 7B; 10B; 12B; 13A; 18B; 22A; 23A; 24B; 26A; 29A; avoidance: 1A; 5B; 6A; 7A; 9A; 12A; 15B; 17B; 19B; 20B; 27A; 29B; device: 1B; 3B; 4B; 11B; 15A; 16A; 18A; 21A; 24A; 25B; 27B; 30B. The number of points scored on each scale gives an idea of the tendency to display appropriate forms of behavior in conflict situations.

FRAMEWORK

The work was carried out according to the research work of I. Horbachevsky Ternopil National Medical University "Development of communicative competence of students in the conditions of a medical university" (state registration number 0122U000033).

ETHICS

This work complies with the principles of the Declaration of Helsinki.

RESULTS

The data obtained on the basis of processing the answers of the CG members are accompanied by descriptive names of negotiation strategies that are often found in psychological and sociological studies to express their essence and perception. It turned out that 30.8% of respondents in conflict situations were inclined to take a position of rivalry («Shark»; «Boxer»), which means striving to achieve one's own interests, regardless of the needs of the other. A rigid orientation to victory ("go for the breakthrough") is characteristic of people with certain character traits (egoism, narcissism, selfishness) and social status (formal and informal leaders, managers). In the long run, it is ineffective, because the affected parties to the conflict will sabotage such decisions.

Considering the mental attitudes common among today's youth, a fairly high percentage (28.2%) of supporters of the avoidance strategy ("Turtle";"Kamikaze"), which means refusing dialogue and achieving one's own goals, was expected. Such behavior can be justified if the conflict situation does not affect the direct interests of a person and does not inhibit his development. However, avoiding negotiations often leads to forcing a person to change his attitude to the problem that caused the conflict.

The adaptation strategy ("Teddy Bear"; "Kindness") had the least supporters (11.3%). Modern young people are not always ready to sacrifice their interests for the sake of others. The strategy of settling the conflict at the expense of one's own losses contradicts the instinct of self-preservation and increases the tension in interpersonal relations, especially if one side is not ready for dialogue.

The strategy of compromise ("Fox";"Kleister";), which involves understanding on the basis of mutual concessions, turned out to be unattractive for respondents (13.8%). People with this type of behavior are characterized by caution, balance, and equanimity; they strive to preserve good relations and normalize relations based on mutual concessions. The implementation of this strategy involves a joint analysis of all information regarding the content of the conflict, an open exchange of opinions with the help of an experienced facilitator. It facilitates the reaching of agreements that do not necessarily satisfy all participants in the conflict, but they, taking into account the agreed consensus, are forced to agree with the decisions made.

The strategy of cooperation ("Owl", "Virtuoso"), indicated by 16.7% of respondents, is aimed at a joint search for an alternative and reaching a consensus that satisfies the interests of all participants in the conflict. Discussing the "fan" of alternative proposals, they try to avoid unnecessary accusations and emotions, but make compromises, concessions and look for reasonable solutions that would satisfy the interests and ensure the productive activity of each individual and the entire group.

Therefore, each behavior style has its own strengths and limitations for conflict resolution. From the BGP's point of view, the most effective strategies for achieving

such goals are the strategies of compromise and cooperation, so the educational methodology implemented at the formative stage of the experiment was aimed at forming the knowledge, skills, and abilities necessary for their implementation.

FORMATIVE EXPERIMENT

The formative stage was organized in the form of a seminar. His task was to implement the author's curriculum, which consisted of three parts: theoretical, methodical, and practical.

The theoretical part provided for the development of EG members' understanding of the possibilities of resolving pedagogical conflicts on the basis of BGP. In three classes (lecture, seminar, discussion), students studied blockchain technology, which, thanks to the scheme of visual representation [10; 12], turned out to be interesting, understandable and useful for them.

Having taken into consideration new knowledge the members of the EG formed an understanding of the possibilities of conflict resolution using blockchain technology. To do this, they deepened the skills of diagnosing the psychological conflict (finding out its sources, biography, essence, features; determining the type of behavior of the participants in the conflict) [2; 14]. In the BGP projection, knowledge about facilitation as a model and technology for conflict resolution in medical and educational institutions was expanded [4; 15]. For this, the analysis of principles, values and techniques of mutual understanding and group decision-making proved to be particularly useful [16].

METHODICAL PART

The methodical part (seven seminars and laboratory classes) provided for the mastering by the members of the EG of original methods and techniques that, in the perspective of the principles of BGP, could be effectively used to resolve conflicts in various social and professional environments. A joint research, discussion, selection, as well as testing of such tools were carried out by the experimenters-teachers and students.

About 200 tools for supporting the group thinking process have been reviewed [16]. Knowledge of facilitation techniques in the in a professional environment where a manager or authority figure acts as a mediator in resolving interpersonal and group conflicts, was deepened [17]. The mediator's functions and methods of achieving understanding between conflict participants were studied: introspection (forms the ability to put yourself in the place of another person and understand his thoughts, feelings, motives of activity and behavior); empathy (develops intuitive thinking, the ability to penetrate and understand the psycho-emotional state of another person); logical analysis (develops rational thinking and a system of intellectual representations for a better understanding of the interlocutor).

New and already known consensus technologies for conflict resolution were developed by future specialists: the ability to speak, listen, hear; chart-writing technique (facilitation; "alternative to open discussion"; "brainstorming"; "managing long lists"; "working with complex dynamics";"effective agenda"). The effectiveness of the technology of facilitating viable agreements, which involves gathering and taking into account different points of view, was investigated; development of a common platform of mutual understanding; reconciliation of mutually exclusive interests approaches and search for and achievement of consensus [16; 17]. Different forms of arbitration were analyzed (binding, advisory, mediation, "final offer", arbitration) [4; 8].

PRACTICAL PART

The practical part became the most difficult in the formative experiment. It provided for the approbation of the possibilities of using the specified tools for resolving psychological conflicts through the prism of BGP. For this, the members of the EG, based on the implementation of a complex scientific and educational project, developed the model "Conflict facilitation", which was tested in the form of a simulated role-playing game. It is based on a typical plot: a group of students with deviant behavior carried out systemic bullying against several physically weaker classmates who were successful in their studies. This manifested itself in public bullying (pushing, kicking); social and psychological pressure; verbal threats, mockery, insults; taking and damaging personal belongings; bullying through social networks, etc. The rest of the students silently observed this process: some were on the side of the offenders and played along with them, but the majority condemned such actions, but they were afraid to stand up for their friends, lest they themselves become victims of bullying. Gradually, the latent tension turned into an open conflict.

In the process of the business game, the members of the EG implemented knowledge and skills on conflict resolution in various social and professional environments based on the postulates of BGP as a component of blockchain technology. This was carried out through the performance of role functions of conflict participants: facilitator and students as conflict participants ("offenders", "victims", "initiators", "instigators", "support-

ers"). On this basis the main components of the "Conflict facilitation" stage of the model were implemented.

In the process of diagnosing the conflict, knowledge and skills were improved in determining the types of behavior of its participants. In particular, the emphasis was on finding out how many of them are "Sharks", "Turtles", "Teddy Bears" and how to transform their views and attitudes so that they replenish the ranks of "Foxes" and "Owls". Acting alternately in the role of a facilitator, the members of the EG learned to establish mutual understanding between the participants in the conflict, stimulate their adoption of mutually acceptable compromise decisions, and cultivate a sense of joint responsibility for their implementation.

The participants of the experiment, teachers and students, modified the facilitation tools [16] in the context of blockchain technology and gained experience in their implementation. According to the EG members, the following techniques were the most effective: "active listening" (creating a comfortable atmosphere that facilitates the interlocutors to express their thoughts and views); open discussion and alternative open discussion (allow to find out the positions of each participant in the conflict and compare them with other group and individual positions); "cipher" (allowed participants in the conflict to change their roles as subjects of the conflict) and others. When performing the circular survey technique, each participant received a cardboard image of a "Shark", "Turtle", "Teddy Bear", "Fox", and "Owl", which figuratively reflected the proportion of representatives of the corresponding types of conflict behavior.

Finally, these two tools were tested. The decision-making scale technique, which, based on a differentiated scale (voting pattern) from 1 - "strongly disagree" to 7 - "fully support", made it possible to identify and formalize the general attitude of the participants in the conflict towards the decision on its settlement. The technique of asking open-ended questions developed in coaching [18] proved to be effective for establishing a dialogue between various conflicting parties and convincing the "offenders" that their behavior not only offends the "victims" of bullying, but also does not correspond to the feelings of the majority of students in the class and contradicts humanistic values of modern society.

THE CONTROL STAGE OF THE STUDY

At this final stage, on the basis of the same prognostic toolkit ("Questionnaire" and the key to it), changes in the attitude of the EG members to the strategies of behavior in the conflict were clarified. The obtained results in comparison with the indicators of the ascertainment experiment are presented in Table 2.

DISCUSSION

A comparison of the data of the ascertainment and control stages of the experiment proves the real impact of the experimental program on changing the attitude of the EG members towards behavioral strategies, because they explained their choice through their comparison. Analysis of students' answers allows us to generalize the results of the experiment.

Among the EG members, the number of supporters of the rivalry strategy decreased most significantly (by 4.3 times). They formed a clear conviction that the "shark" type of behavior, strictly oriented towards victory "in any case" and "at any cost" and defending one's interests at the expense of another person, allows to obtain only a temporary victory in the conflict. On the basis of this, the rejection of the authoritarian style of interaction between the teacher and the student was strengthened.

The decrease by 1.8 times among the supporters of the "turtle" behavior, which hides in its shell in a conflict situation, is due to the awareness of the falsity of the victim's "passive-suffering" position. It indicates a person's reluctance, inability to solve a problem, change circumstances. The students understood that authoritarian parents, teachers, managers and entire social systems encourage this, but coercion or voluntary acceptance of the role of a victim does not correspond to the ideals of freedom and democracy, it prevents the free development and realization of personal aspirations and interests of a person.

A slight (by 1.3 times) decrease in supporters of the "Teddy Bear" type of behavior was due to the understanding that the restoration of peace and stability does not come close, but sometimes complicates the resolution of the conflict. Meanwhile, many students admitted that the accommodation strategy can be justified under the conditions, if the subject of disagreement is "more important to the opponent than to me"; it is necessary to admit one's mistake; there is a threat of further escalation of the conflict; maintaining good relations is more important than satisfying certain personal interests.

Evidence of the growth of the conflict-related competence of future teachers is also a 2.2-fold increase in the number of supporters of the compromise strategy, which was emphasized in the implementation of our curriculum. The principle of actions of the "Foxes" ("I will give in if you also give in"), the members of the EG supplemented with the principle of BGP, according to which consensus can be reached, understanding is possible under the conditions, if not all, but most of the participants in the conflict agree with a certain decision. Thus, due to "moral coercion", the minority voluntarily gives up part of its interests for the resolution of the

Table 2. Results in comparison with the indicators of the ascertainment

Type of negotiation strategy (behavior) in conflict	Declarative experiment (CG - 56 people)	Control experiment (EG - 22 people)
Rivalry ("Shark")	30,8 %	7,2 %
Evasion ("Turtle")	28,2 %	15,4
Device ("Teddy Bear")	11,3 %	8,3
Compromise ("Fox")	13 %	28.7 %
Cooperation ("Owl")	16,7 %	40.4 %

Source: compiled by the authors of this study

conflict as a whole. To implement this strategy, the person (specialist of any specialty) must have the special knowledge and skills of a facilitator [5].

The attractiveness of the strategy of cooperation (the number of its supporters increased by 2.4 times) was explained by a purely humanistic view of the conflict: "so that everyone wins." The analysis of the students' answers revealed that they did not draw a clear line between the "Fox" and "Owl" types of behavior, which in both cases required balance, caution and focused on "working with the problem, not with the conflict", that is, on its constructive solution. "Owls", like "Foxes" to some extent, strive for an equal dialogue, therefore they do not exploit the weaknesses of "Turtles" and "Teddy Bears" and categorically do not perceive the aggressiveness of "Sharks". Respondents noted that the consensus is appropriate and justified if the "Owls" fail to take into account the interests of all conflicting parties. The strategy of compromise was considered by them as an intermediate stage of a fair resolution of the conflict and the achievement of results that would satisfy all parties.

CONCLUSIONS

The presented results of the research generally confirmed the hypothesis that BGP, as a component of blockchain technology, can become a productive tool for resolving interpersonal and group conflicts in in various social and professional environments. The conceptual and methodological idea based on the analysis of the BGP phenomenon assumes that in order to resolve the conflict, it is not necessary to reach complete agreement between all its participants. To achieve understanding and consensus, it is enough for the majority to take an agreed compromise position, then the participants in the conflict who are in the minority, due to their internal moral convictions, will be forced to agree and fulfill the agreements reached by the majority.

At the level of experimental practice, this approach is confirmed by the author's educational program implemented in the process of simulation games. It provided for the formation of theoretical aspects of the use of BGP for conflict resolution among the EG members and the approbation of conflict resolution methods developed on the basis of the blockchain. A comparison of the results of the ascertainment and control stages of the experiment showed significant changes in the respondents' attitude to conflict resolution strategies. The number of supporters of the strategies of rivalry, avoidance, adaptation, which are the least productive for achieving positive consequences of conflicts, has significantly decreased, while the number of supporters of more effective strategies of compromise and cooperation has significantly increased.

The results of research work can be useful for the further development of the theory and practice of pedagogical conflictology and find practical use in resolving conflict situations in various social and professional environments.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Improvement of morphofunctional status of students by modern means of motor activity

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ABSTRACT

Aim: To study the dynamics of students' morphofunctional status indicators during their high-intensity interval training (HIIT).

Materials and Methods: The research involved 132 male students divided into three groups: students in Group No. 1 (n = 27) did HIIT; Group No. 2 (n = 69) – various sports (sports games, martial arts, strength sports, athletics, swimming, cycling); Group No. 3 (n = 36) – general physical training. The morphofunctional status was assessed using indicators of height, body weight, lung vital capacity, hand dynamometry, heart rate, and blood pressure. The generalized indicator of students' morphofunctional status was assessed using the methodology developed by Ye. A. Pyrohova.

Results: It has been proven that HIIT effectively improves students' morphofunctional status during their studies. Most of the morphological and functional indicators of students who practiced HIIT were significantly ($p \leq 0.05$-0.001) better in the 3rd and 4th instructional years than those of students who practiced general physical training. The most significant effect of HIIT was an improvement in indicators of body weight and in the functioning of students' cardiovascular and respiratory systems. Compared to Group No. 2, the indicators of Group No. 1 do not have significant differences ($p > 0.05$).

Conclusions: The results obtained allow us to assert the effectiveness of HIIT, as well as any kind of sport, during their studies at higher educational institutions under martial law, to increase students' motor activity, improve their morphofunctional status, physical and mental performance, strengthen their somatic and mental health, and promote harmonious personal development.

KEY WORDS: motor activity, HIIT, morphological and functional indicators, health, students

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INTRODUCTION

It is well known that one of the most critical factors in a healthy lifestyle for modern people is motor activity. Motor activity plays a key role in maintaining human somatic health, improving physical condition (strengthening muscles, the musculoskeletal system, cardiovascular and other body systems), mental well-being (reducing stress and anxiety), and overall performance [1]. Regular physical activity is an effective way to prevent many diseases, including cardiovascular disease and obesity, and also improves quality of life [2]. At the same time, technological advances and the information revolution have significantly reduced motor activity in modern humans. In the last 20-30 years alone, the proportion of physical labor in daily life has decreased tenfold, leading to a sedentary lifestyle [3]. Decreased physical activity is the cause of many diseases, slows down development in childhood and adolescence, accelerates aging, and reduces life expectancy [4]. Students of higher educational institutions are no exception, as their motor activity is limited by several factors: distance learning (due to martial law in Ukraine, frequent air raid sirens, the COVID pandemic, etc.), the digitization of education, large amounts of educational information, psychological difficulties (constant stress, poor health, etc.), lack of family traditions, insufficient awareness of the benefits of motor activity, etc. [5]. In addition, scientists [6] emphasize significant barriers to students' participation in regular physical activity, including a lack of time, motivation, and resources. Researchers [7] also link low levels of motor activity among students to stress, poor daily routines and nutrition, the lack of a systematic approach to promoting motor activity in higher educational institutions, and limited integration of innovative technologies and modern types of motor activity into students' physical education.

One modern type of motor activity that can help solve the problem outlined above is high-intensity

interval training (HIIT), which involves performing intense exercises with weights or your own body weight, with short rest periods between sets [8]. HIIT includes modern physical exercises combined into complexes lasting 10 to 30 minutes, depending on students' physical fitness [9]. HIIT does not require special conditions for training sessions (exercises can be performed in a gym, outdoors, or at home), expensive equipment, and can be done in a short period of time (does not take much time), making it popular among today's youth [10], which is appropriate for their use in the physical education of students during their studies in conditions of martial law to increase the volume of students' motor activity, improve their morphofunctional status, and strengthen their health.

AIM

To study the dynamics of students' morphofunctional status indicators during their high-intensity interval training (HIIT). Objectives: 1) to study the dynamics of morphological and functional indicators of students' bodies under the influence of HIIT during their studies at the university; 2) to conduct a comparative analysis of the studied indicators of students who practiced HIIT and students who practiced other types of motor activity.

MATERIALS AND METHODS

PARTICIPANTS

The research, conducted in 2022-2025, involved 132 male students of Khmelnytskyi National University (KhNU, Khmelnytskyi, Ukraine, specialties: A7 – Physical Culture and Sports, J3 – Tourism and Recreation), who chose a type of motor activity to engage in during their studies at the university while martial law was in effect in Ukraine: Group No. 1 (n = 27), whose students engaged in HIIT during their physical education training sessions; Group No. 2 (n = 69), whose students practiced various sports during their physical education training sessions (sports games, martial arts, strength sports, athletics, swimming, cycling); Group No. 3 (n = 36), whose students practiced traditional methods (general physical training) during their physical education training sessions. Coaches trained the students in Group No. 1 and Group No. 2 in specific sports as part of the university's sports program (sports clubs). In contrast, Group No. 3 was trained by a physical education instructor. The total number of hours of motor activity at the university per week was the same across all groups: 6 hours (3 training sessions of 2 hours each). Limitations of the research: we did not take into account the types of activities and the amount of motor activity of students outside of academic hours (outside the university). Inclusion criteria: all students were male, students belonged to the main medical group (had no health deviations or contraindications for the selected types of motor activity), students participated in sports clubs at their own choice, which are available at KhNU (there was no special selection for the groups; analysis of the initial indicators of the morphofunctional status of students in all three groups showed that they were reliably the same ($p > 0.05$)). The exclusion criterion was the student's voluntary desire to withdraw from the research at any time.

RESEARCH METHODS

Theoretical analysis and generalization of literature sources, biomedical methods, statistical methods. The method of theoretical analysis and generalization of literary sources was used to conduct an analytical review of scientific sources on the outlined range of issues (20 sources from MedLine, Scopus, Web of Science databases were analyzed).

Biomedical methods were used to determine the morphofunctional status of students. The morphofunctional status was studied according to the following indicators: morphological – height (H), body weight (BW), vital capacity of the lungs (VC), hand dynamometry (HD); functional – resting heart rate (HR), systolic blood pressure (SBP), and diastolic blood pressure (DBP). The generalized indicator of the morphofunctional status of students was assessed using the methodology of Ye. A. Pirohova, which involves determining the morphofunctional status index (MSI) [11]. The MSI was calculated using the formula that takes into account the age, body weight, height, heart rate, and blood pressure of students at rest, and was assessed in c. u.:

$$MSI = (700 - 3\times HR - 2.5\times BP\ aver. - 2.7\times A + 0.28\times BW) / (350 - 2.6\times A + 0.21\times H),$$

BW – body weight, kg; H – height, cm; A – age, number of full years; BP aver. – average blood pressure (mm Hg), determined by the formula:

$$BP\ aver. = ((SBP - DBP) / 3) + DBP.$$

The level of morphofunctional status of students is considered low if the MSI is 0.375 c. u. and less; below average – 0.376-0.525 c. u.; average – 0.526-0.675 c. u; above average – 0.676-0.825 c. u; high – 0.826 c. u and above.

STATISTICAL METHODS

Statistical analysis was applied to correctly process the data and identify the difference between the indicators

under study. The significance of the difference in the results of the students was determined based on the Student's t-test. The significance for all statistical tests was set at $p \leq 0.05$. All statistical analyses were performed with the SPSS software, version 11.0.

ETHICS

This research followed the regulations of the World Medical Association Declaration of Helsinki and ethical principles for medical research involving human subjects and was approved by the Academic Council of Khmelnytskyi National University (Protocol No. 7 dated 10.05.2022). Informed consent was received from all students who took part in this research.

FRAMEWORK

The study was conducted according to the research plan of the Khmelnytskyi National University for 2024-2027 within the topic "Formation of Personality as a Subject of Self-Creation" (state registration number 0119U103663).

RESULTS

A study of students' height indicators shows no significant difference among the three groups across all instructional years ($p > 0.05$). The dynamics of students' height indicators during their studies show that the average values increased across all groups. Still, the results for students in the 1st and 4th instructional years do not differ significantly (Table 1).

By studying students' body weight, we found that the average values during the 1st and 2nd instructional years did not differ significantly ($p > 0.05$). In the 3rd instructional year, the body weight indicators of students in Group No. 1 were significantly ($p \leq 0.05$) lower than those in Group No. 3, by 4.4 kg, and in the 4th instructional year, by 5.3 kg. No significant difference ($p > 0.05$) was found between Group No. 1 and Group No. 2 (Table I). During their studies, the students who practiced HIIT showed a non-significant increase in body weight of 2.4 kg ($p > 0.05$), while those who practiced other sports gained 2.8 kg ($p > 0.05$). The students who practiced general physical training gained 5.7 kg ($p \leq 0.01$), which may hurt their health. The analysis of body weight indicates that the physical loads students receive during HIIT and other sports help stabilize their body weight.

The analysis of VC indicators showed that in the 1st and 2nd instructional years, no significant differences were found among the indicators of students in Group No. 1, Group No. 2, and Group No. 3 ($p > 0.05$). No significant difference between VC indicators was found in the 3rd and 4th instructional years; however, in the 4th instructional year, the difference between the indicators of Group No. 1 and Group No. 3 is significant and amounts to 252.3 ml, and between Group No. 1 and Group No. 2 – 185.9 ml. During the studies, the VC indicators improved in all student groups. Still, in Group No. 1 and Group No. 2 in the 4th instructional year, the VC indicators were significantly better than in the 1st instructional year by 338.4 ml ($p \leq 0.05$) and 191.8 ml ($p \leq 0.05$), respectively. At the same time, in Group No. 3, the difference between the VC indicators of the 4th and 1st instructional years is 177.4 ml and is insignificant ($p > 0.05$).

The muscular system of students was assessed using hand dynamometry indicators. In the 1st instructional year, the indicators across all groups did not differ significantly ($p > 0.05$). In the 2nd-4th instructional years, the dynamometry indicators of students in Group No. 1 are better compared to the indicators of Group No. 2 and Group No. 3. Thus, in the 4th instructional year, the difference between the indicators of students in Group No. 1 and Group No. 2 is 2.8 kg, and between Group No. 1 and Group No. 3 – 4 kg. However, the difference is not significant ($p > 0.05$) (Table I), which indicates the greater effectiveness of HIIT training sessions on the strength indicators of students. During the research period, the strength indicators of students in Group No. 1 and Group No. 2 improved significantly ($p \leq 0.05$), whereas those of students in Group No. 3 did not ($p > 0.05$).

The study of HR dynamics at rest shows that in the 1st and 2nd instructional years, the average indicators of Group No. 1, Group No. 2, and Group No. 3 did not differ significantly ($p > 0.05$). In the 3rd instructional year, the difference between the HR indicators of students in Group No. 1 and Group No. 3 was 3.6 beats/min, and in the 4th instructional year, it was 4.9 beats/min, which is significant ($p \leq 0.05$-0.001). In the 4th instructional year, the HR indicators of students in Group No. 2 were also significantly better than those in Group No. 3. At the same time, no significant difference between the indicators of Group No. 1 and Group No. 2 was found in all instructional years ($p > 0.05$), which indicates the effectiveness of both HIIT and other sports in improving the functional capabilities of the cardiovascular system of students during their studies in conditions of martial law. This is confirmed by a significant difference between the indicators of the 4th and 1st instructional years in Group No. 1 and Group No. 2 ($p \leq 0.001$) and the absence of a difference in Group No. 3 ($p > 0.05$) (Table 2).

The study of students' systolic and diastolic blood pressure enabled us to observe positive dynamics and improvements in the average indicators of students across all studied groups during their studies. However, the

Table 1. Dynamics of morphological indicators of students in the process of different types of motor activity during their studies (n = 132)

Year of study	Group No.1 (n=25)	Group No. 2 (n=108)	Group No.3 (n=25)	Significance of the difference		
	X±m	X±m	X±m	t_{1-2} (p)	t_{1-3} (p)	t_{2-3} (p)
Students' height, cm						
1st	176.3±2.11	175.8±1.45	176.1±1.96	0.20 (p>0.05)	0.07 (p>0.05)	0.12 (p>0.05)
2nd	176.5±2.03	175.9±1.39	176.4±1.92	0.24 (p>0.05)	0.04 (p>0.05)	0.24 (p>0.05)
3rd	176.8±1.99	176.2±1.40	176.7±1.91	0.25 (p>0.05)	0.04 (p>0.05)	0.21 (p>0.05)
4th	177.0±1.98	176.6±1.39	176.9±1.87	0.17 (p>0.05)	0.04 (p>0.05)	0.13 (p>0.05)
t_{1-4} (p)	0.24 (p>0.05)	0.40 (p>0.05)	0.30 (p>0.05)			
Body weight, kg						
1st	68.4±1.21	70.2±0.94	70.4±1.16	1.17 (p>0.05)	1.19 (p>0.05)	0.13 (p>0.05)
2nd	69.3±1.28	71.6±0.95	72.5±1.21	1.44 (p>0.05)	1.82 (p>0.05)	0.59 (p>0.05)
3rd	70.3±1.31	72.3±1.07	74.7±1.34	1.18 (p>0.05)	2.35 (p≤0.05)	1.40 (p>0.05)
4th	70.8±1.34	73.0±1.05	76.1±1.38	1.29 (p>0.05)	2.76 (p≤0.05)	1.79 (p>0.05)
t_{1-4} (p)	1.33 (p>0.05)	1.98 (p>0.05)	3.16 (p≤0.01)			
Vital capacity, ml						
1st	4205.4± 112.35	4166.1± 72.49	4114.1± 123.22	0.29 (p>0.05)	0.55 (p>0.05)	0.36 (p>0.05)
2nd	4376.1± 111.47	4234.5± 70.28	4192.9± 119.65	1.07 (p>0.05)	1.12 (p>0.05)	0.30 (p>0.05)
3rd	4452.2± 107.16	4292.6± 64.12	4250.7± 116.37	1.28 (p>0.05)	1.27 (p>0.05)	0.31 (p>0.05)
4th	4543.8± 105.28	4357.9± 58.48	4291.5± 114.84	1.54 (p>0.05)	1.62 (p>0.05)	0.52 (p>0.05)
t_{1-4} (p)	2.20 (p≤0.05)	2.06 (p≤0.05)	1.05 (p>0.05)			
Hand dynamometry, kg						
1st	40.1±2.13	41.3±1.11	40.6±2.44	0.50 (p>0.05)	0.15 (p>0.05)	0.26 (p>0.05)
2nd	42.4±2.04	42.3±0.96	41.8±2.31	0.04 (p>0.05)	0.19 (p>0.05)	0.20 (p>0.05)
3rd	45.4±1.92	44.2±0.83	42.9±2.25	0.57 (p>0.05)	0.85 (p>0.05)	0.54 (p>0.05)
4th	47.9±1.85	45.1±0.78	43.9±2.12	1.39 (p>0.05)	1.42 (p>0.05)	0.53 (p>0.05)
t_{1-4} (p)	2.76 (p≤0.01)	2.80 (p≤0.01)	1.02 (p>0.05)			

Notes: X – arithmetic mean; m – error of arithmetic mean; t – Student's test value; p – reliability value
Source: compiled by the authors of this study

indicators of SBP and DBP in Group No. 1, Group No. 2, and Group No. 3 in all instructional years did not differ significantly from each other (p > 0.05) (Table 2).

The analysis of the students' morphofunctional status index showed that in the 1st and 2nd instructional years, there was no significant difference between the indicators of the studied groups (p > 0.05). In the 3rd and 4th instructional years, students in Group No. 1 showed significantly better MSI indicators than in Group No. 2 and Group No. 3 by 0.047 and 0.077 c. u (p ≤ 0.05-0.01) in the 3rd instructional year and by 0.070 and 0.122 c. u (p ≤ 0.001) in the 4th instructional year, respectively (Table II). The data obtained indicate that HIIT has a positive impact on the functioning of the central life-support systems of students' bodies during their studies at higher educational institutions under martial law.

Table 2. Dynamics of functional indicators of students in the process of different types of motor activity during their studies (n = 132)

Year of study	Group No.1 (n=25)	Group No. 2 (n=108)	Group No.3 (n=25)	Significance of the difference		
	X±m	X±m	X±m	t_{1-2} (p)	t_{1-3} (p)	t_{2-3} (p)
Resting heart rate, cm						
1st	71.0±1.35	70.7±0.55	71.2±1.21	0.21 (p>0.05)	0.11 (p>0.05)	0.38 (p>0.05)
2nd	68.2±1.28	69.5±0.52	70.6±1.16	0.94 (p>0.05)	1.39 (p>0.05)	0.87 (p>0.05)
3rd	66.5±1.24	68.2±0.47	70.1±1.12	1.28 (p>0.05)	2.15 (p≤0.05)	1.56 (p>0.05)
4th	64.8±1.17	66.7±0.44	69.7±1.06	1.52 (p>0.05)	3.10 (p≤0.001)	2.61 (p≤0.01)
t_{1-4} (p)	3.47 (p≤0.001)	5.68 (p≤0.001)	0.93 (p>0.05)			
Systolic blood pressure, mmHg						
1st	123.5±2.08	122.9±0.91	123.7±2.14	0.26 (p>0.05)	0.07 (p>0.05)	0.34 (p>0.05)
2nd	122.5±1.99	122.1±0.82	122.9±2.03	0.19 (p>0.05)	0.14 (p>0.05)	0.37 (p>0.05)
3rd	121.4±1.92	121.5±0.74	122.2±1.93	0.05 (p>0.05)	0.29 (p>0.05)	0.26 (p>0.05)
4th	120.2±1.85	121.0±0.68	121.3±1.86	0.39 (p>0.05)	0.42 (p>0.05)	0.15 (p>0.05)
t_{1-4} (p)	1.19 (p>0.05)	1.67 (p>0.05)	0.85 (p>0.05)			
Diastolic blood pressure, mmHg						
1st	70.2±1.12	70.5±0.54	70.4±1.08	0.24 (p>0.05)	0.13 (p>0.05)	0.08 (p>0.05)
2nd	70.0±1.07	70.4±0.51	70.1±0.97	0.34 (p>0.05)	0.07 (p>0.05)	0.27 (p>0.05)
3rd	69.7±0.98	70.3±0.46	69.9±0.92	0.55 (p>0.05)	0.15 (p>0.05)	0.39 (p>0.05)
4th	69.5±0.96	70.2±0.42	70.1±0.88	0.67 (p>0.05)	0.48 (p>0.05)	0.10 (p>0.05)
t_{1-4} (p)	0.47 (p>0.05)	0.43 (p>0.05)	0.22 (p>0.05)			
Morphofunctional status index, c.u.						
1st	0.675±0.020	0.678±0.013	0.680±0.022	0.13 (p>0.05)	0.17 (p>0.05)	0.08 (p>0.05)
2nd	0.719±0.019	0.694±0.011	0.683±0.020	1.14 (p>0.05)	1.30 (p>0.05)	0.48 (p>0.05)
3rd	0.764±0.018	0.717±0.010	0.687±0.019	2.28 (p≤0.05)	2.94 (p≤0.01)	1.40 (p>0.05)
4th	0.813±0.016	0.743±0.009	0.691±0.018	3.81 (p≤0.001)	5.07 (p≤0.001)	2.58 (p≤0.05)
t_{1-4} (p)	5.39 (p≤0.001)	4.11 (p≤0.001)	0.39 (p>0.05)			

Notes: X – arithmetic mean; m – error of arithmetic mean; t – Student's test value; p – reliability value
Source: compiled by the authors of this study

DISCUSSION

Scientists [12] argue that the problem of low motor activity among students is becoming increasingly relevant in the modern educational environment, especially during the legal regime of martial law in Ukraine. Hypodynamia caused by a sedentary lifestyle, excessive use of digital technologies, and academic workload negatively affects the physical and psycho-emotional health of young people [13].

Scientists [14] identify the main problems in students' motor activity as a lack of motivation for regular physical activity, insufficient awareness of the impact of motor

activity on health and quality of life, and limited or ineffective physical education and health services provided by higher educational institutions. Digitalization also significantly affects student involvement in motor activity, promoting a passive lifestyle through prolonged gadget use.

Experts [15] see prospects for improving the situation in improving the quality of physical education and health services, justifying, developing, and implementing modern programs focused on the needs of students, using innovative technologies and recreational approaches; increasing motivation by organizing events aimed at promoting a healthy lifestyle, active recreation, and regular motor activity; using an individual approach that will facilitate the implementation of personalized recommendations for motor activity based on an assessment of students' health status; and forming a culture of health through educational and awareness-raising activities on the importance of motor activity for disease prevention and health promotion.

Considering the capabilities of the higher educational institution and the availability of appropriate trainers in various types of motor activity (sports) within the Department of Physical Education, we suggested that students independently choose the type of motor activity for their training sessions during their studies. Among the proposed types of motor activity, students were most interested in HIIT as a modern form of motor activity and an innovative approach to physical culture and health technology. In the literature [16], we find that HIIT refers to a type of physical training whose main characteristic is the alternation of short periods of intense anaerobic exercise with shorter periods of rest. Experts [17] consider the "Tabata" method to be the most popular and effective type of HIIT in the world. Back in 1996, Japanese professor Izumi Tabata developed a technique to improve athletes' aerobic capacity, which he used to train the Japanese Olympic speedskating team. The method was based on athletes performing 20-second ultra-intense exercises (pedaling) on an exercise bike, bringing their maximum oxygen consumption to 170 %, followed by a 10-second rest. The professor chose 8 work-rest cycles. Each set lasts only 4 minutes. The number of sets and the rest time between sets depend on the athletes' level of physical fitness [18]. Today, interval training using the "Tabata" technique is widely used among students. The technique allows you to use a series of exercises (100 or more) from modern sports, which can be performed independently or in a group, indoors or outdoors, with additional sports equipment or your own body weight, in pairs, over a short period of time. The advantages of HIIT using the "Tabata" technique also include: a significant increase in students' aerobic capacity, increased oxygen consumption, effective fat burning, compensation for a sedentary lifestyle, and time savings in training [19]. As a result of introducing HIIT into university training sessions, we found that systematic HIIT training, on a par with other sports (sports games, martial arts, strength sports, athletics, swimming, cycling), is an effective means of increasing students' MA, improving their morphofunctional status, strengthening their health, and improving their psycho-emotional state. Our results confirm, complement, and expand on the conclusions of many scientists [8, 9, 18, 20], who have studied students' motor activity and the effectiveness of HIIT and other modern methods for strengthening young students' health.

CONCLUSIONS

The research shows that HIIT effectively improves students' morphofunctional status during their studies. Most morphological and physiological indicators of students who practiced HIIT were significantly ($p \leq 0.05$-0.001) better in the 3rd and 4th instructional years than those of students who practiced general physical training during their physical education training sessions. The most significant effect of HIIT was an improvement in body weight indicators and in the functioning of the cardiovascular and respiratory systems of students. Compared to Group No. 2, the indicators of Group No. 1 do not have significant differences ($p > 0.05$), which also allows us to assert the effectiveness of sports activities during studies in higher educational institutions under martial law to increase the volume of students' motor activity, improve their morphofunctional status, physical and, accordingly, mental performance, and strengthening their somatic and mental health.

PROSPECTS FOR FURTHER RESEARCH

It is planned to investigate the dynamics of students' somatic health indicators during HIIT training sessions.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Assessment of interpersonal communications between doctors and patients in a military hospital: Factor analysis of the hcahps survey

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ABSTRACT

Aim: The aim of the study was to assess interpersonal communication between doctors and patients in a military hospital using factor analysis of the HCAHPS questionnaire.

Materials and Methods: A cross-sectional study was conducted, during which military personnel who were undergoing inpatient treatment in Poltava and Kremenchuk hospitals were surveyed. The HCAHPS questionnaire consists of 21 individual-level questions that are presented as 8 hospital-level indicators to reflect specific aspects of the healthcare experience. The process of extracting factors from the correlation matrix using principal component analysis led to the identification of factors. Factors were removed until then, regardless of whether the factor's influence on the variance was significant or not.

Results: The percentage of variance is the largest in the first factor – 36.566%. The higher the percentage of variance, the greater the weight of this factor. Therefore, the first factor has the largest percentage of loading. The cumulative percentage accumulated to the last factor is 69.351, which indicates an appropriate factor solution. Factor analysis allowed us to identify 4 main components that determine the main features of patient satisfaction with medical care: "Responsibility for one's health", "Adherence to treatment", "Deontology" and "Mercy".

Conclusions: Based on the fact that patient satisfaction with care is a predictor of his future behavior regarding the attitude towards treatment, it can be stated that all the established components are extremely important for further obtaining a positive result of the patient's recovery. Therefore, organizational work aimed at improving the established predictors is of great importance for the organization of medical care.

KEY WORDS: factor analysis, military hospital, interpersonal communications, HCAHPS, quality of medical help

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INTRODUCTION

Since 2008, the Centers for Medicare and Medicaid Services (CMS) has been surveying patients using the Hospital Consumer Assessment of Healthcare Providers and Systems (HCAHPS) survey to assess patients' perceptions of care [1, 2]. Conducting such a study in Ukraine will allow to evaluate communications with medical personnel and the quality of medical care in the conditions of a full-scale Russian invasion of the country, which will significantly change the access and organization of medical care to the population. The HCAHPS questionnaire allows you to assess the result of complex human behavior, which culminates in communication between the service provider - a healthcare professional - and its consumer - a patient [3, 4].

As a result of such communication, feelings and needs on both sides are revealed. On the patient's side, this may include the prognosis of the disease, its severity, cost, emotional support, side effects of treatment, preservation of autonomy and respect. Communication itself contains the process, context, and purpose of the meeting. Since medical personnel, by virtue of their professional knowledge and skills, own the situation, they are the subject in this relationship. On the one hand, this gives the right to manage the patient and prescribe treatment. On the other hand, the patient acts as an object and also has the right to treatment with respect and empathy from the medical staff towards his person [5].

The needs of the provider may be clinical, logistical, or resource-related due to shortages. Often the needs of the patient and the healthcare professional coincide, but they may also conflict. Furthermore, needs are often complex and vary significantly between patients [2]. Ultimately, it is necessary to make a joint decision, the proposal of which initially comes from the medical worker. It is necessary to take into account that communication takes place in the current mode, that is, during the stay in the hospital, as well as after discharge. Therefore, it is important to take into account all components of the context of the conversation.

The success of treatment will depend on how much the patient agrees to carry out the doctor's prescription. Therefore, understanding in the relationship of the doctor-patient, nurse-patient pair is of extremely great importance [6].

AIMS

The aim of the study was to assess interpersonal communication between doctors and patients in a military hospital using factor analysis of the HCAHPS questionnaire.

MATERIALS AND METHODS

A cross-sectional study was conducted, during which military personnel who were undergoing inpatient treatment in Poltava and Kremenchuk hospitals were surveyed. Military personnel who were undergoing inpatient treatment there were surveyed. A Google form questionnaire was sent to patients.

The HCAHPS questionnaire consists of 21 individual-level questions that are presented as 8 hospital-level indicators to reflect specific aspects of the healthcare experience [7-9]:

I. Nurses' communication:
1. How often during your hospital stay did the nurses treat you with courtesy and respect?
2. How often did the nurses listen to you carefully during your hospital stay?
3. During your hospital stay, how often did the nurses explain things in a way that you could understand?

II. Doctors' communication:
4. How often during your hospital stay did the doctors treat you with courtesy and respect?
5. How often did the doctors listen to you carefully during your hospital stay?
6. During your hospital stay, how often did the doctors explain things in a way that you could understand?

III. Responsiveness of suppliers:
7. How often during your hospital stay, after you pressed the call button, did you receive help as soon as you wanted it?
8. Do you need help from nurses or other hospital staff to get to the toilet or use the bedpan while you are in the hospital?
9. How often were you helped to get to the toilet or use the bedpan whenever you wanted?

IV. Cleanliness of the hospital environment and silence of the hospital environment
10. How often were your room and bathroom clean during your hospital stay?
11. How often was it quiet around your room at night during your stay in the hospital?

V. Communication about medications:
12. Were you given any medications during your hospital stay that you had not taken before?
13. Before giving you any new medication, how often did the hospital staff tell you what the medication was for?
14. How often did hospital staff describe possible side effects in a way you could understand before giving you any new medication?

VI. Checkout information
15. During your hospital stay, did doctors, nurses, or other hospital staff talk to you about whether you would get the care you needed when you left the hospital?
16. Did you receive written information during your hospital stay about what symptoms or health problems to look out for after you leave the hospital?
17. During my stay in hospital, the staff took into account my preferences and those of my family or carer when deciding what my health care needs would be after I was discharged.
18. When I left the hospital, I had a good understanding of the things I was responsible for in managing my health.
19. When I left the hospital, I clearly understood the purpose of taking each of my medications.

VII. Overall satisfaction with the hospital:
20. We would like to know your overall assessment of your stay at [Hospital Name]. This is a stay that ended around [discharge date]. Please do not include any other hospital stays in your response.

VIII. The likelihood that they would recommend the hospital to a close friend or family member
21. Would you recommend this hospital to your friends and family?

Patients provide answers to the proposed questions on a Likert` scale: 1 - "never", 2 - "sometimes", 3 - "usually", 4 - "always". Only one question "Were you given any medication that you had not taken before" contains 2 answer options: "yes" and "no". [10].

STATISTICAL ANALYSIS

Data entry and statistical analysis were performed using IBM SPSS version 25.0. The process of extracting factors from the correlation matrix using principal component analysis led to the identification of factors. The first task of factor analysis was to select interacting variables whose mutual correlations accounted for the largest share of the variance. These variables formed the first factor. The first factor was then eliminated from the remaining variables and those whose interaction accounted for the largest proportion of the remaining

Table 1. Socio-demographic profile of military personnel who were inpatients in the hospital.

Demographic characteristics		Number of respondents	
		Abs.	%
Gender	Man	83	94.3
	Woman	5	5.7
	Total	**88**	**100.0**
Marital status	Lonely	14	15.9
	In a steady relationship	9	10.2
	Married	53	60.2
	Divorced	11	12.5
	Widower/widow	1	1.1
	Total	**88**	**100.0**
Place of residence	City	68	77.3
	Village	17	19.3
	Urban-type settlement	3	3.4
	Total	**88**	**100.0**
Education level	Average education	16	18.2
	Secondary special education	38	43.2
	Higher education	34	38.6
	Total	**88**	**100.0**
Profession	Builder	9	10.2
	Military	38	43.2
	Driver	11	12.5
	Teacher	2	2.3
	Economist	4	4.5
	Electric gas boiler	4	4.5
	Engineer	3	3.4
	Medical worker	6	6.8
	Mechanic	4	4.5
	Worker	4	4.5
	Lawyer	3	3.4
	Total	**88**	**100.0**
Military status	Sewage service soldier	4	4.5
	Contract soldier	12	13.6
	Professional military	8	9.1
	Mobilized	64	72.7
	Total	**88**	**100.0**
Rank	Cadet	1	1.1
	Ordinary	38	43.2
	Sergeant	23	26.1
	Officer	10	11.4
	Other	16	18.2
	Total	88	100.0
Hospital location	Poltava	30	34.1
	Lubny	58	65.9
	Total	88	100.0

Source: compiled by the authors of this study

Table 2. KMO-value and Bartlett criterion

Indicator	Value
Kaiser-Mayer-Olkin sampling adequacy measure (KMO)	0.762
Sphericity test - approximate Chi-square	901.132
Barletta criterion	171
p-value	<0.001

Source: compiled by the authors of this study

variance were again selected. These variables formed the second factor. The procedure for removing factors was continued until all the total variance of the variables was exhausted independently. Factors were removed in descending order of their influence on the variance of the variables. Factors were removed until then, regardless of whether the factor's influence on the variance was significant or not.

The next step after extracting the factors was to rotate them. The goal of rotation was to obtain a simple structure, which corresponds to a larger loading value of each variable on only one factor and a smaller one on the other factors. The loadings reflected the relationship between the variable and the factor, being similar to the correlation coefficient. The loading value lies in the range from -1 to 1. The ideal simple structure assumes that each variable has zero loading values on all factors except one, for which the loading of this variable is close to 1/-1 [11].

To identify the most significant factors and their structure, we used the principal components method. The mathematical model of principal components is based on the logical assumption that the values of a set of interdependent features generate some general result, which helps to identify hidden indicators (factors) that are responsible for the presence of linear statistical relationships (correlations) between them[12].

When using a conventional correlation matrix, the number of common factors was considered equal to the number of eigenvalues greater than or equal to one (Kaiser criterion), and only those factors were selected that caused a "separate" variance equivalent to at least the variance of one variable. The eigenvalues were plotted on the graph depending on the ordinal number of the factor (having previously ranked them in order of "decreasing importance"), by selecting the number of factors at the "inflection point" of the steep section of the graph into the gentle section. This technique, proposed by Cattell, is called the "rocky scree criterion" [13]. A probability <0.05 was considered statistically significant.

ETHICS

This work complies with the principles of the Declaration of Helsinki.

RESULTS

The medical and demographic characteristics of the respondents are presented in Table 1, and as indicated therein, the majority of patients were married (60.2%), lived in rural areas (19.3%), were male (94.3%), and most of them were military personnel (43.2%) (the profession they had before the war is indicated here). The average age of patients was 39.5±8.7% (max -59.0%; min - 24.0%).

As shown in Table 2, the KMO (Kaiser-Meyer-Opkin) value demonstrates acceptable sample adequacy for factor analysis. Bartler's sphericity test shows a statistically significant result (p<0.00). Therefore, the selected number of factors is sufficient to explain the sample coefficients [14].

By default, in the factor analysis procedure, each variable has a unity value. This indicator is equal to the fraction of the variance of a variable due to the cumulative influence of factors. In this case, "Understanding responsibility for one's health" – 0.923 makes the greatest contribution to the community of other variables. "Understanding responsibility for one's health" is a guarantee that the patient will continue treatment related to the underlying disease after discharge (Table 3).

The percentage of variance is the largest in the first factor – 36.566%. The higher the percentage of variance, the greater the weight of this factor. Therefore, the first factor has the largest percentage of loading. The cumulative percentage accumulated to the last factor is 69.351, which indicates an appropriate factor solution (Table 4, Fig.1).

A correlation matrix was created to identify relationships between respondents' responses in order to assess the quality of communication between medical personnel and military patients. The correlation matrix of responses is presented in Table 5.

When determining the correlations between responses regarding medical staff communication, it can be noted that the average level of correlation is between:

• **"Polite attitude of nurses"** was related to " Attentive listening by nurses", "Polite attitude of doctors ", "Nurses' clear explanations" and "Doctors' clear explanations",

Table 3. Matrix of components and commonalities

Question	Primary	Withdrawal
I. Nurses' communication		
Polite attitude	1.000	0.661
Attentive listening	1.000	0.551
Clear explanation	1.000	0.577
II. Doctors' communication		
Polite attitude	1.000	0.852
Attentive listening	1.000	0.766
Clear explanation	1.000	0.791
III. Responsiveness of suppliers		
Getting help immediately after the call	1.000	0.494
Do you need help getting to the toilet?	1.000	0.733
Did they help you get to the toilet or use the bedpan?	1.000	0.814
IV. Cleanliness of the hospital environment and silence of the hospital environment		
Bathroom cleanliness	1.000	0.440
Silence at night	1.000	0.549
V. Communication about medications		
Medicines were given that had not been taken before	1.000	0.490
Explanation of the use of new medications	1.000	0.821
Explanation of side effects	1.000	0.864
VI. Checkout information		
Did the doctors and nurses talk about getting care after discharge?	1.000	0.454
Did you receive any recommendations after discharge?	1.000	0.845
Taking into account personal needs in medical care	1.000	0.742
Understanding responsibility for one's health	1.000	0.923
Understanding the purpose of taking each medication	1.000	0.875
VII. Overall satisfaction with the hospital		
Overall assessment of hospital stay	1.000	0.677
Recommending the hospital to friends	1.000	0.586

Source: compiled by the authors of this study

Table 4. Dispersive characteristics of the identified factors

Component	Initial eigenvalues			Sums of squares of extraction loadings			Sums of squares of rotation loadings		
	Total	% variance	Cumulative %	Total	% variance	Cumulative %	Total	% variance	Cumulative %
1	6.947	36.566	36.566	6.947	36.566	36.566	3.731	19.636	19.636
2	1.836	9.665	46.231	1.836	9.665	46.231	2.887	15.196	34.833
3	1.815	9.553	55.784	1.815	9.553	55.784	2.383	12.540	47.373
4	1.539	8.098	63.882	1.539	8.098	63.882	2.267	11.933	59.306
5	1.039	5.469	69.351	1.039	5.469	69.351	1.909	10.045	69.351

Source: compiled by the authors of this study

"Quietness at night", and "Did you receive recommendations after discharge".

• **"Nurses' attentive listening"** correlated with "Doctors' polite attitude", "Doctors' attentive listening", and "Doctors' clear explanation".

• **"Clear explanation from nurses"** was associated with "Clear explanation from doctors".

• **"Doctors' polite attitude"** is associated with "Doctors' attentive listening", "Doctors' clear explanation", "Bathroom cleanliness", "Quiet at night", "Explanation

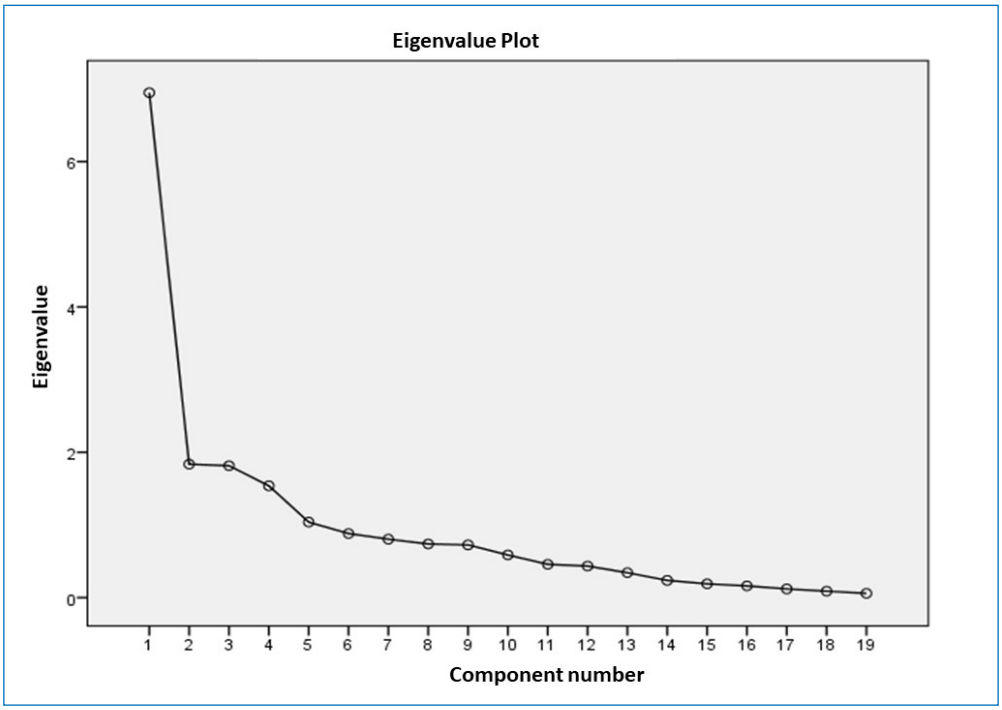

Fig. 1. Distribution of significant factors of satisfaction with communication
Picture taken by the authors

of side effects", "Taking into account personal needs in medical care", "Understanding responsibility for one's health". An inverse correlation was found with "Did doctors and nurses talk about receiving care after discharge". Obviously, this option does not depend on "Doctors' polite attitude", care after discharge is provided regardless of the doctor's manners.

• **"Listening attentively to doctors"** refers to "Clear explanation by doctors", "Cleanliness of the bathroom", "Overall assessment of the hospital stay".

• **"Clear explanation from doctors"** – with Cleanliness of the bathroom, "Quietness at night, with "Taking into account personal needs in medical care", "Understanding responsibility for one's health", "Understanding the purpose of taking each medication", "Overall assessment of the hospital stay", "Recommendation of the hospital to friends".

• **"Did they help you get to the toilet or use the bedpan?"** correlates with "Overall Hospital Stay Rating".

• **"Bathroom cleanliness"** with "Explanation of side effects", "Overall assessment of hospital stay".

• **"Quiet at night"** correlates with "Explanation of side effects", "Overall assessment of hospital stay".

• **"Explanation about the use of new medications"** with "Explanation about side effects", "Overall assessment of the hospital stay", "Recommendation of the hospital to friends".

• **"The explanation of side effects"** is related to "Overall assessment of hospital stay", "Recommendation of the hospital to friends".

• **"Taking into account personal needs in medical care"** correlated with "Understanding responsibility for one's health,""Understanding the purpose of taking each medication," and "Overall assessment of the hospital stay."

• **"Understanding responsibility for one's health"** with "Understanding the purpose of taking each medication", "Overall assessment of the hospital stay".

• **"Overall assessment of the hospital stay"** with "Recommendation of the hospital to friends."

Factor analysis allowed us to identify 4 main components that determine the main features of patient satisfaction with medical care (Table 6).

The first factor was the largest and included the following components that were of greatest importance:

- *«Polite attitude of doctors»*
- *«Clear explanation from doctors»*
- *«Polite attitude of nurses»*
- *«Silence at night»*
- *«Attentive listening by nurses»*
- *«Attentive listening by doctors»*
- *«Getting help immediately after the call»*
- *«Did they help you get to the toilet or use the bedpan?»*
- *«Explanation of the use of new medications»*
- *«Explanation of side effects»*
- *«Bathroom cleanliness»*
- *«Taking into account personal needs in medical care»,*

Table 5.

№		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	Polite attitude of nurses	1.000																				
2	Attentive listening by nurses	**0.501**	1.000							**zz**												
3	Clear explanation from nurses		0.318	1.000																		
4	Polite attitude of doctors	**0.567**	**0.678**	0.370	1.000																	
5	Attentive listening by doctors	0.289	0.409	0.184	**0.661**	1.000																
6	Clear explanation from doctors	**0.547**	**0.590**	**0.408**	**0.850**	**0.637**	1.000															
7	Getting help immediately after the call	0.312	0.326	0.207	0.390	0.203	0.325	1.000														
8	Do you need help getting to the toilet?	0.164	0.117	0.163	0.155	0.172	0.144	0.216	1.000													
9	Did they help you get to the toilet or use the bedpan?	0.252	0.231	-0.023	0.220	0.267	0.153	0.309	**0.666**	1.000												
10	Bathroom cleanliness	0.244	0.348	0.212	**0.491**	**0.410**	**0.424**	0.382	0.176	0.261	1.000											

Table 5. cont.

11	Silence at night	1.000									
12	Medicines were given that had not been taken before	0.185	1.000								
13	Explanation of the use of new medications	.384	.376	1.000							
14	Explanation of side effects	**0.406**	**0.393**	**0.860**	1.000						
15	Did the doctors and nurses talk about getting care after discharge?	-0.213	0.028	-0.120	-0.126	1.000					
16	Did you receive any recommendations after discharge?	0.060	-0.092	-0.017	-0.005	-0.041	1.000				
17	Taking into account personal needs in medical care	0.327	0.190	0.232	**0.381**	**-0.395**	-0.083	1.000			
18	Understanding responsibility for one's health	0.370	0.180	0.176	0.274	**-0.390**	0.093	**0.841**	1.000		
19	Understanding the purpose of taking each medication	0.377	0.163	0.177	0.226	-0.303	0.070	**0.754**	**0.862**	1.000	
20	Overall assessment of hospital stay	**0.418**	0.107	**0.412**	**0.547**	-0.311	-0.063	**0.428**	**0.412**	0.248	1.000
21	Recommending the hospital to friends	0.336	0.081	**0.408**	**0.481**	-0.296	0.004	0.311	0.193	0.201	**0.572**
		0.358	0.089	.231	0.243	-0.244	-0.016	0.324	0.283	0.242	**0.470**	0.280
		0.183	0.153	.253	0.227	-0.252	0.060	0.237	0.239	0.276	0.106	0.182
		0.126	0.202	.212	0.212	-0.204	0.130	0.190	0.163	0.128	0.120	0.230
		0.394	0.049	.161	0.172	-0.397	0.032	0.162	0.199	0.172	0.321	0.305
		0.530	0.137	.141	0.363	-0.423	0.016	**0.491**	**0.481**	0.433	**0.586**	**0.508**
		0.332	0.119	.180	0.320	-0.415	0.026	0.331	0.268	0.221	**0.561**	0.337
		0.597	0.119	.295	0.440	**-0.408**	0.043	**0.465**	**0.422**	0.359	**0.648**	**0.587**
		0.328	0.030	.175	0.313	-0.164	0.021	0.155	0.181	0.187	0.322	**0.558**
		0.305	0.010	.216	0.263	-0.208	0.019	0.287	0.191	0.198	0.447	**0.601**
		0.526	0.082	.333	0.368	-0.359	-0.005	0.309	0.390	0.395	**0.517**	**0.561**

Table 6. Component matrix

	Component				
	1	2	3	4	5
Polite attitude of nurses	0.702	-0.133	-0.070	-0.036	0.298
Attentive listening by nurses	0.638	-0.330	-0.225	0.109	0.026
Clear explanation from nurses	0.480	-0.326	-0.087	-0.100	0.479
Polite attitude of doctors	0.855	-0.183	-0.232	-0.002	-0.124
Attentive listening by doctors	0.635	-0.096	-0.178	0.124	-0.436
Clear explanation from doctors	0.810	-0.066	-0.324	-0.054	-0.093
Getting help immediately after the call	0.488	-0.083	-0.074	0.402	-0.021
Do you need help getting to the toilet?	0.334	0.162	0.424	0.638	0.120
Did they help you get to the toilet or use the bedpan?	0.400	0.262	0.368	0.655	-0.050
Bathroom cleanliness	0.564	-0.042	-0.077	0.166	-0.355
Silence at night	0.659	-0.056	0.033	-0.097	0.102
Medicines were given that had not been taken before	0.248	0.117	0.591	-0.198	-0.216
Explanation of the use of new medications	0.505	-0.189	0.693	-0.207	0.014
Explanation of side effects	0.626	-0.189	0.585	-0.282	0.001
Did the doctors and nurses talk about getting care after discharge?	-0.520	-0.198	0.244	-0.273	0.070
Did you receive any recommendations after discharge?	0.029	0.088	-0.042	0.282	0.498
Taking into account personal needs in medical care	0.663	0.568	-0.044	-0.244	-0.052
Understanding responsibility for one's health	0.637	0.673	-0.099	-0.217	0.114
Understanding the purpose of taking each medication	0.583	0.669	-0.078	-0.206	0.197
Overall assessment of hospital stay	0.750	-0.221	-0.042	-0.168	-0.188
Recommending the hospital to friends	0.690	-0.402	0.017	-0.007	0.263

Source: compiled by the authors of this study

when the staff decided, «Did you receive any recommendations after discharge?»

- *«Understanding responsibility for one's health»*

The second component was less loaded and included the following variables:

- ***«Taking into account personal needs in medical care», when the staff decided, «Did you receive any recommendations after discharge?»***
- ***«Understanding responsibility for one›s health»***
- ***«****Understand the purpose of taking each medication****»***
- ***«****Did you receive any recommendations after discharge?****»***
- ***«****Taking into account personal needs in medical care****»***
- ***«****Overall assessment of hospital stay****»***
- ***«****Recommending the hospital to friends****»***

The third component was less loaded and included:

- ***«****Did they help you get to the toilet or use the bedpan?****»***
- ***«****Medicines were given that had not been taken before****»***
- ***«****Explanation of the use of new medications****»***
- ***«****Explanation of side effects****»***

The fourth component included the fewest variables:

- ***«****Getting help immediately after the call****»***
- ***«****Do you need help getting to the toilet?****»***
- ***«****Did they help you get to the toilet or use the bedpan?****»***

DISCUSSION

Considering the correlations that were significant, it can be argued that the understanding of medical staff at all levels affects how they communicate with patients, how they can effectively explain the need to observe a silence regime, provide recommendations, explain the importance of taking medications, take into account the wishes and responsibilities of patients. Attention and respectful treatment of patients, such as getting to the restroom, affects the hospital rating and recommendations of the hospital to consumers.

Based on the results of the factor analysis, it was found that the first component was the largest and included ingredients that spoke about the attitude of medical staff towards the patient, namely, how communication between doctors and nurses can influence the patient to feel comfortable, despite the conditions of the hospital. All these variables speak about adherence to ethical rules within the framework of communication in the "doctor-nurse - patient-doctor" model, about the moral duty, ethical obligations and ethical norms of behavior of medical personnel, which ensure the optimal quality and effectiveness of their work to restore and preserve people's health [15]. Therefore, this

component was called "Deontology." According to the German philosopher Immanuel Kant, deontology is an ethical approach focused on rules and professional duties [16]. Kant's deontological philosophy stems from his belief that humans have the ability to reason and understand universal moral laws that they can apply to all situations. Unlike many other ethical theories, deontology does not focus on the consequences of individual actions [17]. The personal emotions behind actions are also irrelevant in Kantian deontology, as Kant believed that people do not always have rational control over their feelings. Instead, the intention behind the chosen actions is much more important. Therefore, deontologists judge actions based on what most people believe to be morally right, regardless of the actual consequences. [18].

The second component included variables that indicated feedback from the patient as a reaction to the deontological attitude of medical personnel towards him. It is obvious that every person who enters the hospital has many questions about their health, and all these questions are components of the anxiety experienced by a person who has become ill or injured. When these things are explained to a person, they show care for them, inviting them to trust. If medical personnel behave in this way, talking about medications, explaining what side effects may be, and at the same time helping the patient solve urgent, including physiological needs - getting to the toilet, then this component can be called **"Responsibility for one's health".**

The third component deals with medications and providing assistance with physiological needs. Patients noted that they were given medications that they had not previously taken and there is an indicator that indicates information about side effects, which indicates their responsible attitude to treatment. Therefore, the third component was called **"Adherence to treatment"**.

Only the response of medical personnel to patients' requests for help was discussed in the last, fourth component. A person who has begun to recover, feeling a surge of strength, still needs help with basic things so that medical personnel hear the call, the request, and come to support the victim. You can observe, or you can act – help. This is more about the vocation of a medical worker, which is why this component was named **"Mercy"**, because that was the first name of nurses – sister of mercy.

In our work, we examined interpersonal communication between patients and medical staff through the HCAHPS questionnaire. Factor analysis allowed us to identify components related to the attitude of medical staff towards patients: **"Deontology"** and **"Mercy"** and also in the form of feedback from patients - **"Responsibility for one's health"** and **"Adherence to treatment".** By their attitude through adherence to deontological rules and compassion, doctors create satisfaction. Patient satisfaction is one of the most important indicators of quality outcomes in assessing the productivity of health care systems and personnel [16]. As stated in the Institute of Medicine report, communication is at the heart of patient satisfaction and is essential for providing patient-centered care that respects the individual's unique characteristics, needs, and values [19, 20]. Satisfaction with services, communication between patient and provider are associated with important health outcomes such as: adherence to treatment, medication errors, and readmissions [21-24]. Moreover, effective communication is vital for meaningful information exchange, such as obtaining a complete and accurate medical history for diagnosis and treatment plans [19]. Satisfaction is always patient-centered, driven by the relationship between patient and healthcare professional, and ultimately influences treatment outcomes [20]. Although outcomes and satisfaction are related, the concept of "satisfaction" encompasses more than just outcomes. When determining their satisfaction with the care they receive, patients are more likely to focus on their current health status than on the level of improvement they have experienced [21, 24]. Patient satisfaction with healthcare is considered a measure of the quality of care. Pascoe defined patient satisfaction as "the response of the recipient of healthcare to important aspects of context, process, and outcome."

Patient satisfaction measures focus on patient reports or treatment evaluations, reflect the patient's perspective, and target parameters of care that patients can evaluate (i.e., patient-centered components of care).

From a hospital perspective, clinical staff and managers should be interested in patients' perceptions of care because diagnosis and treatment depend on clear, understandable communication with patients and the provision of necessary information, as well as on patients' participation in the treatment process. Patient satisfaction with care is a predictor of future behavior (e.g., adherence to doctor's appointments and intention to return to the same hospital).

Patient preferences and satisfaction can be used by providers to help make choices about how care is organized and delivered (e.g., scheduling visits and discharges), patient satisfaction can be a direct or indirect measure of outcome (e.g., how well the patient functions/acts toward their health), and as Donabedian noted, "the achievement and retention of health and satisfaction is a primary validator of the quality of care" [24]. The development of medical knowledge, especially

rehabilitation services, is historically linked to times of war [19, 20]. The disciplines of physiotherapy, physical and occupational therapy, rehabilitation engineering, and vocational rehabilitation were largely formed in response to the needs of wounded soldiers returning from World Wars I and II [19, 21, 22].

From the perspective of the patient, who in our case is a serviceman who has been injured as a result of hostilities and requires special attention, adherence to deontology and the manifestation of compassion on the part of medical professionals is of great importance, since, as noted by the UKRSOF surgeons, victims present with multiple potentially fatal injuries at the same time, requiring several emergency damage control interventions before clinicians can begin caring for the next patient [22, 23].

Statistics published by Ukrainian doctors show that over 70% of all Ukrainian combat casualties were caused by artillery and missile attacks by Russian troops, which led to significant polytrauma of many organ systems [24]. So, the wounded arrive at the hospital from the battlefield in serious condition, requiring intensive treatment and care with adherence to deontology and compassion. As a sign of the achieved goal, patients will demonstrate **"Responsibility for one's health"** and **"Adherence to treatment"** in satisfaction [25].

CONCLUSIONS

Thus, according to the results of the factor analysis of satisfaction with medical care among military personnel, it can be stated that the greatest influence falls on the nature of communication with patients, how they can effectively explain what needs to be done, taking into account the wishes of patients. In addition, this also affects the formation of a responsible attitude towards their health in the patient. It has also been established that satisfaction with medical care in the institution is influenced by the internal atmosphere that exists in the hospital, the attitude of medical staff towards the patient and towards each other. Based on the fact that patient satisfaction with care is a predictor of his future behavior regarding the attitude towards treatment, it can be stated that all the established components are extremely important for further obtaining a positive result of the patient's recovery. Therefore, organizational work aimed at improving the established predictors is of great importance for the organization of medical care.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Functional rehabilitation of the upper limb after right-hemisphere stroke: evaluation of the effectiveness of methods

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ABSTRACT

Aim: To study the impact of physical and occupational therapies on upper limb function and the degree of limitations in patients with right-hemisphere ischemic stroke complicated by neglect.

Materials and Methods: The study involved 58 patients, including 29 females and 29 males. The patients had their first ischemic stroke in the right hemisphere of the brain and unilateral spatial neglect. The average age of the patients was 68.62±10.06 years. The patients were divided into the MG and the CG in 29 individuals. In order to assess the impact of upper limb impairment and the degree of limitations experienced by patients, we used the main section of the DASH questionnaire, which consisted of 30 questions.

Results: The recovery of the upper limb in patients in the MG was better than in the CG. The recovery of the upper limb in patients according to the DASH questionnaire in the CG improved by 22%, and in the MG by 43%. Thus, after six months of using rehabilitation programs in the CG and the MG, the recovery of upper limb activities occurred almost 2 times faster in the MG than in the CG.

Conclusions: Thus, the study confirmed the positive effect of a combined program of physical and occupational therapies conducted with MG patients on the sensorimotor recovery of the upper limb.

KEY WORDS: neglect, sensorimotor recovery, physical therapy, occupational therapy

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INTRODUCTION

Cerebrovascular diseases are the leading cause of disability in the world. 70–87% of patients develop motor disorders after a stroke, while 40–56% develop upper limb impairment, often with partial recovery due to the complexity of its structure [1, 2].

The obtained data indicate the importance of timely detection and rehabilitation of unilateral spatial neglect (USN) syndrome, which often affects the quality of upper limb (UL) recovery after stroke. Motor disorders of UL after injury to the right hemisphere are complicated by a decrease in its activity and the development of inhibitory mechanisms, known as the phenomenon of unlearned use. The prognosis is worsened by disorders of spatial gnosis, orientation and attention, which complicates rehabilitation and reintegration to society. Neglect increases the duration of hospitalization and the risk of falling, significantly increases functional dependence and decreases the quality of life of the patient and their environment. Rehabilitation is complicated by anosognosia, which negatively affects the patients' awareness of their condition [3, 4].

Restoring the function of the UL in patients with USN should include measures to activate the limb in everyday life, normalize muscle tone, improve proprioceptive sensitivity, restore balance, as well as train short-term memory and attention. The success of the process depends on neuroplasticity, conscious involvement, memory, executive functions and the visual analyser [5, 6].

Despite various treatment attempts, including behavioral and motor methods, there is no consensus on their effectiveness [7]. Physical therapy focuses on the performance of exercises that contribute to the gradual restoration of motor control, and the improvement of balance using individual programs of safe activity. Occupational therapy includes functional activity training methods aimed at improving the ability to perform activities and integrate into the social environment, with

an emphasis on adaptive techniques and technologies. However, innovative approaches, such as the use of robotic devices, virtual reality simulation systems and biofeedback, are not available in most rehabilitation departments. Therefore, greater emphasis should be placed on multidisciplinary approach and active involvement of patients in the recovery process through the development of individual rehabilitation programs aimed at a faster and more sustainable return of motor functions and quality of life [8-11].

AIM

The aim of the research is to study the impact of physical and occupational therapies on upper limb function and the degree of limitations in patients with right-hemisphere ischemic stroke complicated by neglect.

MATERIALS AND METHODS

The study involved 58 patients, including 29 females and 29 males who met all the program requirements. The patients had their first ischemic stroke in the right hemisphere of the brain and unilateral spatial neglect. The average age of the patients was 68.62±10.06 years. The patients were divided by randomized sampling into the main group (MG) (29 individuals) and the comparison group (CG) (29 individuals). The study was conducted on the basis of the Vascular Neurology Department of Uzhhorod City Multidisciplinary Clinical Hospital of Uzhhorod City Council.

Based on the obtained results and applying the Canadian Occupational Performance Measure (COPM), an intervention was developed for MG patients, taking into account the individual capabilities and needs of each patient. The Predict Recovery Potential (PREP2) algorithm was used to predict interventions aimed at restoring the upper limb. The COAST format was implemented for goal setting. The SOAP algorithm was used to plan therapy. Therapeutic exercises, elements of PNF therapy (scapular, upper limb patterns), constraint-induced movement therapy (CIMT), and dual tasks were used, combined with methods aimed at correcting neglect.

CG patients received a rehabilitation program that included PNF, balance training, occupational therapy intervention, and fine motor skills exercises. After three months, and in the case of the DASH scale, after six months, a repeated final examination was performed.

To assess the activity and participation of patients with neglect, we used the main section of the DASH (Disabilities of the Arm, Shoulder and Hand) questionnaire, which consists of 30 questions related to the state of upper limb function [12]. 21 questions are devoted to the degree of difficulty in performing physical activities due to limitations in shoulder or hand function; 6 questions concern the severity of certain symptoms and 3 to social/role functions. The assessment of the results of the DASH questionnaire on upper limb movement disorders and the limitations was interpreted as follows:

- 0-20 points — minimum restrictions,
- 21-40 points — mild restrictions,
- 41-60 points — moderate restrictions,
- 61-80 points — significant restrictions,
- 81-100 points — severe dysfunction.

RESULTS

In order to assess the impact of upper limb impairment and the degree of limitations experienced by patients, we used the main section of the DASH questionnaire, which consisted of 30 questions. Data analysis is based on the main statistical characteristics of the sample obtained on 8-9th day after the stroke. According to the results of DASH, we found significant limitations in the use of the upper limb. The ±S indicators were 50.46±3.78 points, and the Me value (25%; 75%) was 50.70 (47.7; 53.7) points.

The average (50.46) and median (50.70) are almost the same, indicating a symmetrical distribution. The lack of significant deviations between the minimum and maximum and the low standard deviation indicate homogeneity of the sample. Most respondents demonstrate a moderate degree of dysfunction, concentrated in the range from 47.7 to 53.7.

These results may be due to low scores in the pain section, as 72.4% of patients scored 4 and 5 points.

The greatest difficulties that neglect patients experienced were caused by actions such as raising the upper limb (placing an object on a shelf above the head, washing and drying the hair, washing the back, etc.).

Actions requiring upper limb muscle strength (opening a can; pushing a heavy door; carrying a heavy object (over 4.5 kg)) were difficult for 53.4% of patients with neglect. 87.9% of patients could not even consider cooking or taking care of their apartment, garden or yard. All patients complained of the inability or difficulty performing actions related to moving various objects.

Analysis of the obtained DASH questionnaire scores before the start of the rehabilitation program in the CG and MG showed almost the same average values at the level of 50.8 (47.5; 54.3) points and 50.09 (48.3; 52.3) points, respectively, in both groups. Comparison of the CG and MG scores using the Mann-Whitney test ($p > 0.05$; U-test = 350, Z = 1.0808) confirms the absence

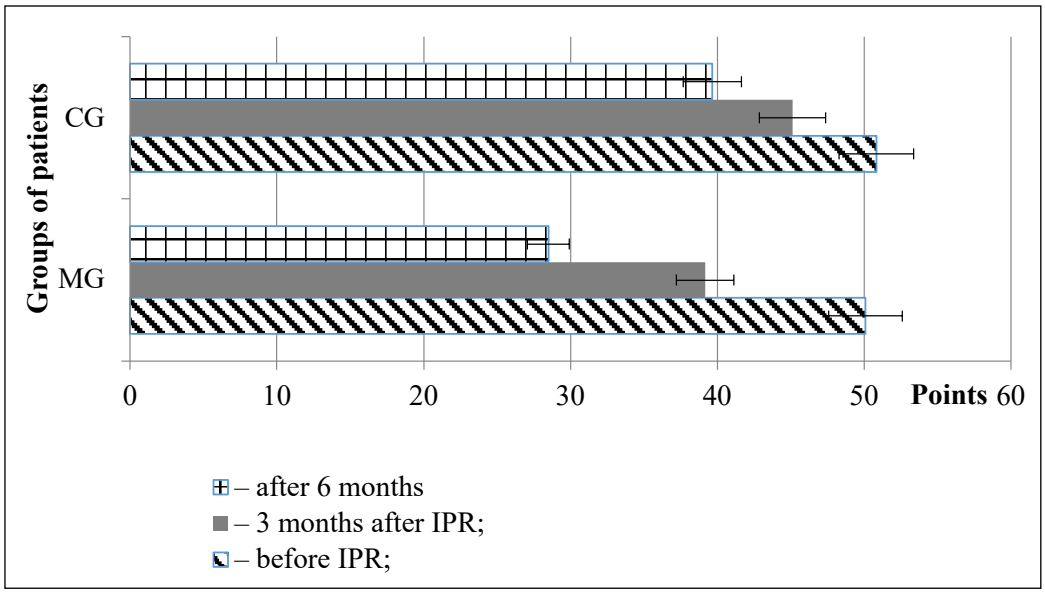

Fig. 1. Dynamics of recovery of upper limb mobility in the CG and MG according to the DASH questionnaire (CG = 29, MG = 29)
Picture taken by the authors

of a significant difference between them. Therefore, the disability of the upper limb in patients of these groups was the same at the beginning of the study.

The use of rehabilitation programs in both groups after 3 months brought certain changes in the condition of the upper limb in patients, which was reflected in the indicators of the second questionnaire in the CG and MG. In the comparison group, the average value was 45.21 (41.3; 47.3) points, while in the main group it was 39.17 (37.1; 41.3) points.

In the CG, the application of the T-test for dependent samples confirmed a significant difference ($p < 0.05$) after 3 months. The T value was 10.26, the difference in average values was 5.71 points at $\sigma = 2.99$. In the MG, a significant difference was also established ($p < 0.05$), where the T value was 12.19, the difference in average values was 10.91 points at $\sigma = 4.81$.

Comparison of upper limb disability scores in the CG and MG after 3 months revealed a significant difference between them according to the Mann-Whitney test ($p < 0.05$; U-test = 105, Z = 4.89). Thus, upper limb recovery in patients in the MG was better than in the CG. The scores in the CG group decreased by 11%, and in the MG group by 21%.

Measurement using the DASH questionnaire after six months showed certain improvement in the condition of the upper limb in the CG, in which the average value was 39.66 (38.5.3; 41.3) points, while in the MG it was 28.49 (26.3; 29.7) points.

After 6 months, CG and MG demonstrated significant changes in the upper limb indicators according to the DASH questionnaire. The application of the T-test for dependent samples confirmed a significant difference ($p < 0.05$). In the CG, the T value was 12.31, the difference in average values was 11.17 points at $\sigma = 4.88$. A significant difference was also established in the MG ($p < 0.05$), where the T value was 20.90, the difference in average values was 21.59 points at $\sigma = 5.56$, Fig.1.

Comparison of the upper limb disability indicators after 6 months revealed a significant difference between CG and MG according to the Mann-Whitney test ($p < 0.05$; U-test = 18.5, Z = 6.24). Thus, the recovery of the upper limb in patients in the MG was better than in the CG. The recovery of the upper limb in patients according to the DASH questionnaire in the CG improved by 22%, and in the MG by 43%. Thus, after six months of using rehabilitation programs in the CG and the MG, the recovery of upper limb activities occurred almost 2 times faster in the MG than in the CG. Thus, the MG patients restored the function of the upper limb much faster, showing the same results after 3 months as the CG patients after 6 months.

DISCUSSION

The 2006 Helsingborg Declaration [1314] set the goal for 2015 that all stroke patients in Europe should have access to a continuum of care: from acute stroke treatment to appropriate rehabilitation in specialized stroke units.

Rehabilitation has been defined by the WHO as "a set of interventions designed to optimize functioning and reduce disability in individuals with health conditions in interaction with their environment" [4, 14]. It is essential to enable individuals with functional limitations to remain in or return to their homes or communities, to live independently without external assistance, and to obtain education, employment, and participation in community life.

There are several promising directions in solving the problem of cerebral stroke:

— prevention [4];
— effective treatment in the acute period [15];
— maximum possible medical and social rehabilitation of people who have suffered cerebrovascular accidents [2], and prevention of repeated vascular disasters [16].

The literature on the third area, namely stroke rehabilitation, is extremely extensive [17]. Dozens of rehabilitation approaches have been studied in recent decades. However, it is sometimes difficult to track new interventions and determine their impact on stroke recovery.

Given the limited health care resources in low- and middle-income countries, effective rehabilitation strategies that can be realistically implemented in such settings are needed. Ekechukwu E.N.D. [3] conducted a systematic literature review (1996 studies, of which 347 (65.22%) of high quality) to identify pragmatic solutions and outcomes that can improve stroke recovery and quality of life for people who have had a stroke in low- and middle-income countries. Most of the innovative high-tech interventions, such as robotic therapy (95.24%), virtual reality (94.44%), transcranial direct current stimulation (78.95%), transcranial magnetic stimulation (88.0%) and functional electrical stimulation (85.00%), were conducted in high-income countries. Several traditional and low-cost interventions, such as constraint-induced movement therapy (CIMT), balance and aerobic exercises, which can be used in resource-limited settings, were found to be effective in enhance recovery in patients with stroke and improving their quality of life.

Early diagnosis and correction of neglect are extremely important for the reintegration of people after stroke to society. USN is unique among the consequences of stroke and affects thinking, mental, visual and sensory functions, and disrupts three-dimensional motor functions [18]. The study confirmed the data of Ungerstedt U. on cases of walking in a circle when trying to move forward, [19]; Riestra AR on turning errors in patients with neglect [20]; on the risk of falling, impaired perception of the vertical axis and safety problems in individuals who have suffered ischemic stroke in the right hemisphere of the brain [21]. This is extremely important, since people with spatial neglect run a sixfold higher risk of falls [22].

Recent studies suggest that USN may be caused by a disruption of the interhemispheric balance of the visual attention network. Based on this hypothesis, non-invasive brain stimulation (NIBS), such as repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS), is used in the rehabilitation of patients with neglect [23, 24]. However, these techniques are not widely used in our country, and therefore rehabilitation care should include available recovery methods with a gradual transition to more modern interventions.

The positive effect of including sensorimotor retraining in the developed IPR was confirmed by the study by Carlsson H, Lindgren 2022 [25], which showed that the SENSUPP protocol (sensory relearning of the upper limb) was a promising method for improving sensorimotor function after stroke. In a study of 15 patients (average age 59 years, > 6 months after stroke), the protocol that included sensory and motor training and home exercises during 5 weeks, was assessed as significant. Patients noted improvements in motor control and performance of daily activities, although performing home exercises was difficult due to lack of support, time and motivation. The intervention included repetitive practices, training in meaningful tasks and the use of feedback based on current neurobiological knowledge. Support from therapists and group classes contributed to a positive perception, while regular monitoring and keeping a diary are recommended to increase the effectiveness of home exercises.

According to the results of the study, the combination of occupational and physical therapies is one of the best means for the recovery of post-stroke patients. Occupational therapy has a positive effect on the degree of recovery of individual functions in patients, as well as on the level of their independence in performing all basic household activities. The rational use of occupational therapy accelerates the recovery of muscle strength, normal amplitude of movements in the joints, and coordination of movements [26].

CONCLUSIONS

Thus, the study confirmed the positive effect of a combined program of physical and occupational therapies conducted with MG patients on the sensorimotor recovery of the upper limb.

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ORIGINAL ARTICLE

CONTENTS

Interrelationship between lipid and bone metabolism in post-menopausal women

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ABSTRACT

Aim: To study the state and interrelationship of calcium-phosphorus metabolism, lipid profile, and bone mineral density in postmenopausal women.

Materials and Methods: A retrospective cohort study was conducted involving 42 women aged 51.9±2.95 years, with a postmenopausal period of 1.69±1.47 years. All patients underwent laboratory and instrumental examinations.

Results: The study found that most women were overweight or obese and had elevated levels of total cholesterol, low-density lipoprotein cholesterol, and atherogenicity index. The average level of 25(OH)D was 26.95±10.58 ng/ml, with more than half of the subjects diagnosed with deficiency or insufficiency. According to ultrasound densitometry, a significant proportion of women had varying degrees of BMD reduction, ranging from osteopenia to osteoporosis. Correlation analysis revealed statistically significant associations between lipid profile indicators, calcium-phosphorus metabolism, and bone tissue condition, in particular inverse correlations between HDL-C levels and phosphorus, parathyroid hormone, and alkaline phosphatase, as well as between the atherogenicity coefficient and vitamin D levels ($p<0.05$).

Conclusions: The data obtained confirm the feasibility of a comprehensive assessment of calcium-phosphorus metabolism, lipid profile, and ultrasound densitometry results. This approach allows for the timely identification of women at increased risk of developing osteodismetabolic disorders and monitoring of bone tissue condition in the postmenopausal period.

KEY WORDS: postmenopause, vitamin D, osteopenia, dyslipidemias, bone density, ultrasonography

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INTRODUCTION

According to the International Menopause Society (IMS), menopause is defined as the point in time 12 months after the last menstrual period, reflecting the loss of ovarian follicular function, which leads to a decrease in estrogen concentration and an increase in follicle-stimulating hormone (FSH) [1]. As a physiological process, menopause is an independent factor that increases the risk of low-trauma fractures due to decreased secretion of estrogen, a hormone that improves bone formation by increasing calcium absorption in the intestines and decreasing reabsorption in the kidneys [2]. Bone mineral density (BMD) is an important parameter for assessing the state of osteometabolism in postmenopausal women, indicating the prevalence of remodeling processes due to osteoclasts or osteoblasts through the activation of systemic inflammation involving the Receptor activator of nuclear factor κB ligand/Receptor activator of nuclear factor κB/Osteoprotegerin (RANKL/RANK/OPG) cascade [3,4]. According to population cohort studies and meta-analyses, bone mass loss increases sharply about a year before the onset of postmenopause and continues for the next five years, reaching about 5% per year [5, 6]. This process leads to the formation of osteopenic syndrome, with an emphasis on women aged 50 and older. The use of methods for screening for BMD disorders, in particular, using ultrasound densitometry, is advisable and allows measures to be taken to prevent the progression of osteoporosis in its early stages [7].

One of the key regulators of calcium-phosphorus homeostasis is vitamin D. Vitamin D comes in several forms, among which cholecalciferol (D_3) and ergocalciferol (D_2) are the main nutritional sources, while 25-hydroxyvitamin D (25(OH)D) and 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) are key metabolites that regulate calcium-phosphorus homeostasis and maintain BMD [8]. As a fat-soluble prohormone, it affects the expression of more than 200 human genes and is used by the body for physiological development and maintenance of

the musculoskeletal system by stimulating calcium absorption from food, osteoid mineralization, bone tissue metabolism, and muscle function [9,10]. According to the recommendations of the Endocrine Society of the United States, a serum 25(OH)D level below 20 ng/mL is interpreted as a deficiency, a value between 20-30 ng/mL as insufficiency, and above 30 ng/mL as sufficient [11].

Postmenopausal women have low levels of vitamin D, which contributes to increased parathyroid hormone (PTH) secretion, reduces calcium absorption in the intestine and serum calcium levels, while increasing bone resorption, which increases the risk of low-trauma fractures [12,13].

In addition to endogenous influences on vitamin D synthesis, exogenous influences can also occur due to insufficient sun exposure and food consumption [14,15]. Thus, dietary strategies can help improve vitamin D status by consuming foods enriched with vitamin D in combination with calcium [16].

In addition to impaired calcium-phosphorus metabolism, the postmenopausal period is associated with the formation of an atherogenic lipid profile, one of the mechanisms of which is endothelial dysfunction [17,18], which increases the risk of developing cardiovascular diseases compared to women of reproductive age [19, 20].

That is why the study of calcium-phosphorus homeostasis, BMD, and lipid profile in the postmenopausal period will allow for a prospective assessment of the risks of developing osteometabolic disorders, such as osteopenia and osteoporosis, as well as cardiovascular risks in women of this age group.

AIM

The aim of this study was to investigate the state and interrelationship of calcium-phosphorus metabolism, lipid profile, and bone mineral density in postmenopausal women.

MATERIALS AND METHODS

This study is part of the initiative research work of the Department of Propaedeutics of Internal Medicine «Clinical and pathogenetic features of cardiovascular diseases in conditions of comorbidity, taking into account gender and age aspects and ways to correct their disorders,» state registration No. 0124U003397.

We conducted a retrospective cohort study. Forty-two postmenopausal women were included in the study.

The inclusion criteria were female gender and menopause lasting 1-5 years.

Exclusion criteria were: artificial early menopause, primary hyperparathyroidism or hypercalcemia, impaired kidney or liver function, malignant neoplasms, diabetes mellitus, thyroid dysfunction, connective tissue disease.

Patients were examined at the clinical base of the Department of Propaedeutics of Internal Medicine of Poltava State Medical University at the Municipal Enterprise «3rd City Clinical Hospital of Poltava City Council» in Poltava.

Before the study, all patients signed an informed voluntary consent form to participate in the study. The study was conducted in accordance with the requirements of the Helsinki Declaration and Order of the Ministry of Healthcare of Ukraine No. 690 of 23.09.2009 «On Approval of the Procedure for Conducting Clinical Trials of Medicinal Products and Examination of Clinical Trial Materials.»

The examination of patients included standard anthropometric, laboratory, and instrumental methods of investigation. Anthropometric indicators were interpreted according to the body mass index (BMI). The data were interpreted according to the criteria of the World Health Organization (WHO), with BMI > 25 kg/m^2 and <29.9 kg/m^2 considered indicators of excess body weight, and BMI was defined as BMI >30 kg/m^2 , with obesity classified as grade I: 30.0–34.9 kg/m^2, grade II: 35.0–39.9 kg/m^2, and grade III (morbid): ≥ 40.0 kg/m^2 [21, 22, 23].

Laboratory tests included assessment of calcium-phosphorus metabolism (ionized calcium, magnesium, phosphorus, alkaline phosphatase, 25OHD, parathyroid hormone (PTH) and lipid profile (total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C (HDL), low-density lipoprotein cholesterol (LDL), very low-density lipoprotein cholesterol (VLDL), atherogenicity coefficient, triglycerides (TG)) in the certified medical laboratory «Analytica».

The condition of BMD was assessed using a Sunlight MiniOmni ultrasonic densitometer (BeamMed, Israel), with measurements taken on the radius, which has a spongy structure. The obtained BMD values were evaluated according to the following criteria: normal condition (BMD change from the reference value as a result of measurement in young people within one standard deviation SD), osteopenia (BMD decrease by > 1.0 SD and < 2.5 SD from the reference value), grade I osteopenia (decrease in BMD > 1.0 SD and < 1.5 SD from the reference value), grade II osteopenia (decrease in BMD > 1.5 SD and < 2.0 SD from the reference value), Grade III osteopenia (decrease in BMD > 2.0 SD and < 2.5 SD from the reference value), osteoporosis (decrease in BMD > 2.5 SD from the reference value) [24].

Statistical calculations were performed using KyPlot 6.0 software and Microsoft Excel. The Shapiro-Wilk test

Table 1. Lipid profile indicators of the study cohort of women

Indicators, units of measurement	Reference range	Statistical indicator	Study cohort (n = 42)
TC, mmol/L	< 5.77	M±SD	6.21±1.13
LDL-C, mmol/L	< 3.24		4.15±1.09
HDL-C, mmol/L	< 0.78		0.70±0.57
HDL-C, mmol/L	1.0		1.62±0.41
TG, mmol/L	< 2.3		1.32±0.84
Atherogenicity coefficient, c.u.	< 2.39		3.0±1.18

Note: M – mean value, SD – standard deviation
Source: compiled by the authors of this study

Table 2. Calcium-phosphorus metabolism indicators in the studied cohort of women

Indicators, units of measurement	Reference range	Statistical indicator	Study cohort (n = 42)
Total calcium, mmol/l	2.25	M±SD	2.21±0.45
Ionized calcium, mmol/l	1.13		1.15±0.25
Parathyroid hormone, pg/ml	11		42.91±20.39
Magnesium, mmol/l	0.7		0.87±0.09
Phosphorus, mmol/l	0.8 – 1.45		1.18±0.15
Alkaline phosphatase, <U/l	<105	Me (Q1; Q3)	81 (73.63; 102.18)

Note: M – mean value, SD – standard deviation, Me – median, Q – quartile
Source: compiled by the authors of this study

Table 3. Correlations between bone metabolism biomarkers

	Mg, mmol/L	Ca^2+, mmol/L	P, mmol/L	ALP, U/l	25(OH)D, ng/ml	PTH, pg/ml
Magnesium, mmol/L	–	r=0.210; p=0.182	r=0.277; p=0.076	r=-0.121; p=0.446;	r=0.194; p=0.218	r=0.060; p=0.708
Ionized calcium, mmol/L	r=0.210; p=0.182	–	r=0.283; p=0.069	r=0.045; p=0.779	r=0.092; p=0.564	r=0.226; p=0.150
Phosphorus, mol/L	r=0.277; p=0.076	r=0.283; p=0.069	–	r=0.127; p=0.424	r=-0.041; p=0.795	r=0.079; p=0.617
Alkaline phosphatase, U/l	r=-0.121; p=0.446	r=0.045; p=0.779	r=0.127; p=0.424	–	r=-0.269; p=0.085	r=0.215; p=0.172
25(OH)D, нг/мл	r=0.194; p=0.218	r=0.092; p=0.564	r=-0.041; p=0.795	r=-0.269; p=0.085	–	r=-0.275; p=0.078
PTH, pg/ml	r=0.060; p=0.708	r=0.226; p=0.150	r=0.079; p=0.617	r=0.215; p=0.172	r=-0.275; p=0.078	–

Note: r – correlation coefficient, p – significance level
Source: compiled by the authors of this study

was used to assess the normality of distribution. To assess the relationships between indicators, we used the correlation analysis method with the calculation of Pearson's correlation coefficients for normal distribution and Spearman's for other types of distribution. The data were presented as M±SD, where M is the mean value and SD is the standard deviation. For distributions that differed from normal, the results were presented as the median (Me) and interquartile range (IQR), (Q1; Q3), where Q1 and Q3 are the first and third quartiles, respectively. The criterion for statistical significance was $p<0.05$.

RESULTS

The mean age of the patients in the study cohort was 51.9±2.95 years, and the duration of menopause was 1.69±1.47 years. Fifty percent (21 individuals) of women were overweight, 23.8% (10 individuals) had grade I obesity, 4.8% (2 individuals) had grade II obesity, and the rest of the patients had a normal BMI.

The study group of women showed higher levels of total cholesterol, LDL cholesterol, and atherogenicity coefficient than the reference values (Table 1).

The mean level of 25(OH)D in the entire study group was 26.95±10.58 ng/ml. In 30.9% (13 people) of women,

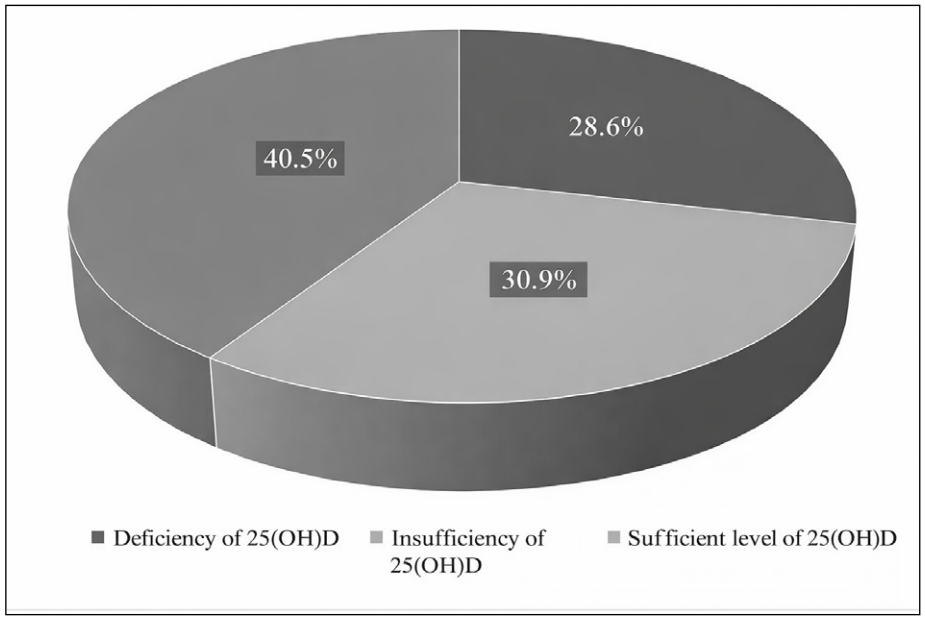

Fig. 1. Distribution of patients by serum 25(OH)D level
Source: compiled by the authors of this study

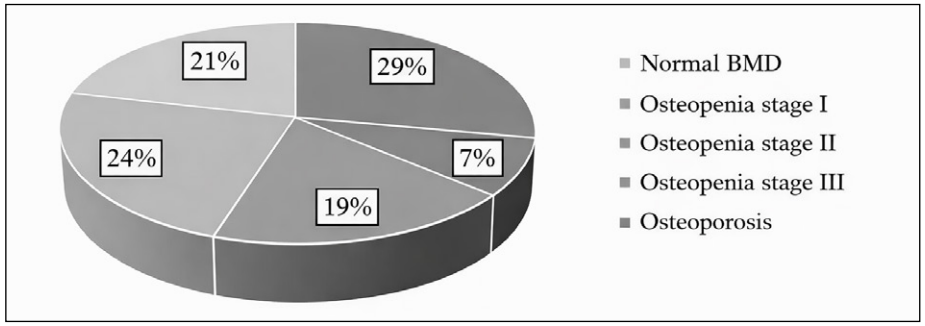

Fig. 2. Distribution of patients according to bone mineral density indicators
Source: compiled by the authors of this study

an insufficient level of 25(OH)D in the blood was determined, in 28.6% (12 people) – a deficiency, and in 40.5% (17 people) – a sufficient level (Fig. 1).

The levels of total and ionized calcium, magnesium, phosphorus, and parathyroid hormone in blood serum were within the reference range in all subjects studied (Table 2). An increase in alkaline phosphatase levels was recorded in 21.4% of women (9 individuals).

Ultrasound densitometry results verified a decrease in BMD, which meets the criteria for grade I–III osteopenia and osteoporosis. In 7.1% (3 individuals) of the women examined, grade I osteopenia was diagnosed, 19.1% (8 people) had grade II osteopenia, 23.8% (10 people) had grade III osteopenia, 21.4% (9 people) had osteoporosis, and the rest of the patients had preserved BMD (Fig. 2).

Correlation analysis between mineral metabolism indicators did not reveal any statistically significant patterns ($p>0.05$) (Table 3).

When conducting a correlation analysis between bone remodeling biomarkers and bone mineral density, a significant negative correlation was found between alkaline phosphatase levels and T- and Z-scores ($p<0.05$), as well as between Z-scores and BMI ($p<0.05$) (Table 4).

When studying lipid profile indicators, calcium-phosphorus metabolism, and BMD, a statistically significant ($p<0.05$) inverse correlation was found between HDL-C and phosphorus levels ($r=-0.3045$, $p=0.0499$), between HDL-C and PTH ($r=-0.3639$, $p=0.0178$), and between HDL-C and alkaline phosphatase levels ($r=-0.3100$, $p=0.0457$). In addition, a significant direct correlation was found between TG and BMI levels, as well as between the atherogenicity coefficient and phosphorus levels ($p<0.05$). At the same time, the atherogenicity coefficient had a significant inverse correlation with vitamin D levels ($p<0.05$) (Table 5).

DISCUSSION

Our results demonstrate the presence of dyslipidemia in women in the study group due to increased levels of cholesterol, LDL cholesterol, and atherogenicity coefficient. This confirms that one of the leading mechanisms in the development of lipid metabolism disorders is estrogen deficiency, which accompanies postmenopause [25,26]. Estrogens exert an antiatherogenic effect by indirectly increasing the expression of LDL receptors in the liver, enhancing LDL clearance from the blood, and thus ensuring their stable levels [27]. In addition, with their antioxidant properties, estrogens promote the synthesis of nitric oxide, which has an anti-inflammatory effect, reducing endothelial cell apoptosis and local inflammation in the vessel wall [28].

Table 4. Correlations between bone metabolism biomarkers and BMD, BMI, and age

	Mg, mmol/L	Ca^{2+}, mmol/L	P, mmol/L	ALP, U/l	25(OH)D, ng/mL	PTH, pg/ml	T-score radius, SD	Z-score radius, SD
T-score radius, SD	-0.134 p=0.397	0.020 p=0.901	0.112 p=0.478	-0.305* p=0.049	0.141 p=0.374	-0.009 p=0.954	-	-
Z-score radius, SD	0.001 p=0.995	-0.040 p=0.806	0.264 p=0.100	-0.354* p=0.025	0.213 p=0.188	-0.062 p=0.702	-	-
SOS, m/s	-0.177 p=0.263	-0.001 p=0.994	-0.017 p=0.916	-0.262 p=0.093	0.234 p=0.136	-0.002 p=0.990	-	-
BMI, kg/m2	-0.045 p=0.779	0.067 p=0.673	-0.028 p=0.859	0.107 p=0.500	-0.005 p=0.976	-0.039 p=0.807	-0.252 p=0.107	-0.318* p=0.045
Age, years	0.074 p=0.643	-0.232 p=0.139	0.233 p=0.137	0.153 p=0.332)	-0.001 p=0.993	-0.100 p=0.529	-0.079 p=0.617	0.052 p=0.752

Note: r – correlation coefficient; * – statistically significant correlation ($p<0.05$)
Source: compiled by the authors of this study

Table 5. Correlations between lipid profile indicators, BMD, BMI, and age

	Mg, mmol/L	Ca^{2+}, mmol/L	P, mmol/L	ALP, U/l	25(OH)D, ng/mL	PTH, pg/ml	T-score radius, SD	Z-score radius, SD	BMI, kg/m2	Age, years
TC, mmol/L	0,05997 p=0,7060	-0,1328 p=0,4019	-0,02454 0,8774	-0,1143 0,4711	-0,2729 0,0803	-0,2632 0,0922	-0,1427 0,3674	-0,1782 0,2587	0,1143 0,4710	-0,09713 0,5406
LDL-C,mmol/L	-0,08487 0,5931	-0,2423 0,1221	-0,1704 0,2807	0,08440 0,5951	-0,2336 0,1365	-0,1635 0,3009	-0,2996 0,0539	-0,3030 0,0511	0,1451 0,3592	-0,03001 0,8504
VLDL-C, mmol/L	0,06655 0,6754	-0,04146 0,7943	0,1535 0,3318	-0,01223 0,9387	-0,2735 0,0797	0,04385 0,7828	-0,02144 0,8928	-0,07549 0,6347	0,2466 0,1153	0,05601 0,7246
HDL-C, mmol/L	0,03452 0,8282	0,02912 0,8547	-0,3045 0,0499*	-0,3100 0,0457*	0,09742 0,5394	-0,3639 0,0178*	-0,08176 0,6067	-0,1412 0,3724	-0,2407 0,1247	-0,2302 0,1425
TG, mmol/L	0,1216 0,4432	0,02920 0,8543	0,1558 0,3244	0,09163 0,5639	-0,2815 0,0710	0,1202 0,4483	-0,08838 0,5778	-0,09835 0,5355	0,3092 0,0463*	0,1364 0,3891
Atherogenicity coefficient, c.u	0,02471 0,8880	-0,08032 0,6465	0,3471 0,0411*	0,2435 0,1587	-0,3992 0,0175*	-0,0006273 0,9971	-0,1142 0,5135	-0,1056 0,5461	0,2845 0,0976	0,1171 0,5030

Note: r – correlation coefficient; * – statistically significant correlation ($p<0.05$)
Source: compiled by the authors of this study

That is why women before menopause are more protected from atherosclerotic heart disease than men, with approximately half the risk of cardiovascular disease [29,30].

At the same time, in the study group of women, 59.5% of the subjects had reduced levels of 25(OH)D, which meets the criteria for deficiency and insufficiency (Fig. 1). It is known that about 64% of women worldwide in the postmenopausal period have serum 25(OH)D levels below 30 ng/ml [31]. Vitamin D deficiency, despite its high prevalence worldwide, is still not adequately corrected. According to researchers, an increase in age by 10 years leads to a 13% decrease in vitamin D synthesis, which is due to a decrease in the formation of 7-dehydroxycholesterol in the epidermis [32]. A decrease in estrogen levels leads to a disruption in the regulation of enzymes involved in vitamin D metabolism, in particular 1α-hydroxylase, reducing the formation of the active form of 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), which is involved in the regulation of calcium-phosphorus metabolism and bone mineralization. Also, in postmenopause, the proportion of adipose tissue in which vitamin D is deposited increases, which reduces its bioavailability in the blood [33, 34].

The vast majority of patients in the study group showed a decrease in BMD, which meets the criteria for grade I-III osteopenia and osteoporosis, consistent with scientific understanding of the effect of hypoestrogenism on bone tissue (Fig. 2). The ratio between osteoblasts, which are involved in the synthesis of new bone mass, and osteoclasts, which ensure bone resorption processes, plays an important role in the remodeling of bone tissue. Estrogens suppress osteoclast activity, promoting osteoblast differentiation by increasing OPG formation and decreasing RANKL, which provides osteoprotection [35, 36]. In the postmenopausal period, estrogen deficiency leads to an increase in the

RANKL/OPG ratio due to increased RANKL expression and decreased OPG synthesis, which causes excessive osteoclast activation and enhanced bone resorption through stimulation of signaling pathways involving NF-κB. It should be noted that estrogen deficiency is also accompanied by an increase in pro-inflammatory cytokines, in particular interleukin (IL) 1, IL-6, and tumor necrosis factor α (TNF-α), which stimulate RANKL expression and further suppress OPG, thereby enhancing osteoclastogenesis [37, 38].

An increase in alkaline phosphatase levels in 21.4% of the subjects may indicate a compensatory response of osteoblasts to increased bone resorption, i.e., it is an indicator of enhanced bone remodeling, where resorption predominates, but some bone formation activity is still present [39]. Our study found that higher alkaline phosphatase levels are associated with a decrease in BMD, as confirmed by a negative correlation with T- and Z-scores (Table 4). Thus, alkaline phosphatase levels can be considered a biochemical marker of bone remodeling in this cohort of women [40].

In 78.6% of women in the study group, an increased BMI was found, which has a negative correlation with the Z-score, consistent with current data on the negative effect of visceral obesity on bone metabolism through mechanisms of chronic low-intensity inflammation [41, 42].

The results of the correlation analysis indicate the existence of relationships between the lipid profile and calcium-phosphorus metabolism. In particular, the negative correlation between HDL-C levels and phosphorus, PTH, and alkaline phosphatase does not indicate a causal relationship but reflects the interrelationship of metabolic processes caused by the postmenopausal period. An increase in HDL levels may occur compensatorily in conditions of metabolic disorders and changes in the inflammatory background due to a decrease in the effect of parathyroid hormone on bone tissue and osteoclast activity, which is simultaneously manifested in a decrease in alkaline phosphatase levels. Thus, high HDL may be an indirect marker of bone mass preservation, at least through a decrease in parathyroid hormone-mediated resorption [43].

The direct correlation between TG and BMI levels confirms that an increase in body weight is associated with increased triglyceride synthesis in the liver, leading to dyslipidemia. That is why an increase in BMI is a risk factor for the development of metabolic syndrome, including dyslipidemia [44].

The established inverse relationship between the atherogenicity coefficient and vitamin D levels in the women studied demonstrates risk factors for both calcium-phosphorus metabolism imbalance and lipid profile. This result confirms that low vitamin D levels are associated with an atherogenic lipid profile, accompanied by increased systemic inflammatory activity [45].

CONCLUSIONS

The vast majority of women in early postmenopause have dyslipidemia with elevated total cholesterol and low-density lipoprotein cholesterol levels, low blood vitamin D levels, and varying degrees of bone mineral density disorders, ranging from osteopenia to osteoporosis.

It has been found that high-density lipoprotein cholesterol levels may indirectly reflect the condition of bone tissue.

The results of the study demonstrate the importance of a comprehensive approach to assessing bone metabolism, taking into account not only traditional markers of bone remodeling, but also the lipid profile, which is especially relevant for postmenopausal women who are at increased risk of developing osteopenia and osteoporosis.

Determining the state of calcium-phosphorus metabolism and lipid profile in combination with ultrasound densitometry allows identifying groups at increased risk of developing osteodystrophic syndrome, as well as monitoring bone tissue condition in registered cases of osteopenia or osteoporosis.

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REVIEW ARTICLE

CONTENTS

The effect of aluminum oxide air abrasion on composite adhesion to enamel and dentin: Literature review 2020–2024

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ABSTRACT

Aim: To evaluate the influence of airborne-particle abrasion with aluminum oxide on the adhesion of resin composites to enamel and dentin, focusing on technical parameters, adhesive strategies, and long-term outcomes.

Materials and Methods: A narrative review was performed according to PRISMA guidelines. PubMed, Scopus, and Web of Science were searched for studies published between 2020 and 2024 reporting bond strength to enamel or dentin after aluminum oxide abrasion. Only peer-reviewed full-text articles with shear or microtensile outcomes were included. Twenty studies were analyzed, comprising in vitro experiments and systematic reviews.

Results: For enamel, most studies confirmed improved retention after abrasion, particularly when combined with phosphoric acid etching. The effect was most evident for self-etch adhesives, which otherwise underperform on aprismatic enamel. For dentin, findings were more variable. Abrasion enhanced adhesion with etch-and-rinse protocols and universal adhesives in etch-and-rinse mode but often reduced bond strength with self-etch adhesives, likely due to smear-layer removal without adequate demineralization. Optimal parameters included 50 µm particles, 3–4 bar pressure, and ~5 seconds of application. Higher pressure or prolonged use risked collagen damage. In composite repair, abrasion consistently increased bond strength, especially when combined with silanization and an adhesive. Evidence on long-term durability was mixed; bioactive particles may improve stability and biocompatibility.

Conclusions: When applied with optimized parameters, aluminum oxide abrasion improves bonding to enamel and dentin, is particularly useful in etch-and-rinse protocols and composite repair, and should be avoided on dentin with self-etch adhesives. Further in vivo studies are needed to clarify long-term performance.

KEY WORDS: air abrasion, dental, aluminum oxide, composite resins, dental enamel, dentin

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INTRODUCTION

Bonding of composite resins to tooth hard tissues is a key factor in restorative and esthetic dentistry. Achieving durable adhesion remains challenging, particularly under difficult clinical conditions such as sclerotic or carious dentin. Conventional approaches to increase micromechanical retention include phosphoric acid etching or self-etching adhesive systems. Airborne-particle abrasion with aluminum oxide (Al_2O_3) has been proposed as an adjunctive technique to enhance adhesion. This review summarizes the latest evidence regarding the effect of Al_2O_3 sandblasting on enamel and dentin bonding, including technical parameters, adhesive strategies, and clinical implications.

AIM

The aim of this review is to evaluate the impact of abrasive sandblasting with aluminum oxide particles (Al_2O_3) on the adhesion of composite materials to enamel and dentin. Particular attention is given to the technical parameters of the procedure (particle size, pressure, application time) as well as the types of adhesive systems and bonding protocols used (total-etch vs. self-etch). Another goal is to formulate practical clinical guidelines for using Al_2O_3 air-abrasion and to discuss the limitations and potential risks of this technique in light of the latest research.

MATERIALS AND METHODS

The review was conducted according to PRISMA guidelines. PubMed, Scopus, and Web of Science databases were searched for publications from 2020–2024 concerning the effect of Al_2O_3 sandblasting on composite bonding to enamel and dentin. The search was limited to peer-reviewed papers available in full text that

reported quantitative bond strength data (e.g., shear bond strength – SBS, or microtensile bond strength – μTBS). Excluded were studies focusing solely on other materials (e.g., ceramics, metals), papers without numerical bond data (descriptive articles, case reports), and commentaries or letters. After removing duplicates and an initial title/abstract screening, 20 publications meeting the criteria were included for analysis, comprising both in vitro studies and relevant systematic reviews. From the selected studies, data were extracted on the sandblasting protocols used (Al_2O_3 particle size, pressure, and application time), the type of bonding system (total-etch vs. self-etch technique, universal adhesives), and test conditions (immediate bond strength vs. after aging). The results of individual studies were compiled, and a qualitative discussion was undertaken to identify consistencies or discrepancies among the findings.

REVIEW

BONDING TO ENAMEL

Most studies confirm that additional preparation of enamel by Al_2O_3 sandblasting (typically with 30–50 μm particles) enhances the retention of composite resins, especially when it is combined with traditional phosphoric acid etching. Enamel after sandblasting exhibits higher roughness and surface energy, which facilitates resin wetting and penetration. For instance, scanning electron microscopy (SEM) confirms the creation of a roughened enamel surface after air abrasion, and contact angle measurements indicate increased surface wettability (i.e., higher surface free energy) on sandblasted enamel. These changes translate to improved resin retention, as reflected in higher bond strength values. In vitro, it has been shown that using sandblasting before 37% phosphoric acid etching leads to higher microtensile bond strength to enamel – for example, Rifane et al. reported an increase in μTBS to intact enamel from ~15 MPa with acid etching alone to ~20 MPa when Al_2O_3 sandblasting was included prior to etching [1]. Similarly, Zhang et al. found a significant increase in bond strength in non-carious cervical enamel/dentin lesions: sandblasting (110 μm Al_2O_3 at 75 psi) raised the μTBS from ~14.2 MPa (no sandblasting) to 17.9 MPa, which is about a 25% improvement compared to no air-abrasion [2]. These findings are consistent with other studies comparing conventional etching to alumina air-abrasion, which also reported superior bond strengths when enamel was sandblasted before bonding [3,4].

BONDING TO DENTIN

Findings on the effect of sandblasting on dentin bonding are more varied than for enamel. The general trend is that with total-etch (etch-and-rinse) adhesive systems, sandblasting dentin with 50 μm alumina can increase bond strength or at least does not decrease it [5,6,7]. Mechanically removing the smear layer and widening the dentinal tubule orifices tends to promote primer and resin infiltration, as long as the dentin is subsequently properly conditioned with acid (exposing the collagen network) and primed. Sinjari et al. recorded higher bond strength in dentin that was sandblasted (5 s, 50 μm, ~0.25 MPa pressure) and then acid-etched and bonded with a universal adhesive, compared to a control without sandblasting (mean μTBS ~31 MPa vs ~25 MPa) [7]. Likewise, a meta-analysis by Lima et al. confirmed that Al_2O_3 sandblasting does not have a negative effect on adhesion to dentin – the pooled bond strength results were not significantly different between sandblasted dentin and conventionally prepared dentin [5].

SANDBLASTING PARAMETERS

An analysis of the literature shows that 50 μm Al_2O_3 particles are the most commonly used and they produce favorable adhesive outcomes on dentin [6]. Several studies have compared different particle sizes – for example, 27 μm vs. 50 μm – and the results are not entirely consistent. Melkumyan et al. found no significant difference in bond strength to dentin between sandblasting with 27 μm particles versus 50 μm particles (mean SBS ~27–28 MPa for both, compared to 28.3 MPa in the control group, indicating no improvement with either size) [8]. On the other hand, some researchers have suggested that smaller particles might polish the surface rather than distinctly roughening it, whereas others observed that smaller grains can create more micro-retentions due to the greater number of particles impacting the surface per unit time.

REPAIR OF COMPOSITE RESTORATIONS

Al_2O_3 sandblasting is widely recommended as part of the procedure for repairing aged composite restorations. An old composite filling undergoes surface degradation over time – the number of available unreacted methacrylate groups capable of chemical bonding is reduced, and the surface becomes smooth and coated with various deposits. The purpose of surface pretreatment is to create micro-mechanical retentions and refresh the composite surface, enabling a strong chemical union through the use of silane and adhesive

resin. Among the available methods (acid etching, roughening with a diamond bur, laser etching), abrasive sandblasting with alumina achieves the highest repair bond strength values [9,10].

BOND DURABILITY AND BIOLOGICAL ASPECTS

An important question is the long-term durability of the bond achieved with sandblasting. The results from aging studies present a mixed picture. On one hand, some research has observed that sandblasted dentin specimens can exhibit a faster decline in bond strength after artificial aging than non-sandblasted controls [11]. On the other hand, recent studies with bioactive modifications indicate a path to potentially improve long-term bond stability. Spagnuolo et al. showed that substituting Al_2O_3 with bioactive glass particles in the air-abrasion process prevented degradation of the resin–dentin interface over time [12].

DISCUSSION

The findings summarized above indicate that air-abrasion with aluminum oxide plays a variable but clinically relevant role in adhesive dentistry.

For enamel, the evidence consistently shows that sandblasting enhances retention by increasing surface roughness and energy, particularly when phosphoric acid etching is combined. Importantly, the greatest benefit is observed with self-etch adhesives, where mechanical roughening compensates for their limited etching capacity on aprismatic enamel.

For dentin, results depend strongly on the adhesive protocol. With etch-and-rinse adhesives, sandblasting generally improves or maintains adhesion by facilitating resin penetration. However, when used with self-etch adhesives, sandblasting can be detrimental. Removal of the smear layer leaves collagen exposed without sufficient demineralization, leading to weaker hybrid layers and reduced bond durability [13,14].

Regarding parameters, most studies point to 50 µm particles, 3–4 bar pressure, and 5 seconds application as the optimal conditions [6,15]. Deviations, such as prolonged time or higher pressures, can cause collagen damage or fail to add benefits. Additionally, after abrasion, the surface should be thoroughly cleaned by rinsing and/or etching to remove residual alumina powder that could interfere with bonding [16].

In composite repair, sandblasting is clearly superior to acid etching or mechanical roughening alone. It creates the most favorable conditions for silane coupling and adhesive bonding, improving repair longevity. Furthermore, studies indicate that combining alumina air-abrasion with silane application and a bonding agent yields the highest composite repair bond strength and durability [9,17].

Concerning durability, the literature shows heterogeneity. While some studies report sustained improvements after aging, others (e.g., in composite repair scenarios) have noted that the initial advantages of sandblasting may diminish over time, with bond strength declining toward that of non-abraded controls [18]. Residual alumina particles and hydrolytic degradation may also compromise long-term performance [19]. Promisingly, bioactive alternatives (e.g., bioactive glass particles) show potential to enhance both bond durability and biocompatibility.

It should be noted that the studies included in this review were heterogeneous in their methods and conditions. Sample sizes varied between experiments, and there were differences in substrates, bonding protocols, and test methods (for example, some used microtensile vs. shear bond testing, with or without aging). This methodological heterogeneity makes direct comparisons difficult and may partly explain the inconsistent outcome trends observed [20]. Greater standardization in future research would help in drawing more definitive conclusions.

Overall, the variability in results highlights that clinical success depends on correct protocol selection: sandblasting is valuable in enamel preparation, total-etch adhesive strategies, and composite repair, but it should be avoided with self-etch adhesives on dentin.

CONCLUSIONS

Aluminum oxide air abrasion can significantly improve the adhesion of composite materials to both enamel and dentin, provided that an appropriate protocol is followed. In enamel, air abrasion provides a clear benefit – particularly if self-etch adhesive systems are being used – by increasing surface roughness and enabling better resin penetration. In dentin, the effect of sandblasting depends on the bonding strategy: it is recommended primarily for use with etch-and-rinse (total-etch) adhesive protocols, whereas in self-etch approaches it should be avoided because the lack of a proper etching step after smear-layer removal can lead to reduced bond effectiveness.

For optimal results, alumina sandblasting should be carried out under controlled conditions (using approximately 50 µm Al_2O_3 particles at ~3–4 bar pressure for about 5–10 seconds per area). Excessively prolonged or high-pressure application offers no added benefit and may even damage the exposed dentin surface. After

sandblasting, the abraded surface must be thoroughly rinsed (or etched) to eliminate residual abrasive particles before the adhesive steps.

In the context of repairing aged composite restorations, alumina air abrasion is highly beneficial. It consistently yields higher repair bond strength than either acid etching or roughening with a bur. To maximize the durability of the repair, the procedure should be combined with the application of a silane coupling agent and a compatible adhesive resin before placing the new composite. During intraoral sandblasting, any nearby exposed dentin should be protected (for example, by covering it with a bonding resin) and the abrasive jet directed mainly at the old composite and enamel margins.

When used correctly, Al_2O_3 air abrasion is a quick and safe technique, although standard precautions are advised (such as using a rubber dam for isolation and protecting soft tissues). Improper use of sandblasting – for instance, prolonged blasting of bare dentin without subsequent etching, or using it in a self-etch scenario – can negate the benefits or even decrease the bond strength. Adhering to a well-defined protocol is therefore crucial for success.

The long-term performance of bonds created with alumina abrasion remains a subject for further research. In many laboratory studies, the initial improvements in bond strength have persisted after artificial aging, but some reports have noted a loss of the benefit over time. High-quality in vivo studies are needed to determine the clinical longevity of alumina-enhanced bonds. Additionally, emerging approaches like bioactive glass abrasives may further improve the durability and biological outcomes of sandblasting in the future.

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CONTENTS

Cortisol and testosterone: Which is more important in metabolic syndrome men

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ABSTRACT

Aim: To review information resources on this problem for the provision of modern knowledge in the pathogenesis of this pathology.

Materials and Methods: An analysis of data from literary sources and medical articles in Pub Med was carried out in order to clarify the influence of cortisol and testosterone on the development of metabolic syndrome. The search was conducted over the last 5 years, but there is material from 2001, 2018, and 2019. Total volume of the number of sources: 17.

Conclusions: The metabolic syndrome (MetS) is a collection of abnormalities that predispose individuals to diabetes, atherosclerosis, and cardiovascular disease (CVD). Patients with metabolic syndrome exhibit hyperactivity of the hypothalamic-pituitary-adrenal (HPA) system, resulting in «functional hypercorticism.» Stress is thought to play a significant role in this interaction by increasing the sensitivity of the hypothalamic-pituitary-adrenal axis. The enzyme 11-beta-hydroxysteroid dehydrogenase type 1 (11HSD1), which is involved in glucocorticoid metabolism in peripheral tissues (particularly adipose tissue and liver), has been implicated in metabolic syndrome and central obesity. Overexpression of 11HSD1 in adipocytes is observed in metabolic syndrome and central obesity, leading to increased conversion of cortisone to cortisol and excessive tissue-specific glucocorticoid activity

KEY WORDS: metabolic syndrome, cortisol,testosterone,obesity,diabetes

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INTRODUCTION

The concept of metabolic syndrome was first described in 1923 by Kylin, a Swedish physician, who observed a clinical association between hypertension and gout. In 1947, the definition was expanded to include upper body adiposity [1]. However, it was Reaven, in 1988, who emphasized the significance of metabolic syndrome [2]. He referred to it as *Syndrome X*, characterized by insulin resistance, hyperglycemia, hypertension, low levels of high-density lipoprotein cholesterol (HDL-C), and elevated levels of very-low-density lipoprotein (VLDL) and triglycerides (TG). Since then, metabolic syndrome has been recognized as a major risk factor for coronary artery disease, drawing considerable attention in the field of cardiovascular medicine.

AIM

The aim is to review information resources on this problem for the provision of modern knowledge in the pathogenesis of this pathology.

MATERIALS AND METHODS

An analysis of data from literary sources and medical articles in Pub Med was carried out in order to clarify the influence of cortisol and testosterone on the development of metabolic syndrome.

The search was conducted over the last 5 years, but there is material from 2001, 2018, and 2019.

Key words for search were: metabolic syndrome, insulin resistance syndrome, obesity, atherogenic dyslipidemia, diabetes mellitus, estrogen, gut microbiota, testosterone, human glucocorticoid receptor, adipose tissue dysfunction, adrenal and gonadal steroid hormone, angiogenesis, arterial hypertension, cardiovascular diseases, gender, heart failure, hypercholesterolaemia.

Total volume of the number of sources: 17.

INCLUSION CRITERIA

Recency, empirical research, articles in English, the object of the study is a male patient with metabolic

syndrome, peer-reviewed articles from scientometric databases.

EXCLUSION CRITERIA
Duplication, incomplete data, insufficient quality.

ETHICS
All sources used in this literature review are publicly available.

REVIEW AND DISCUSSION
Metabolic syndrome (MetS) is a cluster of common abnormalities that include elevated blood sugar levels (hyperglycemia), excess abdominal fat (abdominal obesity), reduced levels of HDL-C, and increased levels of triglycerides (TG) and blood pressure (BP). It was initially referred to as "Syndrome X" or "insulin resistance syndrome" by Reaven in 1988. The components of MetS are associated with endothelial dysfunction and the development of atherosclerosis, increasing the risk of type 2 diabetes mellitus (T2DM) as well as vascular morbidity and mortality. It is estimated that approximately one-fourth of the world's adult population has MetS. Despite its growing global prevalence, there is still no universally accepted diagnostic criterion, and the underlying causes of MetS remain a topic of ongoing debate.

MetS is a complex risk factor resulting from insulin resistance combined with abnormal accumulation and dysfunction of adipose tissue [3]. It poses a significant risk for coronary heart disease (CHD), as well as for diabetes, fatty liver disease, and several types of cancer. The clinical features of this syndrome, as mentioned earlier, may include hypertension, hyperglycemia, hypertriglyceridemia, reduced HDL-C levels, and abdominal obesity.

According to the criteria established by the National Cholesterol Education Program (NCEP), MetS is diagnosed when three or more of the following parameters are present:

- Waist circumference exceeding 102 cm in men and 88 cm in women;
- Triglyceride levels of at least 150 mg/dL (1.7 mmol/L);
- HDL cholesterol levels below 40 mg/dL (1.04 mmol/L) in men and below 50 mg/dL (1.29 mmol/L) in women;
- Blood pressure of at least 130/85 mm Hg;
- Fasting glucose levels of at least 110 mg/dL (6.1 mmol/L).

OBESITY
Obesity has become a global epidemic, affecting over 1 billion adults, with at least 300 million classified as clinically obese [4]. This widespread issue adversely impacts blood pressure (BP), lipid profiles, and glucose metabolism due to insulin resistance, leading to a constellation of cardiovascular risk factors collectively referred to as metabolic syndrome (MetS). MetS is strongly associated with a significant increase in cardiovascular morbidity and mortality, making it a leading cause of death in the Western world. Interestingly, men are at higher risk of developing coronary artery disease at an earlier age compared to women, although the underlying reasons for this gender difference remain unclear. Adipose tissue is metabolically active and produces cytokines known as adipocytokines, which play a critical role in linking the various components of MetS to their cardiovascular effects. In particular, central adiposity—as measured by waist circumference or waist-to-hip ratio—is strongly and independently associated with insulin resistance and increased cardiovascular disease (CVD) risk. Visceral adipocytes located in the abdominal cavity are more metabolically active than subcutaneous adipocytes located beneath the skin. Visceral fat is a major source of free fatty acids and adipocytokines, which directly affect the liver. These substances stimulate gluconeogenesis and inhibit insulin binding in the liver, thereby contributing to insulin resistance. Waist circumference is a clinically practical method for assessing abdominal obesity; however, it does not effectively distinguish between visceral and subcutaneous fat. Imaging techniques such as computed tomography (CT), magnetic resonance imaging (MRI), and dual-energy X-ray absorptiometry (DEXA) provide greater accuracy in differentiating fat compartments.

Central obesity is a key component of MetS (Fig. 1). Cortisol plays a role in the pathophysiology of obesity in the context of MetS. One study found an increased urinary cortisone/cortisol ratio in women with elevated abdominal fat compared to those with peripheral fat distribution, suggesting enhanced peripheral cortisol metabolism [5].

GENETIC FACTORS
The glucocorticoid receptor (GR) belongs to the nuclear receptor superfamily and functions as a transcription factor that regulates gene expression. Upon binding with cortisol, the GR undergoes conformational changes that result in dissociation from a protein complex, with heat shock protein 90 being the most notable component. The activated GR-cortisol complex then translocates to the nucleus, where it exerts various genomic effects. Polymorphisms in the GR gene can influence sensitivity to endogenous glucocorticoids.

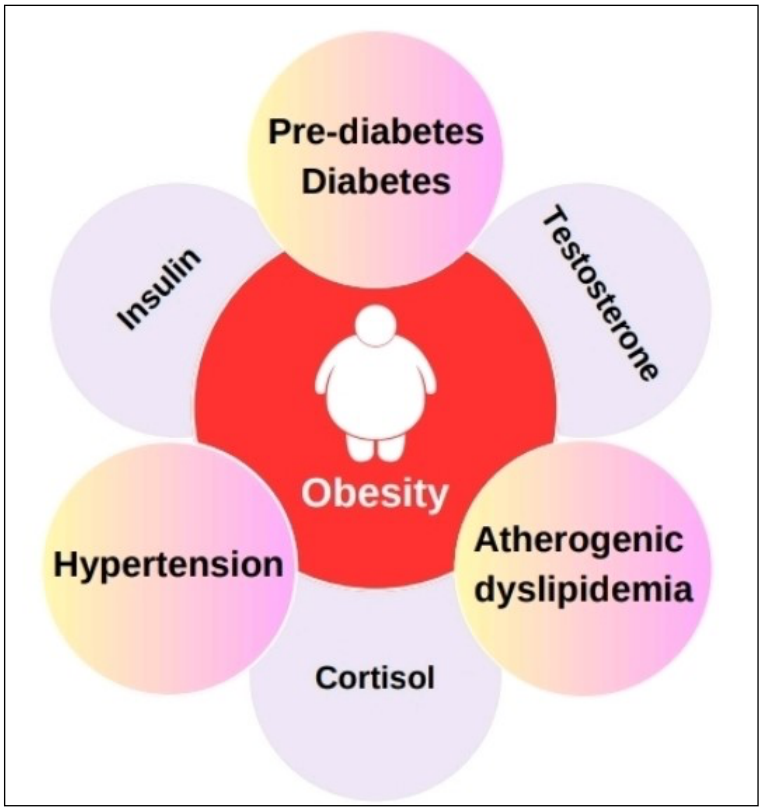

Fig. 1. Components of metabolic syndrome
Source: compiled by the authors based on [5]

Specifically, the N363S and BclI polymorphisms are associated with increased glucocorticoid sensitivity, while the ER22/23EK polymorphism is linked to relative resistance. The N363S variant has been associated with coronary heart disease (CHD), independent of obesity, as well as elevated total cholesterol, triglyceride levels, and the total cholesterol/HDL-C ratio. Both the N363S and BclI polymorphisms may contribute to increased susceptibility to obesity. In contrast, individuals carrying the ER22/23EK variant appear to have a reduced vascular risk [6].

OBESITY AND HORMONAL STATUS

Interestingly, there is an inverse correlation between cortisol clearance and insulin sensitivity, independent of overall body fat levels. It is well-documented that glucocorticoids, such as cortisol, promote the differentiation and expansion of human adipocytes, with glucocorticoid receptors more abundantly expressed in visceral fat compared to subcutaneous fat. Additionally, glucocorticoids facilitate the redistribution of adipose tissue from peripheral to central depots, increase both the size and number of fat cells, and stimulate lipolysis—resulting in elevated circulating free fatty acid levels. A study using MRI to assess central fat distribution found a positive association between excess cortisol and intra-abdominal fat accumulation. In this study, the activity of the hypothalamic-pituitary-adrenal (HPA) axis was evaluated using a single morning cortisol measurement, which supported a link between HPA activity and MetS. Overall, these findings suggest that cortisol plays a significant role in the development of abdominal obesity, a hallmark feature of MetS.

CORTISOL AND WAIST CIRCUMFERENCE: CONFLICTING EVIDENCE

There have been conflicting findings regarding the relationship between cortisol and waist circumference. While some studies report no significant association, others suggest a link between elevated cortisol levels—such as urinary free cortisol and overnight serum cortisol—and insulin resistance, as assessed by the homeostasis model assessment. Higher cortisol concentrations have also been linked to impaired insulin secretion, consistent with previous in vivo and in vitro studies demonstrating the regulatory role of glucocorticoids in insulin dynamics (Fig. 2).

A study in obese children, with and without insulin resistance, showed that weight loss reduced cortisol levels and improved insulin sensitivity only in the insulin-resistant group, but not in those without insulin resistance. Multiple studies have shown an exaggerated HPA axis response to various stimuli in individuals with abdominal obesity. These stimuli include food intake, low-dose tetracosactide, and CRH-arginine vasopressin. Furthermore, abdominal adiposity has been associated

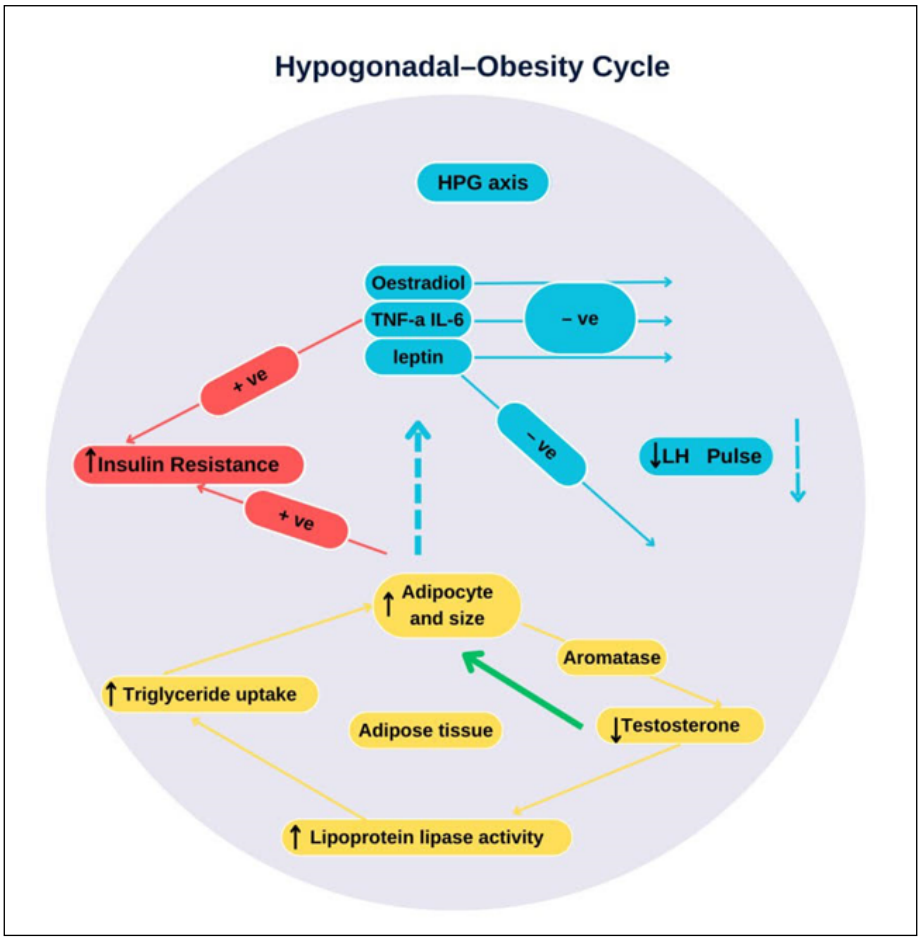

Fig. 2. Hypogonadal-Obesity Cycle
Source: compiled by the authors based on [7]

with impaired negative feedback regulation within the HPA axis and reduced diurnal variation in cortisol levels. Overall, current evidence supports the hypothesis of heightened HPA axis activity in individuals with visceral obesity [7].

Elevated triglyceride levels (hypertriglyceridemia) and low concentrations of HDL-C are commonly observed in metabolic syndrome (MetS). Studies have shown that higher cortisol levels, including urinary free cortisol and overnight serum cortisol, are associated with elevated triglycerides and reduced HDL-C levels. Genetic variations in the glucocorticoid receptor (GR) may also impact the activity of the hypothalamic-pituitary-adrenal (HPA) axis and lipid metabolism.

CORTISOL

The underlying cause of MetS and the mechanisms by which its various components manifest are still not fully understood. However, it has been suggested that cortisol, a hormone involved in the stress response, may play a role in the development of MetS. Although MetS is characterized by only mild hypercortisolism compared to Cushing's syndrome (CS)—a condition caused by excess cortisol—both conditions share several diagnostic features. It has been proposed that inhibiting cortisol action could represent a potential therapeutic strategy for MetS. Initial data indicate that individuals with MetS have higher circulating cortisol levels than healthy individuals, both at rest and in response to stress. This difference is more pronounced in MetS patients with hypertension or impaired glucose tolerance. Weight loss has been shown to normalize cortisol levels and improve insulin resistance. Despite having cortisol levels within the normal reference range, individuals with MetS may exhibit increased cortisol activity in peripheral tissues and dysregulation of the HPA axis. Numerous studies have reported an association between cortisol levels and both systolic and diastolic blood pressure. This correlation is likely mediated by the influence of stress, which activates both the HPA axis and the sympathetic nervous system. Individuals with MetS and hypertension have been found to exhibit higher urinary levels of cortisol and catecholamine metabolites compared to healthy controls. Glucocorticoids may contribute to elevated blood pressure through several mechanisms. One potential mechanism involves increased responsiveness to vasoconstrictors, along with decreased production of vasodilators. Some studies have shown that

glucocorticoid-induced hypertension is associated with a reduction in nitric oxide (NO), a key vasodilator. In MetS, it is suggested that hyperinsulinemia and insulin resistance may promote endothelin-1 (ET-1) release, contributing to renal injury frequently observed in these patients and providing another pathway to hypertension [7].

Obesity, a common feature of MetS, is also associated with hypertension. Several mechanisms contribute to this relationship, including volume expansion, increased cardiac output and systemic vascular resistance, enhanced sodium reabsorption, heightened sympathetic nervous system and renin-angiotensin-aldosterone system (RAAS) activity, elevated leptin levels with concurrent leptin resistance, and increased ET-1 along with decreased NO. In patients with MetS, serum cortisol levels are significantly correlated with fasting glucose concentrations. The association between fasting hyperglycemia and cortisol is attributed to glucocorticoid effects on hepatic gluconeogenesis and insulin secretion [8].

TESTOSTERONE AND METABOLIC SYNDROME

Testosterone plays a significant role in MetS, and compelling evidence indicates that low testosterone levels are an independent risk factor for the development of MetS and type 2 diabetes in men. Moreover, emerging research suggests that testosterone deficiency is also associated with an increased risk of cardiovascular disease (CVD). Numerous recent reviews have emphasized the critical link between hypogonadism (testosterone deficiency), MetS, diabetes, and CVD.

In men, there is a strong inverse correlation between body fat and testosterone levels. Abdominal obesity is consistently associated with low testosterone in both cross-sectional and longitudinal studies. Waist circumference and waist-to-hip ratio also show an inverse relationship with sex hormone-binding globulin (SHBG) levels. Imaging studies using CT or MRI to quantify abdominal fat have confirmed that low testosterone concentrations are linked to increased visceral fat accumulation. Central fat depots, in particular, exhibit high aromatase activity, which leads to the conversion of testosterone to estrogen—further exacerbating the hormonal imbalance and metabolic dysfunction.

Central fat depots' elevated aromatase activity results in increased local conversion of testosterone to estrogen, which helps explain why obese men often exhibit higher estrogen levels. Testosterone promotes the development of myocytes (muscle cells) and inhibits the differentiation of adipocytes (fat cells) from pluripotent stem cells, contributing to increased lean mass. In contrast, testosterone deficiency favors fat accumulation. Testosterone also increases beta-adrenergic receptor density, promoting lipolysis and reducing fatty acid synthesis. Studies using androgen receptor (AR) knockout mouse models have shown that androgen deficiency impairs lipolysis and significantly contributes to obesity development.

The hypogonadal–obesity cycle hypothesis [9] proposes that testosterone inhibits the activity of lipoprotein lipase in adipocytes—an enzyme responsible for breaking down triglycerides into absorbable free fatty acids, which are then re-esterified and stored. Low testosterone, due to increased aromatase activity, creates a self-reinforcing cycle that increases fat storage and further decreases testosterone levels. The hypogonadal–obesity–adipocytokine hypothesis further suggests that the body is unable to compensate for low testosterone due to the suppressive effects of estrogen and certain adipocytokines (e.g., TNF-α, IL-6, leptin) on the hypothalamic-pituitary-gonadal axis, resulting in hypogonadotropic hypogonadism.

Additionally, excessive aromatase activity in obese men suppresses gonadotropin-mediated testosterone production. Most of the negative feedback exerted by circulating testosterone on the hypothalamic-pituitary axis is mediated via its conversion to estradiol, either in peripheral adipose tissue or centrally. This supports the known association between type 2 diabetes, MetS, and low gonadotropins. Several studies have reported increased levels of LH, FSH, and testosterone secretion in obese men treated with aromatase inhibitors and selective estrogen receptor modulators [10].

Insulin resistance and hyperglycemia are hallmark features of type 2 diabetes, often linked to obesity. Emerging evidence shows a strong association between low testosterone and diabetes, with testosterone replacement therapy (TRT) showing potential benefits in improving insulin sensitivity and glycemic control.

Multiple studies have demonstrated an inverse relationship between total testosterone and insulin levels, independent of age and obesity, in healthy, non-diabetic men. The San Antonio Heart Study also confirmed this inverse correlation between total and free testosterone and insulin levels. A meta-analysis of 21 clinical studies involving 3,825 men confirmed a high prevalence of low testosterone in individuals with diabetes and/or MetS. Conversely, men with higher testosterone levels had a 42% lower risk of developing type 2 diabetes. Some studies also suggest that low testosterone may precede the onset of diabetes or insulin resistance [11].

Moreover, the third National Health and Nutrition Examination Survey (NHANES III) found that men in

the lowest tertile of free or bioavailable testosterone were significantly more likely to have diabetes than those in the highest tertile—even after adjusting for age and obesity. These findings collectively suggest that low testosterone is closely linked with diabetes and MetS and may play a crucial role in the pathogenesis of insulin resistance.

In a recent cross-sectional study of 355 men with type 2 diabetes, Kapoor and colleagues found that 17% had overt hypogonadism (defined by clinical symptoms and total testosterone <8 nmol/L and/or bioavailable testosterone <2.5 nmol/L), while an additional 25% had borderline hypogonadism. Another study, using equilibrium dialysis to measure free testosterone in 103 men with type 2 diabetes, found that 33% had free testosterone levels below the normal range, indicating biochemical hypogonadism [12].

Longitudinal studies have confirmed that low testosterone is an independent risk factor for the development of diabetes and MetS. Baseline testosterone levels were inversely associated with central fat accumulation (but not with other fat depots) in a cohort of 110 men. The Massachusetts Male Aging Study (MMAS), the Multiple Risk Factor Intervention Trial (MRFIT), and the Rancho Bernardo Study all demonstrated inverse correlations between baseline testosterone and the subsequent development of diabetes. A Finnish study also showed that low baseline testosterone and SHBG levels predicted the onset of MetS and diabetes during an 11-year follow-up period [13].

The precise mechanisms linking testosterone with insulin resistance and type 2 diabetes are not yet fully understood. While testosterone deficiency can lead to increased fat accumulation—thereby contributing to insulin resistance—this alone may not fully explain its overall impact on insulin sensitivity. For example, a study using hyperinsulinemic-euglycemic clamps to assess insulin resistance in 60 men with varying degrees of glucose tolerance (from normal to diabetic) found an inverse relationship between total testosterone and insulin resistance. The researchers also discovered that low testosterone impairs mitochondrial oxidative phosphorylation in muscle biopsies. Since up to 70% of the body's insulin sensitivity is attributed to skeletal muscle, reduced insulin sensitivity in the hypogonadal state may significantly contribute to systemic insulin resistance.

Low testosterone levels are closely associated with the development of metabolic syndrome (MetS). Hypogonadal men tend to have increased body fat mass, as shown in a study of 57 men aged 70–80 years, where testosterone levels were negatively correlated with percentage body fat. Several epidemiological studies have also demonstrated associations between testosterone and MetS. For instance, a Finnish study of 1,896 nondiabetic men found that both free and total testosterone levels were significantly lower in individuals with MetS. The Quebec Family Study reported that higher testosterone levels were associated with a reduced risk of MetS and improved insulin sensitivity. Conversely, another study involving 803 men found that hypogonadism was highly prevalent among individuals with MetS [14].

The Baltimore Longitudinal Study of Aging, which included 618 community-dwelling healthy men with a mean age of 63 years, showed that age alone did not predict the development of MetS. However, both total testosterone and SHBG levels were inversely associated with the development of MetS over a mean follow-up period of 5.8 years, while the free testosterone index and body mass index (BMI) were positively associated with MetS incidence. Similarly, the Massachusetts Male Aging Study analyzed 950 non-obese men without MetS and found that low testosterone levels predicted the subsequent development of MetS [15].

Moreover, follow-up data from the aforementioned Finnish study indicated that men with MetS at baseline were at increased risk of developing hypogonadism over an 11-year period [16]. A recent cross-sectional study from Australia involving 2,502 community-dwelling men aged 70 years without diagnosed diabetes reported that men with hypogonadotropic hypogonadism had a high prevalence of MetS. These findings further support the strong association between low testosterone levels and the development of MetS.

In summary, there is substantial evidence linking low testosterone levels and clinical hypogonadism to a higher prevalence of MetS and type 2 diabetes in men. Hypogonadism negatively influences several components of MetS, especially cardiovascular risk factors. Testosterone deficiency itself is recognized as a significant risk factor for the development of MetS and type 2 diabetes [17].

CONCLUSIONS

Metabolic syndrome (MetS) is a cluster of metabolic abnormalities that predispose individuals to the development of diabetes, atherosclerosis, and cardiovascular disease (CVD). It is important to determine whether MetS is accompanied by diabetes, as many individuals with MetS may already have established diabetes and/or vascular disease. Due to the overlapping characteristics between MetS and Cushing's syndrome (CS), it has been proposed that the pathogenesis of MetS and central obesity may involve chronic exposure to elevated glucocorticoids.

Emerging evidence suggests that patients with MetS exhibit hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis, resulting in a state referred to as "functional hypercortisolism." Stress is thought to play a key role in this process by increasing the reactivity of the HPA axis. Furthermore, low birth weight has been associated with elevated circulating cortisol levels, indicating a possible long-term dysregulation of the HPA axis due to early-life stress or epigenetic programming.

The enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), which regulates glucocorticoid metabolism in peripheral tissues—particularly adipose tissue and the liver—has been implicated in both MetS and central obesity. Overexpression of 11β-HSD1 in adipocytes has been observed in individuals with MetS, leading to enhanced conversion of inactive cortisone into active cortisol, thereby increasing local glucocorticoid activity. Experimental studies using 11β-HSD1 inhibitors support the involvement of this enzyme in the pathogenesis of MetS and suggest that targeting 11β-HSD1 may offer novel therapeutic strategies for managing MetS and obesity.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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REVIEW ARTICLE

CONTENTS

The impact of COVID-19 on dental practice and care: Adapting to unprecedented times

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ABSTRACT

Aim: This review aims to shed light on the ways dental practices and patient care strategies have evolved in response to the pandemic. It also investigates how patients' perspectives and dentist-patient dynamics have shifted, highlighting lessons for the future of dental healthcare systems.

Materials and methods: The study is based on a comprehensive analysis of previously published research articles and clinical reports on how dental practitioners adapted their practices during the COVID-19 pandemic. It includes qualitative and quantitative data reflecting both professional and patient experiences. The pandemic led to the rapid adoption of new technologies, heightened hygiene protocols, and increased mental health burdens on both patients and practitioners. Tele-dentistry, limited in-person visits, and stricter sterilization practices became the norm. Patients expressed both fear and appreciation for enhanced safety, altering their expectations of dental care, resilience and adaptability in dental settings.

Conclusions The lessons learned from COVID-19 experience underline the importance of incorporating dentistry into broader public health strategies. Moving forward, there is a need to invest in innovative technologies, uphold rigorous hygiene standards, and provide mental workers and patients. These steps are essential to prepare for future health emergencies and ensure the sustainability of dental care delivery.

KEY WORDS: COVID-19 and dentistry, dental practices, dentist-patient relationship dynamics, dentists' attitudes, dental care

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INTRODUCTION

The appearance of the COVID-19 virus and subsequent global pandemic completely changed the face of medicine and dentistry [1-2]. Because dentists are in close quarters with patients and are constantly exposed to respiratory droplets from patients, dentists have ended up at a much higher risk of contracting COVID-19 than the general public [3]. Dentists' perspective and attitudes towards the virus shape the security of dentists, their staff, and patients – in addition to the public health response [1]. In the context of a highly infectious illness, the inherent dangers of direct patient contact and close proximity these patients make it a dangerous occupation. This makes the dental industry one of the first in the healthcare system to have to change previous health suggestions for the virus and revise virus containment practices [4]. The quality of patient care and patients' confidence in these vital healthcare services are directly affected by dentists' perception of these risks as well as their ability to adapt and manage the dynamic situation. In addition, the pandemic has given dentists greatly increased hesitation over the best approach for containment practices [5]. Better understanding of the necessary containment practices, for instance, the use of better PPE (Personal Protection Equipment), adjustments to patient care, and stricter sanitization protocols, are vital in determining how the dental community responds to the COVID-19 pandemic [6-8]. Dental professionals, who have a responsibility for their patients while also securing their own and their employees' safety can easily get emotionally and psychologically impacted by such attitudes. When it comes to public health advocacy, dentists are indispensable, mainly by getting people to receive the COVID-19 vaccine and adhere to public health measures. There are several carriers that have revealed promising results in the development of COVID-19 vaccines using artificial intelligence in the past [9]. This study gathers data from a diversity of sites for a clearer image of how dentists dealt with the encounters of presenting care through a comunnity health emergency. The current study provides detailed insights into the pandemic consequences for dental health services by studying changes in patient attendance, the adoption of strict biosafety measures,

and the psychological influence on both providers and recipients of dental care. Correspondingly covered in this study are the more far-reaching impacts, which include dramatic changes to the dental industry's educational system and economic system. In addition to being a crucial device for assessing rapid reactions to COVID-19, this study plays a vital role in guiding the upcoming policy of dental healthcare and preparedness doctrines – which makes the industry a whole more resilient to future epidemics or crises of a similar kind.

AIM

This review aims to shed light on the ways dental practices and patient care strategies have evolved in response to the pandemic. It also investigates how patients' perspectives and dentist-patient dynamics have shifted, highlighting lessons for the future of dental healthcare systems.

MATERIALS AND METHODS

This systematic review was conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The methodology was designed to identify and synthesize all relevant literature on the impact of COVID-19 on dental practices, patient perceptions, and the resulting adaptations.

INFORMATION SOURCES AND SEARCH STRATEGY

A comprehensive systematic search was performed across multiple electronic databases to identify relevant published literature. The databases included PubMed/MEDLINE, Scopus, Web of Science, and EMBASE. To ensure thorough coverage, the search was supplemented by a manual review of the reference lists of included studies and key review articles. Furthermore, a search of grey literature was conducted through Google Scholar and the websites of major dental associations and public health organizations (e.g., American Dental Association, World Health Organization) to identify clinical practice guidelines, reports, and other relevant documents.

The search strategy was designed to encompass the period of the COVID-19 pandemic, specifically from March 2022, up to March 2023. This timeframe was selected to capture the entire evolution of the pandemic's impact on dentistry.

SEARCH KEYWORDS

The search utilized a combination of Medical Subject Headings (MeSH) terms and free-text keywords related to the core concepts of the review included.

Population/Context: dentist, dental practice, dental care, dental patient, oral health.

Intervention/Phenomenon of Interest: COVID-19, SARS-CoV-2, coronavirus, pandemic.

Outcomes: adaptation, perception, attitude, anxiety, stress, tele dentistry, infection control, guidelines, personal protective equipment.

These terms were combined using the Boolean operators "AND" and "OR" as needed.

ELIGIBILITY CRITERIA AND STUDY SELECTION

The study selection process followed pre-defined eligibility criteria.

INCLUSION CRITERIA

1. Original research articles (e.g., cross-sectional surveys, cohort studies, qualitative studies) and clinical practice guidelines
2. Studies focusing on the impact of COVID-19 on dental professionals (e.g., knowledge, attitudes, psychological stress, practice adaptations) or dental patients (e.g., perceptions, fears, expectations)
3. Studies reporting on adaptations in dental care delivery, such as the implementation of tele dentistry, revised infection control protocols, or new triage systems. Publications in the English language.

EXCLUSION CRITERIA

1. Letters to the editor, opinion pieces, narrative reviews without systematic methodology, and conference abstracts.
2. Studies focusing exclusively on non-COVID-related topics in dentistry.
3. Studies for which the full text could not be retrieved.

The study selection was performed in two stages. Initially, two reviewers independently screened the titles and abstracts of all retrieved records against the eligibility criteria. Subsequently, the full texts of potentially relevant articles were assessed independently by the same two reviewers. Any disagreements at either stage were resolved through discussion or by consulting a third reviewer.

DATA EXTRACTION AND SYNTHESIS

From the final included studies, data were extracted using a standardized data extraction form. The extracted information included:

Study characteristics: first author, publication year, country, study design, and study period.

Table 1. Comparative overview of global studies on dentists and patients

Country/Region	Study Focus	Key Findings
Iran	Dentists' Knowledge & Attitude	Good knowledge about COVID-19, positive attitude towards dental care, challenges like PPE need and financial difficulties [16].
Mexico	Dentists' Awareness & Behavior	Awareness of COVID-19, knowledge gaps in symptoms and PPE use, willingness to provide emergency treatment [17].
Italy	Dentists' COVID-19 Perception	Informed about transmission, concerns about health and economic impact, need for clear guidelines and effective PPE use [18].
Libya	Online Dental Education	High satisfaction with online courses, despite challenges in e-learning infrastructure [20].
USA	Dental Patients' Perceptions	Concerns about contracting COVID-19 in dental settings, impact of demographic factors on perceived risk, conditions for returning to care [21].
International	Dental Specialists' Knowledge	Good overall knowledge and perception, variance based on age, knowledge source, and specialization [22].
Brazil	Dentists and Dental Undergraduates	General recognition of symptoms and transmission routes, knowledge variations among demographics [23].
China	Orthodontists' knowledge and attitudes	Development of knowledge, sureness, and preparedness of orthodontic professionals [24].
Poland	Dental Practitioners' Responses	Reduction in patient numbers, decision-making in clinic operations, impact of PPE shortages [19].
Kosovo	Dental Students' Knowledge	High awareness of COVID-19 symptoms, transmission, preventive measures, and related stress [25].
Jordan	Dentists' COVID-19 Awareness	Knowledge of symptoms, transmission modes, attitudes towards treating COVID-19 patients, importance of continuous education [26].
Austria	Dentistry Students' Attitudes	Dental students have a good grasp of COVID-19 basics but show gaps in hygiene protocols and infection control knowledge [27].

Source: Author's own work based on [16–27])

Population: sample size and characteristics (e.g., dentists, dental staff, patients).

Key findings related to the review's aims, including quantitative data (e.g., percentages, prevalence rates) and qualitative themes (e.g., perceived challenges, adaptive strategies).

Given the heterogeneity in the study designs and outcomes of the included literature (encompassing both quantitative and qualitative data), a narrative synthesis approach was adopted. The findings were organized thematically to address the key objectives of the review, such as changes in clinical practice, psychological impacts, shifts in the dentist-patient relationship, and lessons learned for future preparedness.

REVIEW AND DISCUSSION

PANDEMIC IMPACT AND ADAPTATIONS IN DENTAL PRACTICES

In the early phases of COVID-19, when the virus began to spread the most, a negative phenomenon was observed regarding dental attendance. Various dental offices, clinics, and health centers observed a sharp decrease in patient appointments. As a result of stringent public health measures and the closure of numerous dental clinics, the number of dental appointments has declined by a shockingly high ratio - 80%. Patient anxiety spiked (concurrent with this decline in dental serves), and with 60% of patients reporting increased worries [10]. The decline can be attributed to the implementation of lockdown techniques, the widespread panic from the virus among patients, and the advice to pause non-urgent dental appointments. As a result, dental offices had to reschedule patients' visits and many even placed an end to certain non-emergency treatments [11]. Decreasing patient flow and following social distancing norms both required this change, causing more stringent sterilization procedures and increased cleaning of surfaces and floors. These were the premier infection control strategies put in place by dental clinics worldwide [12-14]. Plexiglass barriers, distinct entry and departure methods, and rearranging waiting areas were used to impose social distancing and slow the spread of the virus. Staff received additional training on COVID-19 safety measures. The use of enhanced PPE became standard, including N95 masks, face shields, gowns, and gloves [15]. A study focused on the impact of COVID-19 on dental practices in the region of northern Iran and investigated the

dentist's knowledge about COVID-19, attempting to promote a positive attitude towards dental care. The study also highlights challenges such as the need for personal protective equipment (PPE), financial difficulties in relation with buying PPE during national shortages, and the necessity of health training programs for dental professionals for better containment of infectious diseases [16]. Another study indicated that most dentists were aware of COVID-19 and its transmission, but their knowledge of signs, symptoms, and consistent use of PPE was limited, and a significant portion of dentists were willing to continue providing emergency treatment during the pandemic [17]. A study that evaluated Italian dentists' knowledge about COVID-19 was conducted via a questionnaire in April 2020 and involved roughly 1,500 dentists who were informed about the mode of transmission but less knowledgeable about symptoms and patient presentation. The study highlighted concerns about health, economic impacts on practices, and the need for precise operating guidelines and effective use of PPE [18]. A Cross-Sectional Survey examined the response of Polish dentists to the pandemic, it highlights the significant reduction in patient numbers, the dentists' decision-making factors regarding clinic operations, and the importance of access to PPE. The study found a major impact on dental services due to PPE shortages and the need for enhanced infection control protocols [14]. In most countries, including Italy, Brazil, Jordan and many other, dental professionals demonstrated good general knowledge about COVID-19's symptoms and transmission as presented in Table 1.

DENTAL PATIENT PERCEPTIONS AND RESPONSES TO COVID-19

The COVID-19 pandemic has significantly impacted the dynamics of dentist-patient relationships, communication, and patient attitudes towards dental care. These changes are a direct response to the challenges posed by the pandemic and have reshaped the way dental care is delivered and received [19]. During the pandemic there has been a shift towards digital modes of communication, such as emails, text messages, and social media, to convey information about changes in practice operations, safety protocols, and appointment scheduling. Teledentistry has become more prominent, allowing for remote consultations, follow-ups, and triaging of dental emergencies, thereby reducing the need for in-person visits [20-21]. The shared experience of navigating the pandemic may lead to stronger dentist-patient relationships, with patients valuing the efforts made by dental practices to ensure safety. Both dentists and patients are adapting to a 'new normal' in dental care, which may include continued use of enhanced safety measures and a greater reliance on digital communication even post-pandemic [22]. The immediate need to adapt to new circumstances and manage care amidst the pandemic has significantly increased stress and workload for patients. Adhering to strict infection control measures, rescheduling their appointments, and managing a backlog of postponed treatments have been challenging [20]. Patients may demand higher standards of infection control and become more cautious about routine dental visits, affecting how dentists engage with and educate their patients [23]. Understanding dental patients' perceptions in the United States regarding the risk of COVID-19 transmission in dental settings, utilizing an electronic survey, the study gathered responses from 464 adults on their attitudes, beliefs, and perceived susceptibility to contracting COVID-19 in dental offices. Key insights include concerns about contracting the virus in dental settings, the impact of patients' demographic factors on their perceived susceptibility, and the factors influencing their willingness to return to routine dental care [23]. The role of Dentistry technologies in revolutionizing dental education and care amidst the COVID-19 pandemic, with twenty notable enhancements has been discussed in [20]. These advancements range from improved healthcare device connectivity to the implementation of smart transportation systems within hospitals. They have streamlined operational processes, boosted productivity, and enhanced treatment efficacy. This digital transformation underscores a significant shift in dental practices, marking a step forward in healthcare innovation. In a study with a 57% response rate, 86.2% of female and 57.8% of male dental students preferred offline learning, especially those over 22 years. A year-wise increase in offline exam preference peaked at 100% in the 4th year. Overall, 77.7% of students opposed continuing e-learning, highlighting a strong preference for traditional, in-person dental education [24]. WhatsApp application has been explored in dental care, with systematic searches yielding 327 articles, of which six met the criteria for randomized clinical trials. These articles highlight WhatsApp's effectiveness in remote consultations, oral hygiene maintenance, and disease diagnosis in underprivileged areas. The findings emphasize its role in enhancing doctor-patient interactions and knowledge sharing in orthodontics, leading to better patient compliance and engagement [25]. In a study involving 12 public dental practitioners from Sydney, teledentistry's adoption in Australian public oral health services was evaluated. Participants generally accepted teledentistry, appreciating standardized consultation templates and

Table 2. Challenges and Acceptance of Enhanced Safety Measures in Dental Practices during COVID-19

Aspect	Challenge	Acceptance
PPE Shortage	Significant initial shortage of masks, gowns, and face shields, posing challenges for dental practices.	Routine PPE use grew as supply chains stabilized, with widespread recognition of its necessity for safe practice.
Financial Burden	High costs for additional PPE and safety equipment, particularly burdensome for small or independent clinics.	Most dentists have accepted these costs as necessary for patient and staff safety and practice continuation.
Adaptation to New Protocols	Significant changes to practice operations were required to adapt to new safety protocols.	Growing integration and proficiency in these protocols among dental staff over time.
Physical Discomfort and Communication Barriers	Extended PPE use led to physical discomfort and communication issues with patients.	Adaptations like communication aids and regular breaks to alleviate discomfort.
Training and Compliance	Challenges in training staff and ensuring consistent compliance with new protocols in busy environments.	Higher compliance and acceptance achieved through continued education and importance reinforcement.
Patient Management and Scheduling	Changes in scheduling and patient interaction protocols needed to manage patient flow and implement social distancing.	Patients and staff adapting well to these changes, understanding their importance for safety.
Psychological Impact on Staff	High-risk work environment and stringent protocols lead to stress and psychological impact on dental professionals.	Ongoing challenge, with increasing support systems for mental well-being.
Public Perception and Patient Confidence	Ensuring patient safety and understanding new protocols in high COVID-19 areas was challenging.	Rebuilding patient confidence through effective communication and demonstration of safety measures.

Source: Author's own work based on [16–27]

communication among clinicians. Despite this, challenges in patient contact and technological difficulties had been noted – the study's result mentions the potential of teledentistry, indicating that more studies are needed to verify how to make it work best in the context of a public health setting.

PSYCHOLOGICAL IMPACT OF THE PANDEMIC

Dentists and their patients have been greatly affected psychologically by the COVID-19 pandemic [26]. Panic about contracting the virus, keeping up with ever-evolving standards, and fears about the future of their profession and their income all contributed to dentists' already high levels of stress and anxiety when COVID-19 was at its peak. Patients also reserved fears of contracting COVID-19 during their appointment, so several approaches were used to decrease the impact of these mental health issues [27]. Stress-controlling measures, regular and open contact with patients to calm their anxieties, and mental health care for dental staff were all part of the package. In this context, the role of antioxidants becomes increasingly relevant. Antioxidants have been shown to combat oxidative stress, which can be exacerbated by chronic psychological stress, thus potentially mitigating some of the negative health outcomes associated with heightened anxiety during the pandemic [28]. Addressing these psychological challenges also requires fostering a supportive work environment and advocating for a balanced approach to work and life [29]. The research investigated the perceived risk and preventive behaviours of dental students in Kosovo regarding COVID-19 [23]. The study included using a questionnaire that covered topics such as fear of COVID-19, symptoms, transmission, and stress connected to the virus. It involved 157 students from different study years. According to the findings, students are quite concerned about contracting COVID-19 and are well informed about the virus. This study looked at how the epidemic affected these students emotionally [29]. Despite dentists' best struggles, the mental toll of the pandemic implies they could use further assistance in adjusting their practices. Because the pandemic is still going strong, dentists need to be willing to deal with the emotional and mental toll as well as the physical ones [30]. The heightened emphasis on safety has had a psychological impact on dental professionals, while some express a sense of security due to these measures, others experience increased stress and anxiety related to the potential for infection and the responsibility of protecting their staff and patients. Several studies highlighting the psychological impacts including fears of infection, financial concerns, changes in work protocols, and the overall uncertainty

surrounding the pandemic. A study reported that dental professionals experienced significant anxiety, particularly related to the use of PPE and the effectiveness of infection control measures in preventing the spread of COVID-19 [31]. Numerous dentists feel overwhelmed by the demands of ensuring safety while providing effective treatment. This highlights the urgent requirement for supportive measures to address these mental health challenges [32]. Because of not knowing how long the epidemic would last and how it would affect the feasibility of dental practices, a survey published in "Community Dentistry and Oral Epidemiology" stated that dental professionals experienced increased burnout and emotional strain [33]. There was a correlation between Chronic Obstructive Pulmonary Disease COPD and OSA in meta-analyses, but no such correlation with asthma. The study revealed a correlation between periodontitis and COVID-related mortality as well as the necessity of assisted ventilation. Periodontal cure improved symptoms in COPD, asthma, and CAP. This section, where dental health and lung health, requires additional investigation and intervention trials [34]. The financial viability of dental practices was harshly affected by the temporary closure of practices and lower patient volume, which contributed to overall stress levels. The pandemic disproportionately affected younger dentists and those working in private practice, leaving them financially vulnerable [35]. Furthermore, dental practitioners' mental health and well-being were damagingly impacted by the pandemic. Challenges with handling patient care considering inexperienced regulations, concerns about infecting loved people, and interference with work-life stability were among the factors mentioned [36]. Despite these challenges, some research, such as a study by [37] that focused on the adaptability of dental practitioners during the pandemic. Many dentists employed various coping strategies, such as seeking assist from colleagues, remaining informed about COVID-19 updates, and prioritizing their health and safety guidelines. These ways reflect the requirement for flexibility in changing dental practices and ensuring compliance with new protocols, as highlighted in the paper examining dentists' experiences during the COVID-19 pandemic. The dentistry community's reactions to the COVID-19 safety measures, and it's clear that they were resilient and adaptable in the face of adversity as summarized in Table 2.

This summary highlights the continuing necessity for adaptation and support in the field, while also reflecting the dedication of dental professionals to maintaining safety standards during a worldwide health crisis.

LESSONS LEARNED AND FUTURE PREPAREDNESS

Imposing the vital constraint of strict infection control measures in dental settings is only one case of how the dentistry sector can use the lessons learned from the COVID-19 pandemic to future situations. Along with stressing the implication of flexibility, specifically when it comes to transitioning to emergency treatment and applying tele-dentistry [38]. Dentists should always be knowledgeable about new health hazards and they should communicate visibly with their patients. Furthermore, it highlighted the importance of mental health assistance for practitioners and patients and the requirement for robust emergency response plans, comprising the storage of personal protective equipment and the development of contingency operational methods. Many people in the dental sector around the world are working together to share their experiences and best practices because of the pandemic. The dentistry industry can use these findings to better handle future crises of a similar kind. Managing the epidemic has brought home to many dentists the magnitude of being ready for comparable public health emergencies in the future. Staying aware of emerging health concerns, having a plan for handling infectious diseases, and maintaining a stockpile of necessary supplies are all part of being prepared. Sights on the new precautions have altered as the pandemic has proceeded. As dentists have gained more experience with these procedures, their initial feelings of concern have mostly evaporated [39]. The capacity to promptly assimilate new safety norms and specifications is an extraordinary quality in numerous dentists. Their flexibility reflects their dedication to the wellbeing of patients and their sense of professional duty. Several dentists are selfassured about the effectiveness of their infection control procedures. This certainly stems from the fact that, before the pandemic, dental practices adhered to stringent hygiene and sterilizing measures. Improved personal protective equipment (PPE) and patient screenings, which are special to COVID-19, have added to this assurance [40]. The current literature supplies helpful information regarding the effects of COVID-19 on dentistry, but it also highlights the requirement for further comprehensive and varied research. The findings should be more broadly applicable if future research seeks to incorporate more countries, especially those with low or medium incomes. Research into the long-term consequences of the pandemic on dentistry education, practice, and policy is urgently required. Also, less-studied areas of dentistry and orthodontics should get additional research results

so that we can learn about the specific complications and solutions they faced during the pandemic. In addition, one of the most important ways to tackle the problems caused by the COVID-19 pandemic in dentistry is to incorporate new nanomaterials. The properties of nanomaterials are supreme [41] to assist in the advancement of cutting-edge dental procedures and improve procedures of infection prevention. Their usefulness covers a wide range of areas, including orthodontic biofilm prevention and the avoidance of microbial infections [42-43]. Dentists' flexibility in applying nano biomaterials and their dedication to patient safety have proven critical. This alteration in thinking highlights the consequence of ongoing training for dentists so they can keep up with new health technology. Nano biomaterials are a potential tool for strengthening infection control procedures, which could help the dentistry community adapt to future emergencies and remain strong in the face of global health issues. To understand the complete economic effect on dental offices and to guide sustainable company models in the aftermath of the epidemic, thorough economic evaluations are essential. At the end, filling these gaps will enhance our knowledge and make the dentistry sector more prepared to handle future global health emergencies.

CONCLUSIONS

To conclude, our review succinctly summarizes the far-reaching impact of the COVID-19 pandemic and how it transformed numerous avenues of dental policy and practice. Our review examines how the pandemic brought about a sweeping alteration in safety regulations and standards - centered on the importance of infection prevention. A critical development, like the rapid adaptation to digital instruments like tele-dentistry, guarantees continuity of care while reducing the risk of infection. The psychological toll on dental workers is considerable and highlights the necessity of improved mental health services in the sector. Due to the pandemic's impact on the global economy, management practices have been reevaluated to make sure that business models are stronger in case more economic shocks are witnessed. Crucial for sustaining trust and continuity of care, the pandemic has rethought the dentist-patient relationship, elevating the need for openness and education. The pandemic also prompted major shifts in the industry, which has rewarded the ingenuity and innovation needed to adapt to the new challenging conditions. To warrant patient safety and resilience in the future, learning from this experience is an imperative for the dental community to be better prepared for emergencies. The prospects of building a stronger and more efficient (yet patient-centered) model in the future will depend on these outcomes.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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REVIEW ARTICLE

CONTENTS

In-office *vs* at-home tooth bleaching: A narrative review of efficacy and safety

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ABSTRACT

To compare in-office (chairside) and at-home (dentist-supervised) vital tooth bleaching techniques in terms of efficacy, longevity, safety, sensitivity, and patient-reported outcomes. A narrative review of the literature was conducted using PubMed, Scopus, and Cochrane Library, including randomized trials, systematic reviews, and meta-analyses published between 2000 and 2025. Studies directly comparing in-office and at-home bleaching with peroxide-based agents were selected. Outcomes of interest included immediate color change, relapse over time, incidence and intensity of tooth sensitivity, and adverse effects on enamel or pulp. Emphasis was placed on high-quality evidence from the last five years. Both techniques achieved significant, perceptible whitening. Numerous meta-analyses showed no clinically meaningful difference in bleaching efficacy when treatment protocols were followed. At-home bleaching (10–16% carbamide peroxide) and in-office bleaching (25–40% hydrogen peroxide) produced comparable ΔE and shade guide improvements. Some studies suggested slightly greater long-term color stability with at-home bleaching, likely due to longer contact time and ease of touch-ups. Sensitivity occurred frequently with both methods (incidence 37–90% at-home, 17–100% in-office), but was typically more intense in-office and milder but more recurrent with at-home use. No irreversible pulp or enamel damage was reported. Light activation did not enhance whitening outcomes or reduce sensitivity. Both methods were well accepted by patients. In-office and at-home bleaching are equally effective and safe. In-office offers faster results but a higher risk of acute sensitivity; at-home provides gradual whitening with lower intensity side effects and better long-term maintainability. Choice of technique should be individualized based on sensitivity profile, desired speed, compliance potential, and lifestyle.

KEY WORDS: tooth bleaching; hydrogen peroxide; carbamide peroxide; tooth sensitivity; color stability; cosmetic dentistry

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INTRODUCTION

The desire for whiter teeth has become increasingly common in recent years, paralleling growing public demand for cosmetic dentistry [1]. Tooth bleaching – the lightening of tooth color using chemical agents – is now a routine procedure in dental practice. Tooth discoloration can be extrinsic (surface stains from foods, beverages, smoking, etc.) or intrinsic (internal tooth pigment changes due to factors like aging, fluorosis, tetracycline exposure, or trauma). Bleaching is most effective for extrinsic and age-related discoloration, penetrating enamel and dentin to oxidize pigmented molecules, thereby producing a lighter tooth shade. Two principal techniques for vital tooth bleaching are widely employed: in-office bleaching, done by dental professionals in the clinic, and at-home bleaching, in which patients apply dentist-prescribed bleaching agents using custom-fitted trays at home [1].

In-office (chairside) whitening typically uses high concentrations of hydrogen peroxide (H_2O_2) gel (approximately 25–40% H_2O_2) applied to the teeth under direct supervision. The gums and soft tissues are carefully isolated (e.g. with rubber dam or protective gels) to prevent chemical burns from the strong oxidizing agent. The bleaching gel may be applied in one or more sessions of about 15–30 minutes each, often during a single appointment. Sometimes a light or laser is used to heat or "activate" the peroxide, although extensive evidence indicates that light activation does not significantly enhance whitening efficacy or longevity of results [1]. Meta-analyses have concluded that use of light does not improve the bleaching outcome in terms of final shade, nor does it affect the risk or intensity of tooth sensitivity, regardless of the peroxide concentration [1]. (In fact, one umbrella review found that in-office bleaching with a light-activated low-concentration gel

achieved similar results to in-office bleaching with a high-concentration gel without light [2]). Therefore, the presence or absence of a light should not be mistaken as a fundamental difference between at-home and in-office techniques—rather, it is a variable within in-office techniques. In contrast, at-home bleaching involves lower concentrations of bleaching agents, most commonly carbamide peroxide (CP) in concentrations of about 10–20% (which releases ~3–7% hydrogen peroxide) or equivalent low-strength hydrogen peroxide gels (around 6–10%). Patients perform the treatment themselves, wearing a custom-made tray or other delivery device containing the gel, typically for several hours daily or overnight, over a span of 1–3 weeks (depending on the formulation and protocol). This approach was first introduced by Haywood and Heymann in 1989 as "nightguard vital bleaching" with 10% carbamide peroxide in a tray[9]. It quickly gained popularity for its ease of use and proven effectiveness.

Both methods rely on the same fundamental chemistry: peroxide compounds break down into free radicals (reactive oxygen species) that penetrate through enamel prism structure and oxidize organic pigmented compounds in the dentin and enamel. By cleaving the double bonds of chromophores (the molecules responsible for tooth color), these agents render the pigments lighter or colorless, resulting in a whiter appearance. Notably, hydrogen peroxide is the active bleaching radical in both systems (carbamide peroxide decomposes into hydrogen peroxide and urea). The difference lies in concentration and contact time: in-office techniques deliver a strong dose in a short time, whereas at-home techniques use a weaker dose over an extended period to achieve a comparable effect.

Safety is an important consideration with any bleaching. Hydrogen peroxide, especially at high concentrations, is a potent oxidizer that can cause tissue irritation or damage if misused. Professional in-office systems have built-in safety measures, such as professional isolation of soft tissues and controlled application by trained personnel. Carbamide peroxide (urea peroxide) is a stabilized compound that breaks down slower; its use in custom trays allows the saliva to buffer and dilute the peroxide, generally resulting in a gentler treatment per session. Numerous studies and reviews have established that bleaching, when properly conducted, does not cause permanent structural damage to teeth. Transient changes like slight decreases in enamel microhardness or mineral content have been noted in some laboratory studies, but saliva and remineralization can readily reverse these changes, and no clinically significant enamel loss or damage occurs under recommended use [3]. Pulpal health is likewise maintained: while the bleaching agents can diffuse to the pulp and cause transient inflammation (the cause of sensitivity), no irreversible pulpitis or necrosis has been directly linked to vital bleaching in healthy teeth at typical concentrations [2]. Indeed, a recent systematic review on at-home bleaching reported that most patients maintain stable tooth color for 1–2.5 years after treatment with no long-term adverse effects, and only cases of severe initial discoloration show higher relapse over time [4]. Modern bleaching products often incorporate desensitizing agents (e.g. potassium nitrate, fluoride, amorphous calcium phosphate) to help mitigate sensitivity and protect the enamel during treatment [5]. For example, many in-office kits include fluoride or calcium to offset demineralization, and at-home kits may be accompanied by desensitizing gels for use in the tray after bleaching [5].

Given the widespread use of both in-office and at-home whitening, it is important to understand how they compare in outcomes and side effect profiles. Patients often ask which method will make their teeth whiter or which results last longer. Earlier studies and anecdotal perceptions sometimes presumed that the prolonged exposure of at-home bleaching might yield better long-term whitening, whereas in-office offers immediate gratification. To address these questions, a growing body of evidence from clinical trials and systematic reviews has compared the two techniques directly. This review will summarize the scientific evidence on the efficacy, longevity, and safety of in-office vs. at-home tooth bleaching, highlighting any advantages or disadvantages of each approach.

AIM

The aim of this review is to evaluate and compare the effectiveness of in-office (professional) versus at-home (take-home) tooth bleaching techniques, as well as to assess differences in post-bleaching sensitivity and other relevant clinical considerations (such as color relapse, patient satisfaction, and safety to hard tissues). The goal is to determine whether one technique offers superior outcomes or fewer side effects than the other, based on current evidence, and thus to guide clinicians in evidence-based recommendation of bleaching options.

MATERIALS AND METHODS

A literature search was performed to collect relevant publications comparing in-office and at-home tooth whitening. The databases PubMed/MEDLINE, Scopus, and Cochrane Library were queried using keywords and MeSH terms such as "tooth bleaching," "teeth whiten-

ing,""in-office,""at-home,""take-home,""vital bleaching," "hydrogen peroxide," and "carbamide peroxide." The search included articles published in English without an upper date limit, focusing primarily on the last two decades as tooth bleaching techniques became mainstream. Both randomized controlled trials (RCTs) and controlled clinical trials comparing at-home and in-office whitening were sought, as well as systematic reviews and meta-analyses on the topic. Key inclusion criteria were studies on vital (living) teeth bleaching that directly compared an in-office bleaching regimen to a dentist-supervised at-home regimen. Studies evaluating combination techniques (e.g., in-office followed by at-home), over-the-counter products (unsupervised home use), or non-vital tooth bleaching were excluded or considered only for background information.

Titles and abstracts were screened to identify comparative studies. Full texts of eligible studies were obtained and analyzed. Data extracted included sample size, bleaching agents and concentrations used, treatment protocols (number of sessions or days of use), outcome measures (such as color change quantified by shade guides or spectrophotometry, immediate and long-term color stability), and reported side effects (especially the incidence and severity of tooth sensitivity). Because the included evidence spans heterogeneous study designs and outcome reporting, a narrative (descriptive) synthesis is provided rather than a quantitative meta-analysis. However, findings from high-level evidence like systematic reviews are given particular weight. The results are organized around the primary outcomes of whitening efficacy and color stability, and the secondary outcomes of tooth sensitivity and safety. Only findings pertinent to the comparative question (in-office vs. at-home) are highlighted. In-text reference numbers correspond to the bibliography, which is formatted in Vancouver style and contains only the works cited in this review.

REVIEW

WHITENING EFFICACY AND COLOR STABILITY

Immediate Bleaching Outcomes: The literature consistently shows that both in-office and at-home bleaching techniques are effective at significantly lightening tooth color. Numerous clinical trials have directly compared the color change achieved by each method. In general, no statistically or clinically significant difference in immediate whitening efficacy is found between at-home tray bleaching and in-office (chairside) bleaching when each is carried out properly [3, 6]. For example, Giachetti et al. conducted a randomized trial with a 9-month follow-up and reported that a 14-day at-home 10% carbamide peroxide regimen produced virtually the same degree of whitening as a single session of in-office bleaching with 38% hydrogen peroxide, with both groups showing comparable color improvements (measured by spectrophotometer) one week post-bleaching [3]. Other studies echo these findings: Mondelli et al. compared different methods (including in-office 35% H_2O_2 and at-home 15% CP) and found all methods yielded effective whitening with similar immediate color changes (no method was clearly superior) [4]. Patients typically achieve an improvement of several shade guide units (SGU) or a notable increase in lightness (ΔL*) and decrease in yellowness (Δb*) with either approach.

It is worth noting that in-office bleaching can provide visible results faster – often within a single dental visit – whereas at-home bleaching attains the result more gradually over one or two weeks. Despite this difference in treatment timeline, by the end of the full treatment course the degree of whitening is comparable. A recent comprehensive meta-analysis (2025 update) confirmed that there is no significant difference in overall bleaching efficacy (measured in shade change) between in-office and at-home techniques. In that analysis, the mean color improvement in standard shade guide units (ΔSGU) was essentially equivalent for the two methods [5]. Interestingly, the same meta-analysis noted that measurements in the CIELab color space (ΔE*, which captures color difference with a spectrophotometer) were slightly in favor of at-home bleaching on average (a small but statisztically higher ΔE for at-home)[5]. This subtle difference might be due to the longer cumulative exposure of the teeth to peroxide in at-home protocols, potentially allowing more thorough penetration of enamel and dentin. However, the difference in ΔE was modest, and the evidence quality was rated low, meaning it may not translate to a perceptible clinical advantage.

LONG-TERM COLOR MAINTENANCE

An important consideration is how well the whitening results last (i.e., relapse or recurrence of staining). Both techniques have shown durable effects over months to years, though some relapse (partial rebound of tooth color toward baseline) is common with time due to dietary stains and aging. Available studies with follow-up periods indicate that color stability is generally similar for in-office and at-home whitening. Giachetti et al.'s trial found that at 9 months post-treatment, tooth color remained significantly improved compared to baseline in both groups, with no significant difference between

the in-office and at-home patients in terms of shade retention [3]. Mondelli et al. observed patients for 2 years after bleaching and reported all methods had maintained a lighter shade than baseline; the slight differences in relapse between techniques were not statistically significant [4].

Some evidence suggests that at-home bleaching may have a slight advantage in long-term maintenance of tooth color, possibly because patients can perform occasional touch-ups or because the longer initial treatment better oxidizes deep stains. A 2024 systematic review noted that across studies comparing the two techniques, there was a lower recurrence of discoloration (less relapse) when using at-home tray bleaching with carbamide peroxide, compared to in-office alone [6]. In practical terms, this means the at-home group's teeth tended to remain a bit whiter over time before any color regression, although both groups still demonstrated significant whitening at final recalls. For instance, one split-mouth RCT reported that after 3 and 6 months, the in-office side showed more color rebound than the at-home side, despite similar initial whitening [1, 4]. In that study, in-office bleaching had significantly greater relapse by 6 months and higher post-bleaching sensitivity, whereas at-home bleaching maintained color better and with less sensitivity [7, 8]. Over longer periods, maintenance strategies become important: regular dental hygiene and avoiding excessive chromogenic foods/drinks can prolong the effect for both techniques. Clinical reports suggest that without any maintenance, roughly 10% of patients may notice some shade relapse by ~2 years post-bleaching, around 25% by 5 years, and up to 50% by 8 years, depending on lifestyle habits [9]. However, many patients opt for periodic retreatment or "touch-ups," especially those who have at-home trays available, which can substantially extend the whiteness. Indeed, most authors agree that on average the whitening effect can remain stable for at least 1–3 years regardless of technique, with gradual fading thereafter [4]. Some clinicians even combine protocols (an initial in-office session followed by at-home bleaching) to capitalize on immediate results and sustained at-home treatment; however, combination approaches have not shown definitively superior long-term whitening compared to at-home alone, and they can increase total exposure and cost. A recent systematic review concluded that all bleaching types are effective in changing tooth color, and no single method is significantly more effective than others; notably, at-home techniques appeared more effective over time, while combined in-office+at-home techniques did not yield greater efficacy than either alone [10]. Both in-office and at-home bleaching, when done correctly, yield long-lasting improvements in tooth shade, and neither has proven to outperform the other decisively in the long run. The longevity of whitening for both methods is favorable, often on the order of 1–3 years or more before a touch-up might be desired, depending on individual factors.

TOOTH SENSITIVITY AND OTHER SIDE EFFECTS

TOOTH SENSITIVITY

The most frequently reported side effect of vital bleaching is tooth sensitivity (also called dentin hypersensitivity during bleaching). This manifests as transient, sharp sensitivity to thermal stimuli (cold air, liquids) or spontaneous "zing" sensations in teeth, typically during the bleaching period. Both in-office and at-home methods can induce tooth sensitivity, as the peroxide penetrates to the pulp and may provoke a mild inflammatory response. A key question is whether one technique causes more or less sensitivity.

In terms of incidence (risk) of sensitivity, studies show that a large proportion of patients experience at least some sensitivity with either method, but the range is broad. Meta-analyses and trials have reported sensitivity occurring in anywhere from one-third to the vast majority of patients. For at-home bleaching, roughly 37% up to 90% of users have reported transient sensitivity in various studies, whereas for in-office bleaching the incidence ranges from about 17% of patients to nearly 100% in some reports (depending on the product and protocol)[6]. Thus, the likelihood of experiencing sensitivity is high with both approaches. Statistically, many comparisons find no significant difference in whether sensitivity occurs or not between the two techniques [11]. In the latest umbrella review of the literature (aggregating multiple systematic reviews), the risk of developing tooth sensitivity was similar for at-home and in-office bleaching (no significant difference in incidence, $p \approx 0.85$) [11]. Likewise, a 2025 meta-analysis reported that the relative risk of experiencing bleaching-related sensitivity did not differ significantly between at-home vs. in-office treatments [5].

However, when considering the severity or intensity of sensitivity, some differences emerge. In-office bleaching, using strong peroxide for a short duration, tends to produce sensitivity that is more acute but short-lived. Patients often report moderate intensity sensitivity or a sharp "zing" during or immediately after an in-office session, which then subsides within 24–48 hours. By contrast, at-home bleaching can cause sensitivity of mild intensity (often described as slight

twinges or tingling), potentially recurring each day after tray use, but generally more tolerable on a pain scale. The 2025 meta-analysis did find a significantly lower mean intensity of sensitivity with at-home bleaching compared to in-office, suggesting that although both cause sensitivity, the pain was on average milder in the at-home groups [5]. This aligns with clinical observations: the lower concentration peroxide over multiple days causes a diffuse, gentle irritation, whereas one-shot high concentration can provoke a sharper pulp reaction initially. It should be noted that the umbrella review mentioned above concluded that not only was the risk similar, but also the overall intensity of sensitivity did not significantly differ between at-home and in-office when considering the evidence as a whole [11]. The apparent discrepancy in findings may be due to how different studies measure pain or the inclusion of various protocols; in practice, many clinicians find in-office sensitivity, when it occurs, tends to be more intense but brief, whereas at-home sensitivity is more intermittent and mild.

It is important to emphasize that bleaching-related sensitivity is transient and resolves spontaneously in virtually all cases. Studies have shown that any tooth sensitivity typically disappears within a few days to a week after completing treatment, with no lasting nerve damage. Strategies to manage sensitivity include using desensitizing toothpaste (with potassium nitrate) before and during bleaching, application of fluoride or calcium-based remineralizing gels after each session, shorter application times, and ensuring a few days gap between in-office sessions if multiple are needed [11]. Using a lower concentration in-office gel or performing fewer applications per visit can also mitigate severity, albeit at the cost of possibly needing more sessions for equivalent whitening. Some trials have tested pre-treatment analgesics (e.g., ibuprofen) to reduce bleaching sensitivity, but results are mixed [11].

GINGIVAL IRRITATION

Another side effect to consider is gingival or mucosal irritation. Because in-office gels are very potent, if isolation is not perfect, the gel contacting the gingiva can cause chemical burns (white blanching or ulceration of the gums). Dentists counteract this by applying protective barriers on the gingiva. At-home bleaching can lead to gum irritation if the trays leak or are overfilled with gel, causing the peroxide to overflow onto the gums. Generally, any soft tissue irritation is temporary; the affected gum areas usually recover within a day or two. Patients should be instructed on proper tray use and gel dosage to minimize this risk. Studies comparing methods suggest no major difference in overall incidence of gingival irritation between well-supervised at-home use and properly performed in-office treatment, as both can be made safe. A slight increase in minor gum irritation might occur with at-home if patients improperly apply the gel, whereas in-office has the advantage of professional control. In any case, no lasting periodontal issues have been linked to bleaching; the changes are superficial and reversible.

EFFECTS ON ENAMEL AND RESTORATIONS

Both techniques have been scrutinized for their effects on enamel surface morphology, mineral content, and existing restorations. Research indicates that neither in-office nor at-home bleaching causes clinically significant damage to enamel. Microscopic evaluations (SEM studies) have shown no visible enamel surface changes after completion of bleaching treatments, whether over-the-counter, at-home, or in-office, compared to unbleached controls [8]. Some in vitro studies do note minor alterations: for example, a slight increase in enamel porosity or roughness and a transient reduction in enamel microhardness immediately after bleaching. These effects are thought to result from peroxide's deproteinizing action and the chelation of calcium by ingredients in some gels (especially if they are highly acidic). Crucially, saliva exposure and fluoride use post-bleaching tend to remineralize and restore enamel hardness within days. Neither method appears to strip away enamel prisms or cause measurable loss of enamel thickness. Recent comprehensive reviews uphold that peroxide-based whitening is safe for enamel when instructions are followed, with only minimal, reversible changes observed [3]. Regarding dental restorations (composites, etc.), peroxide can superficially soften composite resin or alter its surface roughness and color. Both at-home and in-office bleaches can cause this to a similar degree. Thus, patients should be informed that existing tooth-colored fillings or bondings might not bleach (and could even require replacement to match the new tooth shade). There is no evidence that one method is gentler on restorations than the other; any peroxide exposure can affect composite, so it's a general consideration for bleaching.

PATIENT ACCEPTANCE AND CONVENIENCE

Each technique has practical pros and cons that may affect patient preference. In-office treatment offers the convenience of one or two appointments and the immediate gratification of seeing results right away, which some patients find highly motivating. It does not rely on patient compliance beyond showing up to the dental

office. However, it typically incurs higher cost (due to clinician time and materials) and the aforementioned risk of more intense short-term sensitivity. At-home treatment is generally more affordable and allows the patient to whiten at their own pace and in their own home, which many find comfortable. The trade-off is that it requires consistent daily use of trays for one or two weeks, and results are gradual. Some patients may not comply fully or may find wearing trays overnight inconvenient. Interestingly, a clinical study on patient acceptance found that the at-home tray technique was the most accepted method compared to others (including in-office and over-the-counter products) [8]. Patients appreciated the gentle, incremental improvement and the ability to control or pause treatment if sensitivity occurred. In contrast, the high intensity of in-office bleaching's result (and possibly the use of lights or long sessions with mouth open) can be uncomfortable for some. Both methods, when explained properly, have high satisfaction rates because the outcome – a whiter smile – tends to meet patient expectations in the majority of cases. The decision often comes down to individual lifestyle and how quickly the result is desired. Some patients even opt to combine approaches (often called "boost" or "jump-start" whitening): an initial in-office session to rapidly improve shade, followed by an at-home regimen to further enhance and stabilize the result. While combination protocols can be effective, studies have not shown them to dramatically outperform either method alone in final shade change [6]. They can, however, carry a higher total risk of sensitivity since the teeth undergo both procedures [12]. In one systematic review, tooth sensitivity was reported to be highest in groups that underwent combined in-office + at-home bleaching, slightly more so than in-office or at-home alone [12]. Therefore, when using a combined approach, extra care with desensitizing measures is warranted.

In summary, both at-home and in-office bleaching cause transient tooth sensitivity and occasional gum irritation, with no permanent deleterious effects on the teeth or oral tissues documented. At-home treatments tend to have milder sensitivity symptoms and are very safe when dentist-supervised, while in-office treatments produce faster results but with a risk of short-lived, more intense sensitivity episodes. Proper patient education and the use of desensitizing strategies can make either experience relatively comfortable.

DISCUSSION

The comparative evidence reviewed confirms that in-office and at-home tooth bleaching are largely equivalent in their ability to whiten teeth, aligning with the consensus of recent systematic reviews that neither modality is definitively "better" in terms of efficacy [5] [6]. This finding is clinically important: it indicates that dentists can confidently recommend either approach based on patient preferences and case-specific factors, rather than out of concern that one method might fail to whiten as well as the other. Both techniques leverage the same peroxide chemistry, and when concentration × time of exposure is balanced, the outcome in color change converges. In practical terms, an at-home protocol of 10–15% carbamide peroxide for 1–2 weeks can achieve a similar whitening effect to an in-office treatment with 35–40% hydrogen peroxide for about an hour total (often split into 2–3 applications in one visit). The degree of whitening attained depends more on the initial stain level and the total peroxide dose delivered than on the modality of delivery.

One aspect where subtle differences have been observed is color relapse and long-term effectiveness. While both methods maintain results well, the at-home approach might confer a slight advantage in long-term color stability, as noted in some studies [5, 6]. A possible explanation is that the extended exposure in at-home bleaching could better oxidize deeper or more stubborn stains, leading to less rebound over time. Additionally, patients who own bleaching trays can perform periodic touch-up applications (for example, a couple of nights of bleaching every few months) to counteract any staining that reoccurs, thereby prolonging the whiteness. In contrast, after an in-office treatment, a patient would need to return to the office or obtain a take-home kit for maintenance if the color relapses over time. This difference highlights a practical consideration: maintenance of whitening is often easier with an at-home system at hand. Clinicians increasingly integrate this by providing custom trays and gel to patients who underwent in-office bleaching, so they can manage relapse at home subsequently. In effect, the combination approach is popular – using the strength of in-office for a quick initial result and the convenience of at-home for maintenance – even though strictly speaking, the combination hasn't shown significantly greater whitening than at-home alone in controlled research [6]. It does, however, marry the benefits of both: immediate impact and sustained control.

Regarding tooth sensitivity, the discussion corroborates that it is an inherent transient side effect of peroxide use. The findings that at-home bleaching tends to produce less intense sensitivity might influence patient selection: individuals with known sensitive teeth or a lower pain tolerance may be better served with a gentler, at-home regimen where they can stop

or skip days if discomfort arises. Alternatively, if an in-office treatment is preferred (for instance, due to time constraints or the psychological impact of "instant" whitening), the clinician can employ measures to mitigate sensitivity – such as using a desensitizer on the teeth beforehand, avoiding light activation which can heat the pulp, and spacing out sessions. It is also notable that updated evidence suggests no significant difference in the overall risk of sensitivity between methods [11]; earlier assumptions that in-office causes sensitivity more frequently might have been due to older high-intensity techniques or lack of desensitizing agents. Modern bleaching gels often include additives like potassium nitrate or ACP to reduce sensitivity, and these are available for both in-office and at-home products [7]. For example, some in-office kits incorporate fluoride or calcium to help re-mineralize enamel, and many at-home kits come with separate desensitizing gels. Thus, the gap in patient comfort between the two methods has likely narrowed with improved product formulations. Still, in practice, many clinicians observe that in-office treatments, if they cause sensitivity, produce a short, sharp post-operative sensitivity, whereas at-home treatments may cause a lower-grade sensitivity that can persist during the days of treatment. Both scenarios are temporary. The patient's history of sensitivity and preference should guide the choice, and prophylactic use of desensitizing protocols can be employed in either case.

Another discussion point is the role of light activation in in-office bleaching. Dental marketing of in-office systems often touts special lamps or lasers to "accelerate" bleaching. However, current high-level evidence indicates that lights (whether LED, UV, or laser) do not significantly improve the bleaching outcome in terms of final shade or longevity [1]. They may slightly speed the release of peroxide or dehydrate the teeth to give an immediate lighter appearance, but meta-analyses have found no meaningful effect on the degree of whitening, and in some cases lights may increase the risk of post-operative sensitivity due to heat in older lamp systems [13]. Importantly, a 2018 systematic review and meta-analysis by Maran et al. concluded that activating in-office gel with light neither enhanced color change nor affected sensitivity when compared to in-office bleaching without light [1]. The most recent umbrella review (2024) similarly reported no difference in bleaching efficacy between light vs. non-light in-office protocols, and also noted that the use of light did not increase the intensity or risk of sensitivity [14]. Therefore, the presence or absence of a light is largely a matter of product design and marketing, not a determinant of success. Dentists can assure patients that light activation is optional and has no major impact on outcome. The key factor is the chemistry and contact time of peroxide on teeth. It's worth mentioning that some newer approaches have investigated using lights or lasers in novel ways – for example, applying low-intensity laser irradiation in hopes of reducing sensitivity via photobiomodulation [15]. There is preliminary evidence that certain laser protocols (e.g. a combination of infrared and visible light during bleaching) might reduce post-bleaching sensitivity duration and intensity [15], essentially by a biostimulatory effect on the pulp, but these methods do not make the teeth whiter than standard techniques [16]. In summary, light/laser activation is not necessary for effective bleaching and does not dramatically alter results; it should not be a decisive factor when comparing at-home vs in-office, since at-home techniques inherently do not use light and yet achieve equivalent outcomes.

From a regulatory and safety perspective, at-home bleaching is limited in some jurisdictions by the concentration of peroxide allowed without professional oversight. For instance, the European Union regulations permit only up to 6% hydrogen peroxide in products sold directly to consumers (higher concentrations must be applied by a dentist) [2]. In the United States, the American Dental Association (ADA) has given its Seal of Acceptance to dentist-dispensed home bleaching products containing 10% carbamide peroxide, affirming their safety and efficacy for home use [7]. These endorsements underscore that dentist-supervised at-home bleaching is considered a safe treatment when proper protocols are followed. Meanwhile, in-office treatments, using higher peroxide percentages, are restricted to professional application – which provides a safety net through professional technique and isolation. The presence of a dental professional also means any adverse reaction can be promptly managed. It bears repeating that both techniques, as traditionally practiced, have excellent safety records. Even though animal studies have shown that very high concentrations of peroxide can induce pulp inflammation or necrosis in extreme circumstances (e.g. in rat molars or when applied repeatedly to exposed human pulp tissue) [17], these scenarios do not translate to normal clinical use. In vital teeth with intact dentin, no cases of irreversible pulp damage have been reported from bleaching agents in the concentrations and durations used in practice. Nonetheless, clinicians should avoid overuse of high-concentration bleaching and adhere to recommended exposure times to minimize any risk of pulpal irritation.

Patient selection and clinical judgment remain paramount in choosing the bleaching method. Both

techniques have limitations. For example, neither will significantly change the color of restorations (crowns, veneers, fillings), so those might need replacement for color matching after bleaching. Severely discolored teeth (such as tetracycline-stained teeth) often need longer treatment: extended at-home bleaching (several months of nightly use) has been shown to gradually lighten tetracycline stains, whereas in-office alone might not achieve as much change due to the depth of stain. On the other hand, a very quick result might be needed for a special event, in which case an in-office session is justified. Patients with very sensitive teeth might do better with a slower, at-home approach or a lower concentration in-office treatment over multiple shorter visits. Ultimately, the bleaching outcome depends on patient-specific factors (initial tooth shade, type of staining, enamel thickness, etc.) as well as adherence to instructions. The dentist should tailor the treatment plan to the individual – in some cases recommending an at-home kit, in others an in-office treatment, or a combination – knowing that the end result (whiteness) can be reached via either route. Importantly, the dentist should manage expectations and educate the patient that neither method is permanent; good oral hygiene and occasional maintenance are needed to keep teeth white after bleaching.

The evidence base for tooth bleaching is quite robust, with multiple RCTs and several systematic reviews in the last decade. The agreement among high-level studies that at-home and in-office methods are equivalently effective strengthens confidence in our conclusions. However, some limitations exist in the literature: not all studies use the same concentration or regimen, making direct comparisons tricky. For instance, an at-home protocol in one study might involve 2 weeks of nighttime use, whereas another study might use 2 hours/day for 1 week – these could yield different outcomes, yet both are labeled "at-home." Similarly, in-office protocols can vary (one vs. two sessions, with or without light, 35% *vs* 40% peroxide, etc.). These variations introduce heterogeneity. The systematic reviews attempt to account for this by pooling similar studies, but there is always some uncertainty. Moreover, patient subjective factors (like how they perceive sensitivity or satisfaction) can be hard to quantify and compare. Despite these variables, the overarching trends are consistent.

Future research could explore optimizing combination protocols (to see if there is a way to significantly reduce sensitivity while achieving the same whitening in less time) or investigate new bleaching agents (such as non-peroxide whiteners like PAP – phthalimidoperoxycaproic acid – as alternatives). But as of now, hydrogen peroxide-based bleaching remains the gold standard, and the practitioner can choose between an at-home or in-office delivery knowing that each has its place and can produce a brighter smile safely.

CONCLUSIONS

In summary, both in-office and at-home tooth bleaching techniques are effective modalities to achieve significant tooth whitening, and current evidence-based comparisons do not show a clear superiority of one over the other in final outcome. The main points of conclusion are:

- **Bleaching Efficacy:** In-office and at-home bleaching produce equivalent improvements in tooth color. When comparing properly administered protocols, neither method yields significantly whiter teeth than the other. Both can achieve satisfying, natural-looking whitening results across a range of vital tooth discolorations.
- **Longevity:** Whitening results from both techniques are long-lasting (often 1–3 years or more). There is no strong difference in relapse rates, though at-home treatments may have a slight edge in maintaining color over time, possibly due to easier touch-ups. Overall, both require maintenance in the long term (good oral hygiene and periodic re-bleaching) to sustain the brightest shade. Most patients can expect a durable result for at least a year or two before any noticeable color rebound, and even then the teeth remain lighter than baseline in the absence of heavy staining.
- **Safety and side effects:** Both methods are safe for teeth and surrounding tissues when used as directed. Transient tooth sensitivity is the most common side effect in each case, affecting a large portion of patients but resolving after treatment completion. In-office bleaching tends to cause sensitivity of higher intensity immediately post-treatment, whereas at-home sensitivity is typically milder but can persist in low levels throughout the treatment period. The overall risk of experiencing some sensitivity is similar for both. No permanent enamel damage or other serious adverse effect has been documented with either method; any minor enamel changes are reversible and soft tissue irritation is temporary and avoidable with proper technique.
- **Patient factors:** The choice between in-office and at-home should be individualized. In-office bleaching offers rapid results in a controlled setting (ideal for those desiring instant gratification or under time constraints), while at-home bleaching offers convenience, lower cost, and more gradual whitening (suitable for those willing to commit to daily application and seeking a gentler process). Patient preference, compliance likelihood, tooth sensitivity history, and budget are all considerations. Both methods have high patient

satisfaction when expectations are properly managed, and a combination approach can be used to leverage the advantages of each (immediate change plus easy long-term maintenance). It should be noted that use of lights/lasers in in-office treatment is optional and does not fundamentally improve outcomes, so patients should not equate "laser whitening" with a better result.

In conclusion, a dentist can recommend either in-office or at-home bleaching with confidence in its efficacy and safety profile. Both are valuable tools in cosmetic dentistry. The equivalence in outcome means that factors such as patient lifestyle, tolerance for sensitivity, and need for professional supervision can drive the decision. By understanding the nuances of each technique, clinicians can better tailor tooth whitening treatments to individual needs, ensuring effective results with minimal side effects. Continuing research and well-designed clinical trials will further refine these guidelines, but at present, the evidence supports that a bright, white smile is achievable via both routes to nearly the same extent.

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SHORT COMMUNICATION

CONTENTS

Analysis of histopathological results of the endometrium in breast cancer patients treated with tamoxifen. Preliminary report

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ABSTRACT

Aim: The study aimed to compare histopathological outcomes of the endometrium in breast cancer patients treated with tamoxifen. The analysis included asymptomatic women referred for abnormal ultrasound findings and symptomatic women with abnormal uterine bleeding.

Materials and Methods: The study included 85 patients hospitalized between 2013 and 2024 at the Medical University of Warsaw. Group I (n=41) comprised patients with abnormal ultrasonographic findings, while Group II (n=44) included women with abnormal uterine bleeding. All patients underwent hysteroscopy with subsequent histopathological tissue analysis.

Results: The most frequent histopathological diagnosis in both groups was the presence of endometrial polyps (Group I: 51.22%; Group II: 36.36%). Endometrial cancer was diagnosed in 7.32% of Group I and 9.09% of Group II patients, indicating a comparable prevalence of serious pathology. Statistically significant differences ($p < 0.05$) were noted between the groups only for non-atypical hyperplasia and proliferative endometrium.

Conclusions: The findings confirm that endometrial polyps are the most frequent pathology associated with tamoxifen use in this cohort. This preliminary report underscores the critical need for further targeted research focusing on population-specific factors and menopausal status to develop clear, personalized clinical guidelines for screening and surveillance, particularly for premenopausal women, independent of symptomatic presentation.

KEY WORDS: breast cancer, endometrial hyperplasia, endometrial carcinoma, endometrial polyps, tamoxifen therapy

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INTRODUCTION

Breast cancer is one of the most common malignancies in women and is a heterogeneous, phenotypically diverse disease composed of several biologic subtypes that have distinct behavior and response to therapy [1]. A significant proportion (75%) of cases are characterized by positive estrogen receptor (ER) and progesterone receptor (PR) status [2].

One of the most frequently used substance in a patient with positive ER is tamoxifen (TAM), a nonsteroidal selective estrogen modulator (SERM) with mixed antagonist and agonist properties [3]. Tamoxifen was approved by the Food and Drug Administration in 1977 for the treatment of women with advanced breast cancer and several years later for adjuvant treatment of primary breast cancer [3]. It is antiestrogenic in breast cancer and by mechanism of binding to estrogen receptors it blocks tumour proliferation. For premenopausal women at diagnosis, the NCCN Guidelines for Breast Cancer suggest tamoxifen for 5 years, with or without ovarian suppression, or an AI (Aromatase Inhibitors) for 5 years combined with ovarian suppression or ablation (category 1). Women who remain premenopausal can consider tamoxifen for another 5 years (10 total) or no further endocrine therapy. Those who become postmenopausal can consider 5 more years of tamoxifen or switch to an AI for 5 years [4]. The use of TAM as adjuvant hormonal therapy is effective in reducing recurrences and extending survival in premonopausal and some postmenopausal patients with breast cancer. It is also used in chemoprevention in selected patients at increased risk for breast cancer.

Despite tamoxifen's effectiveness in inhibiting estrogen receptors in breast tissue, it also exhibits agonistic effects on the endometrium, leading to adverse proliferative changes. Although premenopausal women with breast cancer are usually treated with tamoxifen as first-line adjuvant hormone therapy, it remains unclear whether the use of tamoxifen in premenopausal women is associated with an increased risk of

several uterine diseases [5]. Over the past decades tamoxifen was correlated with the risk of endometrial carcinoma with an increased risk in postmenopausal women [6, 7]. However, the most common type of pathology are endometrial polyps also in postmenopausal women [8, 9]. Other types of uterine pathology (endometrial hyperplasia and carcinoma) may also occur and the risk depends on many factors such as previously mentioned menopausal status or aim of use (whether the tamoxifen is used for chemoprevention or treatment of breast cancer). Currently, there are no clear guidelines regarding routine screening for gynaecologic diseases in premenopausal women taking tamoxifen, which remains a subject of ongoing debate.

AIM

The study aimed to compare the histopathological results of the endometrium in breast cancer patients who underwent adjuvant tamoxifen therapy among two groups: asymptomatic women, those referred for an ultrasound procedure due to abnormal endometrial imaging, and patients with abnormal uterine bleeding (e.g., postmenopausal bleeding or heavy, irregular menstrual bleeding).

MATERIALS AND METHODS

The population consists of 85 patients hospitalized at the Department and Clinic of Obstetrics, Gynecological Diseases, and Oncological Gynecology at the Medical University of Warsaw between 2013 and 2024. The study included patients with a history of breast cancer who were receiving adjuvant tamoxifen therapy and underwent hysteroscopy, during which histopathological examination was performed and adequate tissue samples were obtained for analysis. The study population was divided into two subgroups: Group I comprised 41 (48.24%) patients whose histopathological verification was prompted by abnormal endometrial findings on follow-up ultrasonography, while Group II included 44 (51.76%) women with abnormal uterine bleeding. Abnormal bleeding was defined as postmenopausal bleeding or heavy, irregular menstrual bleeding. The average age of patients in Group I was 58 years, whereas in Group II, it was 54 years. The mean BMI was 27.85 for bleeding patients and 26.13 for non-bleeding patients. A detailed analysis of histopathological differences in the two subgroups was performed, followed by further data interpretation.

RESULTS

In groups I and II, the most common histopathological diagnoses were endometrial polyps, occurring in 21 (51.22%) women in group I and 16 (36.36%) in group II. Endometrial polyps hyperplasia was found in 8 (19.51%) in group I and 7 (15.91%) in group II. Endometrial cancer was diagnosed in 3 women (7.32%) in group I and 4 (9.09%) in group II. Atypical hyperplasia was observed in 2 patients (4.88%) in group I and 1 (2.27%) in group II. Non-atypical hyperplasia was identified in 1 patient (2.44%) in group I and 3 (6.82%) in group II. Other histopathological diagnoses, such as atrophic endometrium, submucosal fibroids, and proliferative endometrium, were respectively present in group I as 4 (9.76%), 1 (2.44%), and 1 (2.44%), while in group II, they were 1 (2.27%), 1 (2.27%), and 10 (22.73%). Significant differences were found only for non-atypical hyperplasia and proliferative endometrium ($p < 0.05$), while other changes were not statistically significant (Fig. 1-2).

DISCUSSION

Patients treated with tamoxifen may develop uterine pathology, often presenting as asymptomatic or with abnormal uterine bleeding (AUB). Studies show that AUB occurs in over 50% of premenopausal patients and up to 25% of postmenopausal patients on tamoxifen [10, 11]. In our study, AUB was present in 60.0 % of premenopausal patients and 45.5% of postmenopausal patients, corroborating these findings.

Tamoxifen use significantly increases the risk of uterine pathology, including endometrial polyps, hyperplasia, and carcinoma, with reported risks of uterine pathology reaching up to 67% within four years of use [12–14]. In our cohort, endometrial pathology was diagnosed in 83.33 % of patients in group I and 70.45 % in group II, slightly exceeding reported data, possibly due to rigorous diagnostic protocols in our institution.

The most common uterine pathology associated with tamoxifen use is endometrial polyps, reported in approximately 21.1 per 1,000 patients annually in the NSABP P-2 trial [11]. Similarly, our study found endometrial polyps in 50.0 % of group I and 36.36% of group II patients. This aligns with findings from other studies, such as Ryu et al., where tamoxifen users exhibited higher rates of polyps than non-users [15]. However, our slightly higher rates may reflect differences in cohort characteristics or regional variations in care practices.

Endometrial carcinoma (EC) is a well-recognized risk associated with tamoxifen, particularly in postmenopausal patients. The NSABP P-2 trial reported an average annual EC incidence of 2.3 per 1,000 patients [11], and studies like Taponeco et al. observed a 7.8% EC rate among patients with postmenopausal bleeding [14]. In our study, EC was diagnosed in 7.14% of group I and 9.09% of group II patients, indicating a comparable

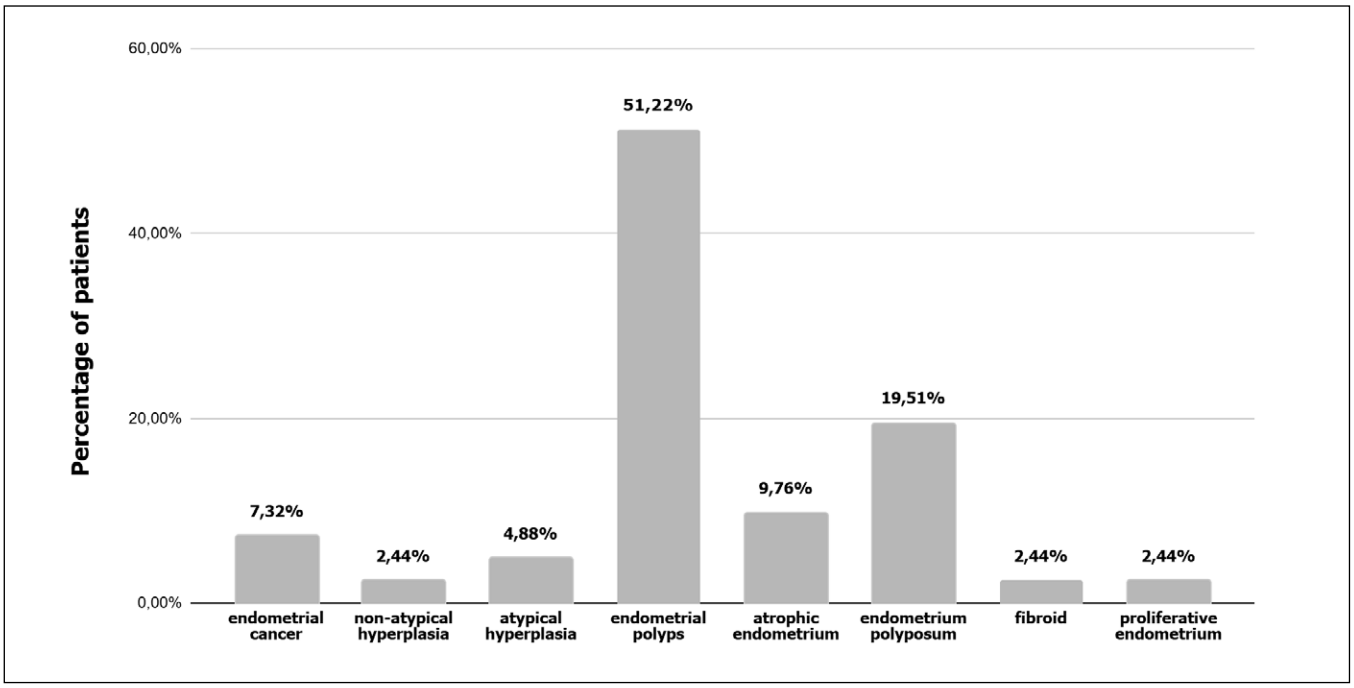

Fig. 1. Histopathological alterations in Group I
Source: compiled by the authors of this study

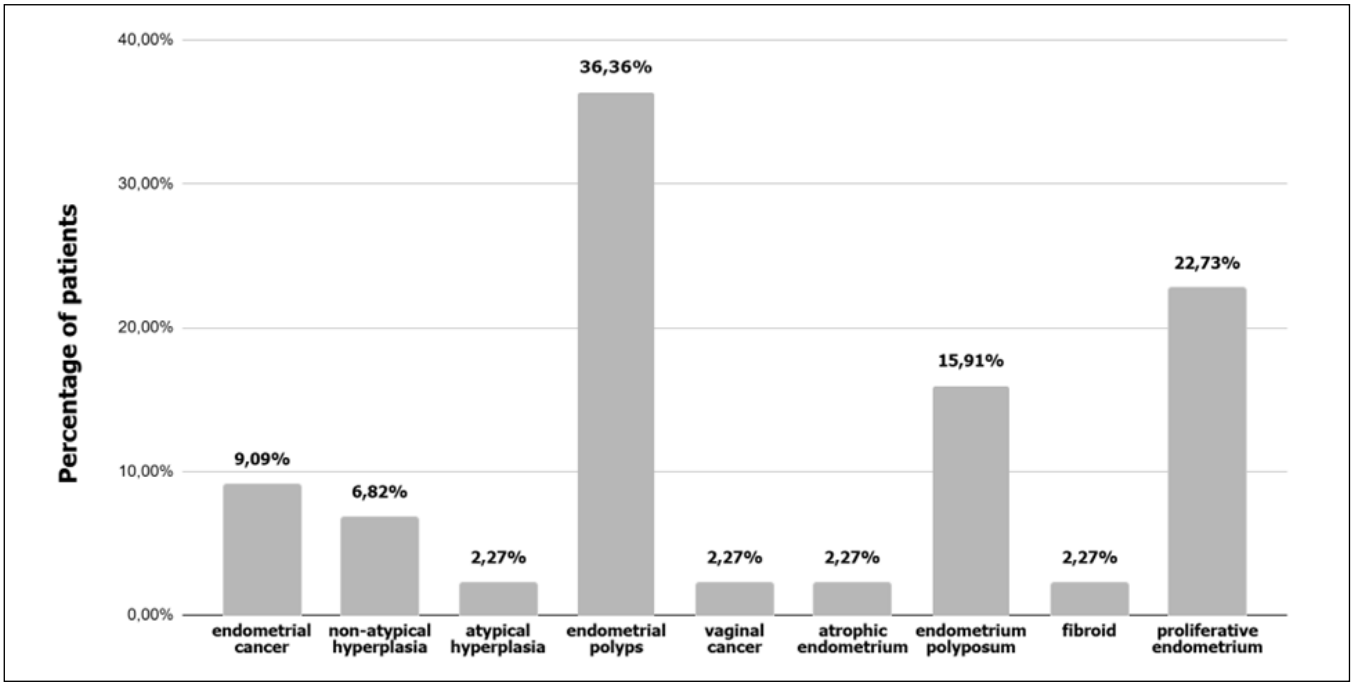

Fig. 2. Histopathological alterations in Group II
Source: compiled by the authors of this study

prevalence. Interestingly, no EC cases were identified in premenopausal patients with AUB in our cohort, consistent with findings by Taponeco et al. [14].

Non-atypical and atypical hyperplasia are other notable pathologies. Cohen et al. reported a time-dependent increase in hyperplasia rates in tamoxifen users [13]. In our cohort, non-atypical hyperplasia was identified in 2.38% of group I and 6.82% of group II patients, while atypical hyperplasia was found in 4.76% of group I and 2.27% of group II patients. These results are within expected ranges but highlight a slight increase in non-atypical hyperplasia in group II, potentially due to longer tamoxifen exposure.

Some cases in our study presented diagnostic challenges.

Given the high prevalence of tamoxifen-associated uterine pathologies, further studies are needed to

refine diagnostic criteria and management strategies. Future research should focus on stratifying risk factors and evaluating alternative therapies to mitigate these adverse effects while maintaining tamoxifen's efficacy.

In the latest NCCN Clinical Practice Guidelines in Oncology for Uterine Neoplasms (Version 1.2025), tamoxifen use is highlighted as a risk factor for uterine neoplasms, alongside increased estrogen levels due to obesity, diabetes, and high-fat diet; early age at menarche; nulliparity; late age at menopause; Lynch syndrome; and ages between 55 and 64 years . These guidelines recommend hormonal therapy predominantly for lower-grade endometrioid histologies, especially in patients with small tumor volume or indolent growth [16].

Tamoxifen is also listed among other recommended regimens for hormonal therapy in recurrent or metastatic endometrial carcinoma. It is used alone or in combination with megestrol acetate as an alternating regimen [17].

CONCLUSIONS

While this study confirms endometrial polyps as the most frequent pathology in tamoxifen-treated patients, it critically highlights the need for targeted research focusing on population-specific factors (especially within the Polish population), menopausal status, and the role of BMI. Developing clear, personalized clinical guidelines for screening and surveillance, particularly for premenopausal women, is essential to optimize the early detection of serious pathologies, independent of symptomatic presentation.

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